Perspective

A Phage Foundry Framework to Systematically Develop Viral Countermeasures to Combat Antibiotic-Resistant Bacterial Pathogens

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SUMMARY

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

PREAMBLE: KNOWLEDGE GAPS IN AMR

AMR has been a consistently growing global problem and has been called the "invisible pandemic" (Tacconelli et al., 2018; Mahase, 2019; Knight et al., 2021). Failure to stem the rising tide of multidrug-resistant (MDR) bacterial and fungal pathogens is estimated to have a real worldwide economic cost running into trillions of USD by severely debilitating agriculture, dairy, aquaculture, livestock, and poultry industries among others, in addition to the tragic human cost (Executive Office of the President, 2014; O'Neill and Others, 2016; Tacconelli et al., 2018; CDC Report, 2019). A number of factors contribute to the selection and spread of antibiotic resistance genes and the known and emerging microbial pathogens that host them (Buckley et al., 2021) (reviewed in detail earlier (White et al., 2005; Payne et al., 2007; Chadwick and Goode, 2008; Mayers, 2009; Fair and Tor, 2014; Holmes et al., 2016; Surette and Wright, 2017; Gotte et al., 2018; Anderson et al., 2020; Andersson et al., 2020)) (Figure 1), for example, (1) misuse of frontline antibiotics and other antimicrobials; (2) climate-driven niche destruction and induced migration of host and pathogens; (3) agricultural intensification; (4) increasing environmental pollution with drugs, industrial chemicals, and pesticides; (5) a denser population of humans along with closer contact with animal reservoirs, and in some places (6) poor public infrastructure. There is an increasing realization that this rise is not just about the individual fitness of the resistant strain but the ecology in which it resides (Brockhurst et al., 2019; Andersson et al., 2020).

Faster dispersal mechanisms among differentially selective reservoirs (Figure 1), rapid adaptation to new zoonotic hosts, increasing "safe passage" opportunities for horizontal transfer and recombination of genetic elements carrying resistance genes to nonpathogens, co-occurring microbial community members that support/trigger transfer of resistance traits and process of adaptation are all being increasingly recognized as significant elements in the rise of fit and resistant infectious agents (Palmer and Kishony, 2013; Hu et al., 2016; Andersson et al., 2020; Antunes et al., 2020). Many of these adaptations lead to easily spreadable cross-resistance toward broad as well as narrow spectrum antibiotics without seeming a fitness tradeoff (Andersson and Hughes, 2010; Palmer and Kishony, 2013; Baym et al., 2016; Tyers and Wright, 2019). In addition, these same mechanisms are used to adopt or fight off pesticides, ionophores, fungicides, metals, biocides/disinfectants, and diverse xenobiotics (White et al., 2005; Baker-Austin et al., 2006; Pal et al., 2017; Kampf, 2018; Andersson et al., 2020; Getahun et al., 2020; Knight et al.,

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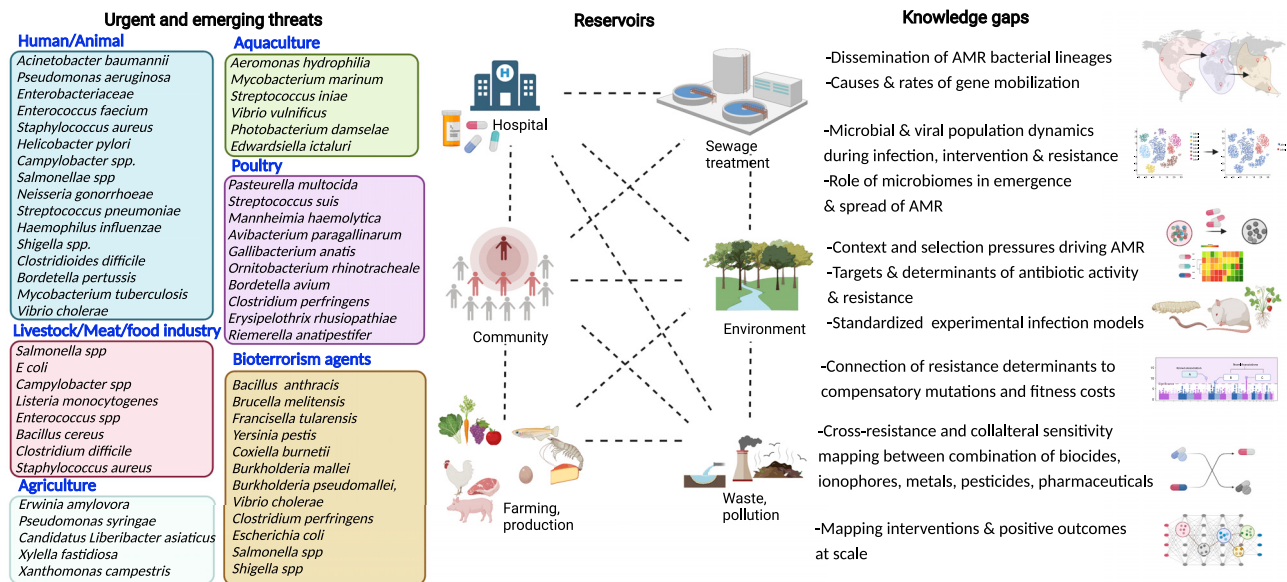
Figure 1. The interconnected web of routes to spread AMR and current knowledge gaps

A nonexhaustive list of urgent and emerging threats is shown (left panel) and are derived from the World Health Organization (WHO) and US-Center for Disease Control and Prevention (CDC) bulletins. One Health initiative aims to achieve optimal health by recognizing the interconnections between people, animals, plants, and their shared environment (middle panel). A list of key knowledge gaps (right panel) that still exist and need research to develop countermeasures to tackle AMR spread.

2021; Lindell et al., 2022). Indiscriminate use of these agents in agriculture, aquaculture, food processing, and industrial processes has further accelerated the emergence of pathogen variants displaying cross-resistance to unrelated but clinically important antimicrobials (White et al., 2005; Davin-Regli and Pagès, 2012; Guardabassi and Courvalin, 2019; Andersson et al., 2020; Elekhnawy et al., 2020; Verweij et al., 2020; Kang et al., 2022).

To compound these aforementioned challenges, no new class of antibiotics have been brought to market in the last five decades (Plackett, 2020; Theuretzbacher et al., 2020). Further, developing new antibiotics to overcome new mechanisms of antibiotic resistance is nontrivial (Årdal et al., 2020; OECD and World Health Organization, 2020). It is time-consuming, expensive, and cumbersome to identify such new molecules either from natural sources or from rational design or screening from synthetic libraries in a short time (Fair and Tor, 2014; Plackett, 2020). The approval process for such new molecules is expensive and often may take longer than the adaptation times of the pathogens (Årdal et al., 2020). Recently developed programs are helping defray some of these costs and establishing new routes to fund the research and development of novel therapeutics (Alm and Gallant, 2020; Årdal et al., 2020).

One of the critical piece in responding effectively to rising AMR is to build the capability to rapidly detect from when and where a particular pathogen variant emerges so that the mechanisms that have allowed its emergence can be identified and the appropriate strategy can be applied with respect to where to intervene (patient, animal, and abiotic pools) and with which agent(s) (Boolchandani et al., 2019). This requires improved infrastructure and appropriate policy changes for establishing surveillance networks at the regional, national, and global levels (Fauci, 2001). In this regard, One Health initiative offers a well-coordinated approach to elucidate and control the rise of AMR through specifically respecting the interconnectedness among humans, livestock, pets, wildlife, and environmental systems with the goal of optimal health outcomes for everyone (National Academies of Sciences, Engineering, and Medicine, 2018; Walsh, 2018) (Figure 1). It has also led to the search for combinatorial therapies and alternative modalities/approaches to control infection/spread of AMR bacteria such as the use of bacteriophages (i.e. bacterial viruses), obligate parasites that infect and kill specific bacterial strains (Clatworthy et al., 2007; Yeh et al., 2009; Villa and Crespo, 2010; Kim et al., 2014; Baym et al., 2016; Czaplewski et al., 2016; Dickey et al., 2017; Mariathasan and Tan, 2017; Baker et al., 2018; Douafer et al., 2019; Heselpoth et al., 2019; Tyers and Wright, 2019; Poolman, 2020; Theuretzbacher et al., 2020; Bottery et al., 2021), and focus

on this opinion piece. We argue below, along with the international network of sophisticated surveillance programs, we need focused and federally funded programs to form a critical scaffolding that could support next generation of more rationally and mechanistically designed, ecologically understood, and more effectively manufactured and deployed antibiotics with phage therapy at its core.

PHAGE THERAPY TO TACKLE AMR

Phages represent the most abundant biological entities in nature—10-fold greater than bacteria (Hendrix et al., 1999). Double-stranded DNA (dsDNA) phages can be readily isolated from the environment and display specificity to their target bacteria (Kutter and Sulakvelidze, 2004). Based on the infection lifestyle, phages are classified either as lytic or as temperate. In the lytic mode, phage infection leads to delivery of genetic material, lysis of target bacteria, and release of progeny. Temperate phages on the other hand can switch between lysogenic to lytic cycle based on conditions, where the lysogenic mode leads temperate phage genomes to persist as prophages integrated on to the target bacterial genomes or exist extrachromosomally. Many recent reviews have outlined the possible uses and advantages of using phage as a therapeutic agent (Hagens and Loessner, 2010; Loc-Carrillo and Abedon, 2011; Frampton et al., 2012; Young and Gill, 2015; Koskella and Taylor, 2018; Svircev et al., 2018; Bull et al., 2019; Gordillo Altamirano and Barr, 2019; Hesse and Adhya, 2019; Kortright et al., 2019; Luong et al., 2020b; Hatfull et al., 2021; Pirnay et al., 2021). Detailed discussions on using phages to treat infections in plants, animals, and humans have been put forth in numerous monographs and books (Kutter and Sulakvelidze, 2004; Sabour and Griffiths, 2010; Hyman et al., 2012; Reinheimer, 2012; Azeredo and Sillankorva, 2017; Jassim and Limoges, 2017; Górski et al., 2019). Recent successes in using dsDNA phages to treat antibiotic-resistant infection under compassionate use protocols have received much attention in the western countries despite nearly a century of anecdotal use in other regions of the eastern world (Kutter et al., 2010, 2015; Abedon et al., 2011; Kortright et al., 2019; Hatfull et al., 2021).

Phages have many unique properties that make them particularly attractive and are increasingly recognized as potentially transformative agents precisely because they address some of the key issues mentioned earlier in the AMR preamble section. Most known MDR pathogens have known phages that can specifically attack them. Phages tend to be bactericidal rather than bacteriostatic and those that are temperate can be often converted to a lytic form by rational genetic manipulation or natural mutant selection (Lenski, 2017b; Dedrick et al., 2019). Phages are readily identified in the environment through sequencing, and the evolutionary patterns of the Red-Queen warfare between target pathogen and phages could possibly be tracked via sequencing as well, thereby identifying the genetic bases of the mechanisms of resistance and counter resistance (Hussain et al., 2021; LeGault et al., 2021). The ability of phages to rapidly evolve to evade target pathogen resistance can be exploited using *in vitro* directed evolution to "train" libraries of phages against panels of targets to create banks of complementary phage antimicrobial agents for cocktails (Rohde et al., 2018; Burrowes et al., 2019; Abdelsattar et al., 2021; Borin et al., 2021; Eskenazi et al., 2022; Torres-Barceló et al., 2022). The small genomic size of phages enable both full genome synthesis and possibly "booting" (producing viable phage particles from synthetic DNA) when isolation is difficult as well as efficient engineering of designed genetic changes (Chan et al., 2005; Ando et al., 2015; Pires et al., 2016, 2021a, 2021b; Kilcher et al., 2018; Lemire et al., 2018; Dunne et al., 2019; Kilcher and Loessner, 2019; Weynberg and Jaschke, 2020; Lenneman et al., 2021). Designer changes include engineered genetic payloads that increase toxicity, counteract defenses, and potentially suppress horizontal gene transfer of resistance genes by, for example, degrading the target bacterial genome rapidly (Lu and Collins, 2007; Yosef et al., 2015, 2017; Barbu et al., 2016; Dunne et al., 2019; Kilcher and Loessner, 2019; Yehl et al., 2019; Lenneman et al., 2021). These approaches allow diversification of antimicrobial targets, leading to more effective cocktail design. Phages' physical form as a proteinaceous particle allows functional and programmed decoration of its surface that can confer the ability to penetrate biofilms, better control of *in situ* targeting, and enhanced pharmacokinetic (PK) and pharmacodynamic (PD) properties (Dąbrowska, 2019; Dąbrowska and Abedon, 2019). Finally, once created, their synthesis and formulation could prove scalable, distributable, and cost-effective and offer paths to alleviate some of the industrial and regulatory hurdles associated with other types of antimicrobials (Pelfrene et al., 2016; Malik et al., 2017; Malik and Resch, 2020; Malik, 2021).

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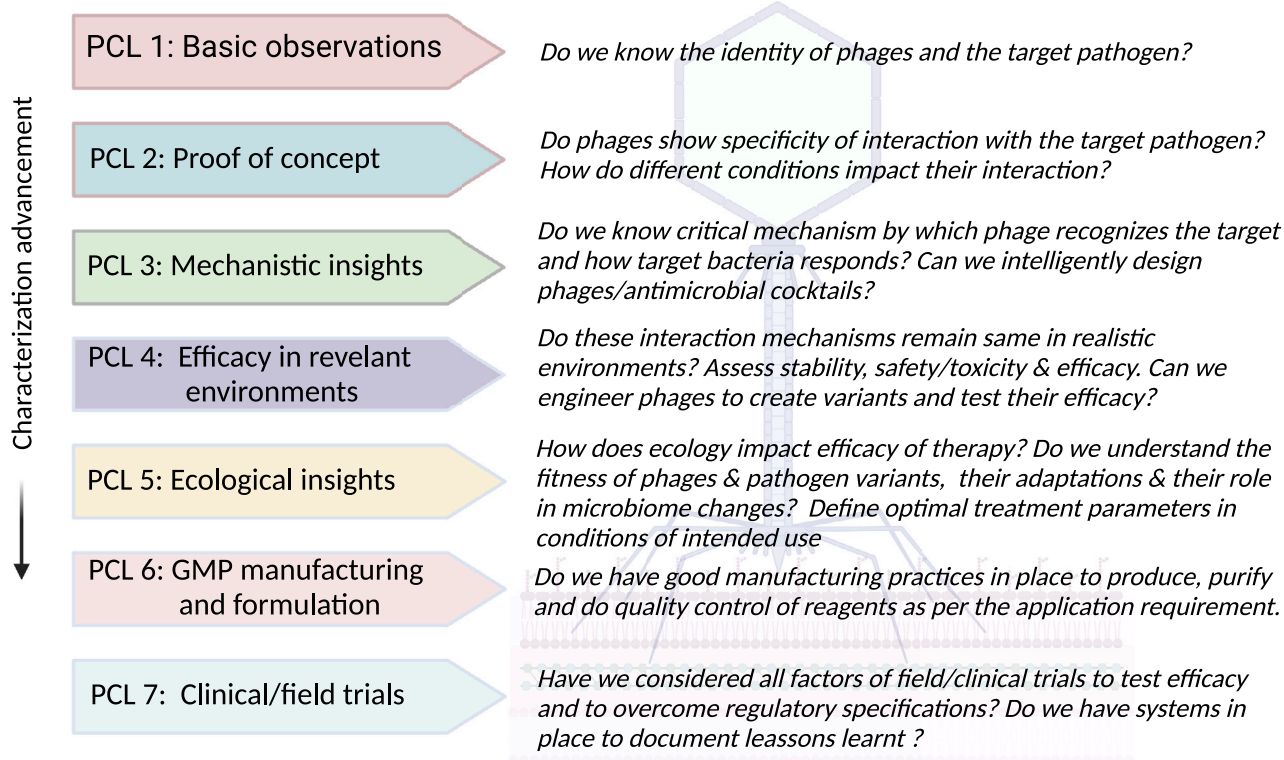
we need to be able to identify and "engineer" effective interventions. This is not realistically possible using our current antibiotic/antimicrobial arsenal. But with the natural and unlimited reservoir of phages and their inherent engineerability, there is hope to develop a flexible and nimble platform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

However, application of phages in therapy and biocontrol present challenges as well, and many of these challenges share a substantial overlap with AMR knowledge gaps listed in Figure 1. Although there is great progress in the technologies per se for characterizing and engineering phages, there is still lack of progress in our ability to rationally and predictably design a phage formulation to effectively eliminate a target pathogen and to ensure it works robustly across the spectrum of the population variation of a given pathogen in its natural or real-world environmental matrices (Meaden and Koskella, 2013; Young and Gill, 2015; Koskella and Taylor, 2018; Brüssow, 2019; Caflisch et al., 2019; Dąbrowska and Abedon, 2019; Hesse and Adhya, 2019; Kortright et al., 2019; Górski et al., 2020; Luong et al., 2020b; Federici et al., 2021; Hatfull et al., 2021; Pirnay et al., 2021); For example, despite the recent progress made in phage-target pathogen matching methods (Henry et al., 2012; Estrella et al., 2016), it remains difficult to rapidly and economically identify phages/phage-antibiotic combinations to which a patient's infections are susceptible or predict which multiple phages in a phage collection ("phage bank") may be synergetic (or not) in their antimicrobial activity due to independent mechanisms of interaction, cross-resistance, and infectivity profile with the target pathogen (Wright et al., 2021; Segall, et al., 2019; Gu Liu et al., 2020; Al-Anany et al., 2021; Gordillo Altamirano and Barr, 2021; Markwitz et al., 2022; Torres-Barceló et al., 2022). There are currently no guidelines for the delivery and dosing of phages that guarantee access to appropriate target infection sites in sufficient numbers so that a self-sustaining replicative cycle can be established. Lack of adequate knowledge on the phage-bacteria ecology in the therapeutic environment makes it difficult to assess or predict how variable host environments (dysbiosis or healthy status) and the extended microbial community impact the therapeutic effect of phages. It still remains a challenge to design natural phage interventions or engineer natural phages we have on hand to respond to variations in pathogens or new threats, in natural contexts. Finally, we are only at the early stages of establishing appropriate environmental surveillance systems that can potentially identify emerging threats early enough to be able to develop an intervention and to deliver it before an outbreak becomes epidemic or pandemic.

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

Here, we argue that a systematic response to emergence of AMR bacteria can be significantly augmented if there is increased and coordinated investment in those aspects that enable phages to be harnessed for therapies in combination with each other i.e., cocktails and/or with classical antimicrobials. We propose this framework as a coordinated "Foundry," or Federation of Foundries, which collaborates with the international AMR infrastructures already in place to coordinate the sampling, observation, and characterization protocols. In this manner, these efforts can drive the discovery, harnessing, and engineering of therapeutic phages in a responsive, knowledge-building, and cost-effective manner. The Foundry would (1) expand and deepen surveillance efforts to track pathogens, their genetic variants and phages, and resistant/defense elements; (2) systematize sampling and isolation and basic characterization of phages along with target pathogens and other critical community members; and (3) for cases where there was sufficient novelty or need, it would be equipped for deep molecular characterization and engineering of environmentally mediated phage/target interaction through advanced genetics and adaptive laboratory

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 ?
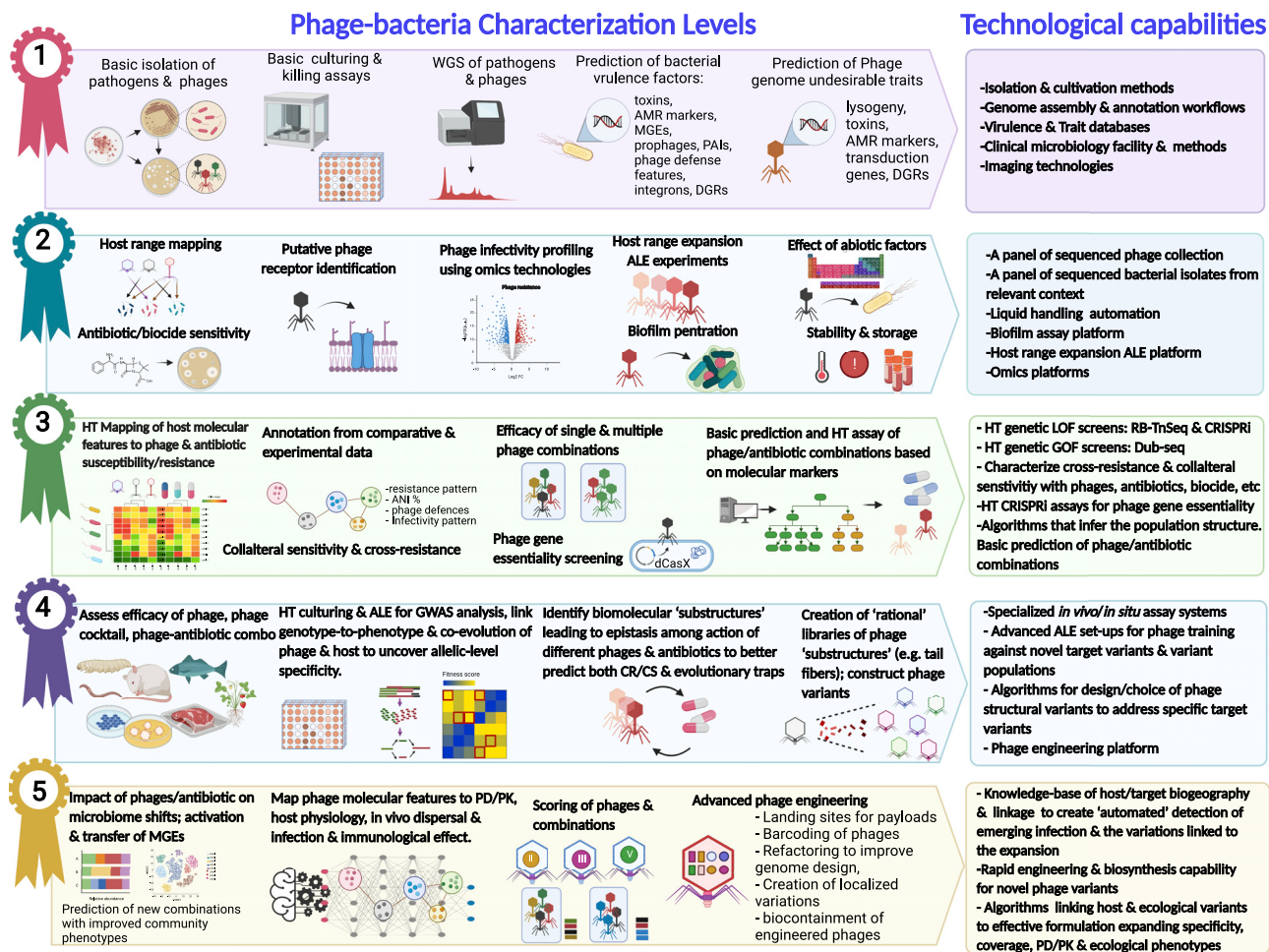
Figure 2. Phage-bacteria characterization level (PCL) scale
The characterization levels of phage-bacteria interactions and key questions to assess the tier level.

evolution (ALE). The Foundry would be designed to bring these phages into increasing readiness for rational deployment as biocontrol agents or pharmaceuticals so that the time to acceptance by regulatory bodies could be shortened. Formal phage characterization, readiness level, and testing criteria would be a product of this effort. Finally, a fully operational Phage Foundry will share phages and bacterial pathogen characterization platforms, engineering technologies, knowledge-base, and characterized phages to researchers worldwide in a fair and equitable manner. Here we propose the concept of the Phage Foundry and a first version of these characterization and readiness level specifications stemming from this body.

RAISING THE READINESS LEVEL OF PHAGE AND COCKTAILS FOR THERAPIES

Here we define an incremental "Phage-bacteria Characterization Level (PCL)" "scale" indicating current practices and capabilities needed to achieve each of those levels (Figures 2 and 3). The PCL scale is inspired by the technology readiness levels (TRL) scale developed by US-NASA and US-DoD that has been adopted widely by diverse industries (Sadin et al., 1989; Banke, 2010; Straub, 2015; Buchner et al., 2019; Hofmann et al., 2020). Similar to TRL, the PCL scale provides a set of characterization metrics for a specific set of reagents in the context of an application and operational environment. Level 1 represents the most basic characterization needed for using phages in an application, whereas Level 7 represents the highest level needed to successfully deploy phages for a specific application in a given scenario/environment. As every biocontrol/therapeutic application has different specifications for characterization, efficacy, safety, stability, formulation, delivery modes, and regulatory requirements, not all applications need phage-target host interactions characterized at top level. The appropriate level of characterization for phages depends on their intended application, their specification, environmental context, and cost and time needed to cross-certify tiers. Here we focus on Level 1 to 5 that represent the core of this categorization roadmap and leave out Level 6 and 7, as these focus primarily on scale up, manufacturing under good manufacturing practices (GMP) and desired clinical/field trials, data collection, and characterization to achieve safety and regulatory certifications. We believe Level 6 and 7 are beyond the scope of this perspective and separable

Figure 3. Phage-bacteria characterization methods and required technological capabilities
Detailed characterization levels of phages/cocktail antimicrobial therapeutics, factors impacting target pathogen interactions, and readiness level of 1–5 with increasing characterization depth are illustrated. A summary of technological capabilities needed to meet the PCL tiers are shown on the right. Abbreviations: WGS, whole genome sequencing; MGE, mobile genetic elements; PAIs, pathogenicity islands; DGRs, diversity generating retroelements; ALE, adaptive laboratory evolution; HT, high-throughput; ANI, average nucleotide identity; LOF, loss-of-function; GOF, gain-of-function; GWAS, genome-wide association study; CR, cross-resistance; CS, collateral sensitivity; PD, pharmacodynamic; PK, pharmacokinetic.

topics that have been reviewed in detail earlier (Verbeken et al., 2014; Jassim and Limoges, 2017; Malik et al., 2017; Pirnay et al., 2018; Gabard and Jault, 2019; McCallin et al., 2019; Bretaudeau et al., 2020; João et al., 2021; Liu et al., 2021; Malik, 2021; Verbeken and Pirnay, 2022). We posit that the standardization and categorization of phage-target host interaction studies through PCL will help in defining and assessing "phage readiness status" for an application. The PCL assessment studies will also identify technology gaps in phage characterization steps, fuel the development of inventory of new technologies, and provide a framework for open collaboration, knowledge-sharing, and partnerships between academic labs, private, public-benefit/philanthropic, and government entities.

Phage characterization level 1

At this most basic characterization level, phages are enriched and isolated from an environment using a diverse panel of target bacterial pathogen strains, some of which have been co-isolated from the same surveillance site assessed by standard plaque assays and isolation procedures (Gill and Hyman, 2010; Henry et al., 2012; Kauffman and Polz, 2018; Hyman, 2019). This collection of phages ("Phage Banks") and bacteria are then archived, and their genomes are sequenced, assembled, and subjected to state-of-the-art functional annotation workflows. Generalized bioinformatic analysis is then performed to identify toxins, AMR markers, mobile genetic elements (MGEs), prophages, pathogenicity islands (PAIs), CRISPR systems,

and phage defense systems in pathogenic bacteria while identifying toxins, integrases, AMR markers, virulent genes, diversity generating retroelements (DGRs), integrons, CRISPR and Anti-CRISPR (Acr) systems, and transduction genes in phages (Biswas et al., 2016; Liu et al., 2019a, 2019b; Ecale Zhou et al., 2019; McNair et al., 2019; Alcock et al., 2020; Ramsey et al., 2020; Cook et al., 2021; Li et al., 2021; Nayfach et al., 2021; Roux and Paul, 2021; Tesson et al., 2021; Wang et al., 2021). In addition to genome sequencing and bioinformatic analysis, host targeting particles are confirmed via imaging using transmission electron microscopy (TEM). This structural information provides valuable detail on phage morphology and taxonomy, arrangement of tail fibers, and size and type of phage particle. This information along with genome sequence allows instant comparison, classification and identification of phage particles, and suitability of phages for downstream applications, for example, temperate versus lytic phages. To obtain infection cycle parameters such as phage adsorption rate, latent period, and burst size, the phage adsorption curve and one step growth experiments need to be performed on a key panel of target hosts (Hyman and Abedon, 2009; Henry et al., 2012; Dennehy and Abedon, 2021). These quantitative parameters help in designing therapies including the timing and dose needed for efficient control of pathogens in a specific environmental context. In summary, PCL1 provides basic isolation and characterization of phages and pathogens, with genome features contributing to their characteristics.

Phage characterization level 2

At this level, a panel of phages from PCL1 are used to perform the phage host-range determination on a collection of genome-sequenced target bacterial strains co-isolated from the same infective environment in a diverse set of relevant conditions. Essentially PCL2 provides a quick killing matrix, phages-by-target-by-condition, that can be used to assess if there are any genomic features (including those identified from PCL1) that have predictive value for which targets can be infected by a given phage. To uncover how particular environments may have impacted the fitness "state" of the target bacteria at the infection site, a collection of bacterial strains are used to map fitness landscape in the presence of antibiotics, disinfectants, pesticides, preservatives, metals, ionophores, and biocides (McDonnell and Russell, 1999; White et al., 2005; Henry et al., 2012; Elekhnawy et al., 2020). As phage infection is dependent on diverse abiotic factors (Jończyk et al., 2011; Díaz-Muñoz and Koskella, 2014), combinatorial phage infectivity assays are performed in different conditions. Basic identification of the target "putative' receptor for a phage is performed by isolating and sequencing the phage-resistant mutants on a standard model target pathogen (Schwartz, 1980; Nobrega et al., 2018; Maffei et al., 2021). Assay systems are established for assessing phage infectivity and accessibility of pathogens in biofilm (Harrison et al., 2010; Azeredo et al., 2017; Pires et al., 2021b). To gain specific insights into the response of target pathogen to phage infection or to understand phage infectivity mode, omics methods (RNAseq, ribosome profiling, proteomics, metabolomics) are used in one of the assay conditions (Liu et al., 2013; Chevallereau et al., 2016; Parmar et al., 2017; Howard-Varona et al., 2018). As these omics approaches can get cost-prohibitive and unscalable to hundreds of samples, specific criteria should be established for assessing the need for such datasets in a specific application. The adaptive landscape of a phage is mapped via low-throughput ALE experiments (Scanlan et al., 2015a; Lenski, 2017a; Akusobi et al., 2018; Sandberg et al., 2019; Favor et al., 2020; Kering et al., 2020), and new functions such as altered receptor identification (Meyer et al., 2012) or evolutionary trade-off traits such as antibiotic sensitivity to phage resistance are evolved (Chan et al., 2016; Chatterjee et al., 2020; Gurney et al., 2020; Mangalea and Duerkop, 2020; Canfield et al., 2021; Gordillo Altamirano et al., 2021).

Phage characterization level 3

At PCL3, high-throughput (HT) genetic tools (Wetmore et al., 2015; Koo et al., 2017; Liu et al., 2017; Price et al., 2018; Rousset et al., 2018; Mutalik et al., 2019, 2020; Peters et al., 2019; Rishi et al., 2020; Carim et al., 2021; Rubin et al., 2021) are developed for a genomically diverse representative target pathogen strain collection that support HT genetic screenings to map molecular mechanisms of phage sensitivity and resistance. Genome-wide genetic screens are performed to uncover phage infection determinants including phage receptor discovery (Rousset et al., 2018; Adler et al., 2020; Chatterjee et al., 2020; Kortright et al., 2020; Mutalik et al., 2020). These phage-host bacteria characterization platforms are further used to map out cross-resistance (CR) and collateral sensitivity (CS) trait profiles of phages in addition to antibiotics, biocides, ionophores, metals, drugs, preservatives, and pesticides (Chan et al., 2016; Allen et al., 2017; Price et al., 2018; Barbosa et al., 2019; Mutalik et al., 2019; Burmeister et al., 2020; Chatterjee et al., 2020; Gurney et al., 2020; Jiang et al., 2020; Mangalea and Duerkop, 2020; Altamirano et al., 2021; Canfield et al., 2021; Kever et al., 2021). The HT genetic tools developed for target bacterial hosts should

also be able to support mapping of gene essentiality in select phages in the phage banks (Marinelli et al., 2012; Dedrick et al., 2013; Thomas et al., 2016; Shen et al., 2018; Meeske et al., 2019; Mageeney et al., 2020; Marino et al., 2020; Vo et al., 2020; Rubin et al., 2021). Infection efficiency of phages or combination of phages is assessed by designing rational formulations using genome-wide phage-host interaction datasets including knowledge of probable phage receptors (Wright et al., 2018, 2019, 2021; Chatterjee et al., 2020; Altamirano et al., 2021; Canfield et al., 2021). Deeper assessment of phage stability, efficacy, competition, and evolutionary changes within phage cocktails and costs of delaying resistance are carried out (Tanji et al., 2004; Chan and Abedon, 2012; Chan et al., 2013; Reyes et al., 2013; Schmerer et al., 2014; Wright et al., 2019). The generated datasets are then used to improve annotations and sensitivity profiles powered with comparative genomics and experimental data (average nucleotide identity %, phage defenses, infectivity pattern) analytics. Thus, established data analytic workflows should be able to help in carrying out basic prediction of phage-antibiotic combinations based on molecular markers (Young and Gill, 2015; Segall et al., 2019; Mutalik et al., 2020).
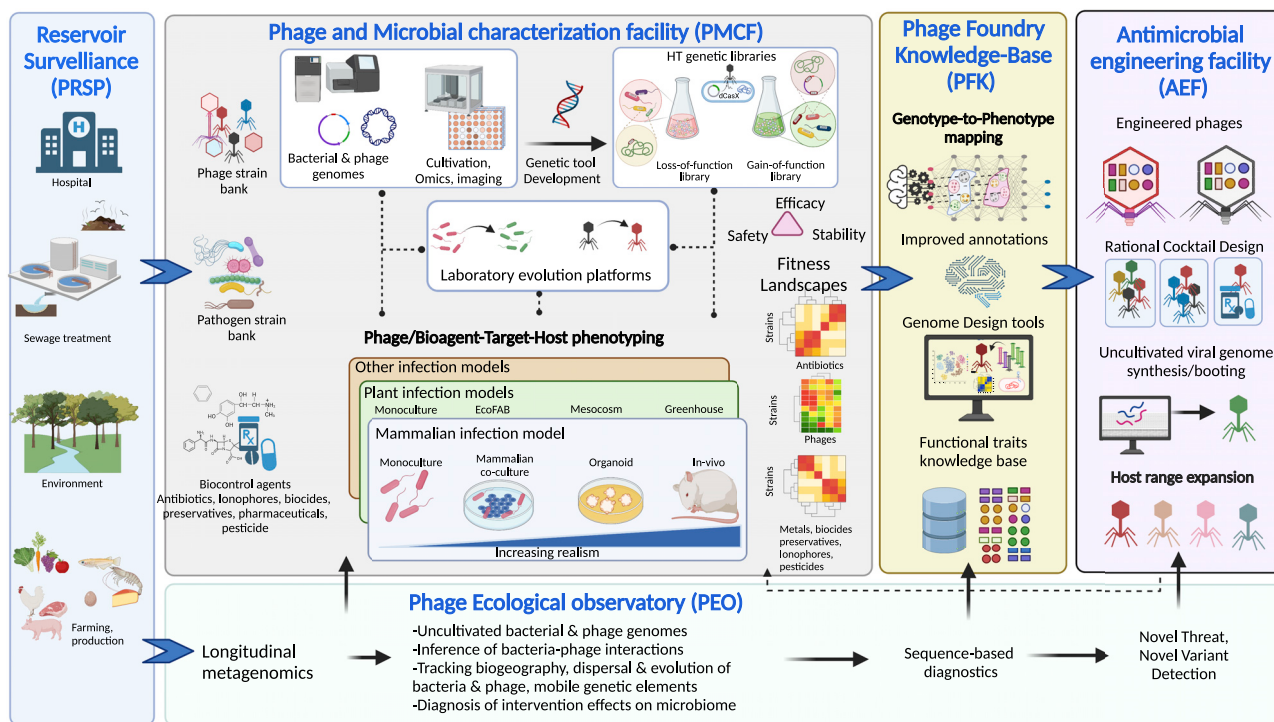
Phage characterization level 4

At this step, phage-bacteria interaction and "state" of the target pathogen (for example, resistance to antibiotic) characterized *in vitro* are extended to the *ex vivo* model to *in vivo/in situ/in planta* systems. Specific selection criteria for choosing a most suitable infection model system should be established that includes the model's complexity, relevance, handling, and operational costs (Alivisatos et al., 2015; Blaser et al., 2016; Douglas, 2018, 2019; Chevrette et al., 2019). A number of model systems such as *Caenorhabditis elegans*, *Galleria mellonella*, *Drosophila melanogaster*, zebrafish and mouse models, organoids, and organs-on-chip for *in vivo* studies (Bulitta et al., 2019; Brix et al., 2020; Aguilar et al., 2021; Cieślik et al., 2021; Penziner et al., 2021), whereas Fabricated Ecosystems (EcoFAB and EcoPODs) for intensive field-scale monitoring of phage-target pathogen-host interactions are brought on-board (Buttimer et al., 2017; Koskella and Taylor, 2018; Zengler et al., 2019). These model systems are useful to quantitatively assess efficacy of phages, phage cocktails and phage-antibiotic, pesticide, biocides, ionophores, or metal combinations. The impact of phage resistance on target pathogen virulence and fitness is measured. Assessment of phage safety, toxicity, and stability is performed along with tests for cross-reactivity, antigenicity, immunomodulation, persistence, and impact on environment to define optimal treatment parameters under the conditions of intended use (Balogh et al., 2010; Chan, et al., 2013; McCallin et al., 2018; Hernandez and Koskella, 2019; Jault et al., 2019; Wang et al., 2019; Liu et al., 2021; Nale and Clokie, 2021; Popescu et al., 2021). Rapid ALE experimental platforms are established to carry out phage training against new conditions, host variants, link genotype-phenotype relationships, and coevolution of phages and host to uncover allelic level specificity (for example, see refs (Burrowes et al., 2019; Favor et al., 2020; Russ et al., 2020; Abdelsattar et al., 2021; Borin et al., 2021; Eskenazi et al., 2022; Torres-Barceló et al., 2022)). Experiments are carried out to identify biomolecular substructures within a panel of bacterial hosts, leading to interaction among different combinations of phages and antibiotic to better predict CR/CS and evolutionary traps (Pál et al., 2015; Scanlan et al., 2015b; Imamovic et al., 2018; Scortti et al., 2018; Burmeister and Turner, 2020; Maltas et al., 2020; Mangalea and Duerkop, 2020). This information further feeds into designing rational phage-antimicrobial cocktail formulations and also aids in creating rationally designed phage variants of substructures such as tail fibers targeting novel host variants. This PCL4 will also have established a phage engineering platform (Jaschke et al., 2012; Pires et al., 2016; Kilcher and Loessner, 2019; Lenneman et al., 2021; Wetzel et al., 2021; Guan et al., 2022) that is amenable to seamless functional trait engineering in phages and phage-tail-like particles (Scholl et al., 2009; Ghequire and De Mot, 2015; Hockett et al., 2015; Scholl, 2017; Heselpoth et al., 2019; Carim et al., 2021; Heiman et al., 2022) to enhance efficacy in an increasingly realistic environment.

Phage characterization level 5

At PCL5, phages, antibiotic, or combination of interventions are applied to the infection host model/environment, different microbiome level outputs of such perturbations, for example, community abundance, shifts, activation, and transfer of MGEs, and if possible strain and gene-level variations are mapped in a reproducible manner (Cobián Güemes et al., 2019; Nelson et al., 2019; Wang et al., 2019; Whelan et al., 2020). The model host phenotypes are used to design and predict new antimicrobial combinations. Phages, target bacteria, host, and microbiome genotype-phenotype dataset are mapped to ecological variants, phage PD/PK, host physiology, phage infection kinetics, *in vivo/ex vivo* pathogen dispersal and infection, and immunological effects (Bevivino et al., 2019; Wang et al., 2019). The development

Figure 4. The Phage Foundry

The Phage Foundry is a distributed set of standardized and quality-controlled capabilities that span surveillance, characterization, design, and formulation of phage-involved therapies for antimicrobial resistant bacteria to enable rapid response to novel pathogens and emerging infections. It would serve as an organizing hub for phage biologists, microbiologists, clinicians, infectious disease experts, bioinformaticians, data scientists, engineers, phage therapy practitioners, manufacturers, and regulatory experts to work with multiple allied efforts in different programs currently operating to respond to the AMR threat.

and extension of genetic tools in phages will open up opportunities for further phage engineering and fine-tuning functional traits (Chan et al., 2005; Lu and Collins, 2007; Ando et al., 2015; Nobrega et al., 2016; Kilcher et al., 2018; Kilcher and Loessner, 2019; Huss and Raman, 2020; Guan et al., 2022) in PCL5. Extension of phage engineering platform is used to barcode phages that enables rapid and efficient identification, tracking, and quantification in complex environmental contexts. Seamless phage engineering is possible at this stage where landing pads are created on phage genomes for incorporation of payloads such as CRISPR systems, biofilm degrdation traits, engineered diversity generating retroelements, phage-growth-promoting factors, and anti-phage resistance traits. Phage engineering to program biocontainment and design onset of timing, release, and lysis of the host cell in an environmental context is possible. Criteria for "scoring" each phage formulation, phage cocktails, and phage-antibiotic combination with efficacy metrics are established.

A PHAGE FOUNDRY FOR KNOWLEDGE-BASED INTERVENTION

Our proposal for a Foundry (Figure 4) is meant to solve a central challenge in harnessing the advantages of phages for treating AMR infection—developing sufficient knowledge to rapidly "predict" when different phages will be effective in treatment of a given infection in a complementary manner or how to quickly and rationally modify phages, produce and deploy formulations to attack the evolved pathogen. Although the universe of phage-target interaction mechanisms is large, it is constrained as the interactions with the host and environment and basic principles and mechanisms of specificity, susceptibility, and evolution have begun to emerge. However, the data and experiments remain largely disorganized, anecdotal, and poorly cross-comparable. We need efforts to (1) systematize observations of phage-pathogen interactions in the environments of relevance; (2) standardize the characterization workflows based on specification of the application, and (3) share physiological and genetic information of phages/target/host/environment interactions to build the knowledge-base for predictable engineering of phage therapies to address the threat of AMR microbes. Scaling these technologies in a One-Health approach to diverse high-priority

pathogens listed in Figure 1 would need committed resources, expertise, and funding. The scale of such an endeavor would need a Phage Foundry program (Figure 4) that leverages on-going efforts for (1) coordinated response to AMR to produce critical observational data, (2) collect and characterize natural reagents (phages and targets) from environmental and patient samples, and (3) define a set of standardized operations that can be performed by any number of collaborative partners to drive phage characterization and engineering into therapies.

Here we highlight five main "activities" that would aid the development of standardized and quality-controlled procedures for their operation when the, possibly distributed but federated, Phage Foundry is fully operational (Figure 4): (1) *The Phage Reservoir Surveillance Program (PRSP)*, which augments current efforts to identify pathogens by adding identification of their phage predators; (2) *The Phage Ecological Observatory (PEO)*, which deploys broader functional metagenomics in key areas to understand the ecology of pathogens, phages, and resistant elements to detect new sequence-based patterns and mechanisms of resistance, uncover broader ecological impact of "treatments," and produce diagnostics to aid in therapy; (3) *The Phage and Microbial Characterization Facility (PMCF)* aimed at determining the mechanisms by which phage and their targets interact and evade each other's defenses and how these impact fitness in different environments; (4) *The Antimicrobial Engineering Facility (AEF)*, which uses the knowledge from the other components to engineer phages and combination formulations of phages, classical antibiotics, and adjuvants for therapies; and finally (5) *The Phage Foundry Knowledgebase (PFK)* serving as the knowledge-base of the entire operation itself. The PFK would serve as a central clearing house of knowledge about the biogeography, clinical presentation, and molecular characterization of phage-therapy relevant information. This, in turn, would provide substrate for Foundry teams to decide that a particular phage-pathogen pair 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, which will develop tools and technologies for large-scale screening, design, characterization, and engineering of phages and phage-tail 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 aforementioned components has its own unique specific challenges.

The phage reservoir surveillance program

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 et al., 2019; Diallo et al., 2020). In the United States, 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 Foodborne 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 that recognizes that cross-source sampling could identify the emergence and spread of AMR pathogens and facilitate a more rapid response (WHO Report 2021). Focused on extended-spectrum beta-lactamase-producing *E. coli*, this organization surveys human samples, poultry, sewage, runoff, and river sites in urban areas that 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, as 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 transfer to the Phages and Microbial Characterization Facility (PMCF) and perhaps deeper investigation by the Phage Ecological Observatory (PEO). Efforts by the PRSP support to bring phages to PCL Level 1.

The phage ecological observatory

The PEO mission (Figure 4) is to (1) characterize the broader ecology in which the target pathogen and phages are found; (2) assess the population variation of the target and phages; (3) study the dispersal of resistance elements and their mobile carriers, and (4) catalog other members of the microbiome and environment that might mediate the impact of the pathogen and its treatment. This would be a largely functional metagenomic effort with some precise environmental measurements depending on the environment to track population composition, activity, and dynamics before, during, and after treatment in some cases. As an augmentation of the PRSP, PEO would provide longitudinal data about the rise and fall of new infectious agents, their viral predators, and resistance elements (Hussain et al., 2021; LeGault et al., 2021). With the knowledge-base, PEO would develop predictive signatures to mark their spread. Any novel sequence elements or uncultivated phages identified in the PEO could be passed to the PMCF for synthesis and characterization. When PEO infrastructure is used to track the effects of therapeutic (or preventative) intervention, the results could be used by the Knowledge-base and Antimicrobial Engineering Facility (AEF) to diagnose failures and design more effective interventions. Because of the fastidious nature of functional metagenomic analysis, it is critical that methods for sample processing and analysis are standardized across the facility members. The criteria and choice of where and when to implement observations is complex due to the cost of these analyses, but critical known reservoirs should likely have observatories with both regular and event (infection/outbreak)-driven sampling schedules. The use of the observatories for interventional studies (e.g. during and after treatment) would be on a case-by-case basis. The PEO produces information for PCL levels 4 and 5.

The phage and microbial characterization facility

The PMCF is an ensemble of many different methods and platform technologies for discovering and characterizing the mechanisms of interaction between phages and target bacteria and how their variation leads to differences in both susceptibility and general fitness in diverse conditions (Figure 4). It is also where general technologies for phage manipulation and engineering may be developed, as these are often necessary during the characterization stage. Standards for measurements, test environments, data representation, analysis, and quality assessment would be set by a PMCF coordinating body. Although there is a great deal of legacy work to do to characterize known phages/target interactions in standardized, comprehensive ways, the choice to characterize a novel phages and/bacterial host using one or more of the PMCF facilities would depend on the novelty of the organisms and/or surprises in the first-line character-ization of the target. The Phage Foundry team would coordinate prioritization and characterization efforts for both legacy and novel pairs so that the most urgent needs would be addressed and the PMK would have maximal coverage of the high-priority systems. The fundamental goal of the PMCF is to (1) create a molecular map of the interactions of phage elements with host elements, such as between tail-fibers and surface receptors, or phage defense and anti-defense systems, so that it is both possible to predict these interactions in new phages/target pairs and to engineer molecular variations in phages to response to target variations more generally; (2) develop HT approaches for ALE/phage training for designing and optimizing treatment parameters under the conditions of the intended use; (3) establish diverse infection simulation model systems; (4) characterization of phages and antimicrobial cocktail efficacy, safety, stability, cross-reactivity, resistance, cross-antigenicity, and immunomodulation capabilities; (5) map the condition-dependent fitness effect of interactions so that their efficacy in therapeutic conditions can be better predicted as data accumulates; and (6) identify and target mechanisms that trade-off fitness under antibiotic/antimicrobial pressure for use by the AEF. In essence, PMCF serves as the core facility that generates characterization data package for phages and its combinations to establish a "data sheet" for each therapeutic phage something similar to the synthetic biology chassis (Arkin, 2008; Canton et al., 2008) or a "master file" (Fauconnier, 2017) detailing characterization methods/protocols, associated datasets, processes, facilities including manufacturing, downstream processing, formulation, packaging, and storage guidelines. The PMCF is aimed at PCLs 2, 3, and 4.

The antimicrobial engineering facility

The AEF mission (Figure 4) is to develop the technology and the practice of engineering phages (when necessary) and composing cocktails of phages and other antibiotic/antimicrobial elements to treat a newly identified infection. Utilizing the reagents from the PRSF and PMCF and information from all other facilities routed through the PFK, AEF labs would seek to develop critical phage as platforms for flexible design and engineering and develop general and specialized payloads for evading phage defenses, allowing penetration of recalcitrant biofilms, preventing mobilization/transduction of resistance elements after infection, and engineering to generate toxic by-products to efficiently kill the target bacteria among other things. The AEF would use the initial knowledge from the PMCF to design cocktails and dosing schedules of phages and other antimicrobials expected to have synergistic effects, which also prevent adaptation by forcing fitness trade-offs in the target during resistance generation. The AEF would also develop methods with the PEO for identifying high-priority pathogen-associated phages that had not been isolated by the PRSF, obtaining high-quality genomes, and "booting" these in the laboratory for use in therapy (and characterization in the PMCF). Member labs, industry, and hospital partners would collaborate with the PEO to track treatment effects in patients/target environments so that the efficacy and persistence of their treatment could be quantified and possible mechanisms of success and failure discovered to aid in future design. The AEF is aimed at PCLs 4 and 5.

The Phage Foundry knowledge-base

The PFK (Figure 4) would be the central clearing house for data obtained from the other Foundry facilities and dedicated to the development of tools for integration and analysis of this information to aid in (1) phage functional annotation and engineering; (2) functional traits database for engineering projects; (3) approaches for phage-therapy relevant diagnostic analysis that suggest which mechanisms and phages would be starting platforms for attack of a new infection; and (4) therapeutic design and diagnosis tools to drive design and optimization and therapeutic cocktails and to track their effects after administration. The PFK system could be built in collaboration with and using existing elements and largely open, frameworks such as Patric (Davis et al., 2020), iVirus (Bolduc et al., 2017), iMicrobe (Youens-Clark et al., 2019), KBase(Arkin et al., 2018), NMDC (Eloe-Fadrosh et al., 2021), and IMG/VR (Roux et al., 2021), thereby bringing these preexisting teams and their user relevant communities together toward a common goal. These frameworks already encode tools directed at understanding target-phage interactions, effective genome annotation, and primitive engineering tools. Open, Findable, Accessible, Interoperable and Reusable (FAIR) data (Wilkinson et al., 2016) and software practice would both drive good hygiene in the allied Foundry facilities and provide a way for the broader community to build upon and add to the Foundry's work effectively in the format of virtual "phage datasheet" or "phage master file." The AEF is aimed at PCLs 3, 4, and 5.

The Foundry does not have to be staged all at once but can be brought online by prioritizing critical development and partnerships. For example, the PRSF could pilot initial collaborations between the existing surveillance infrastructures and the needs of a Phage Foundry through coordinated sampling and new isolation, sequencing, and susceptibility testing efforts. Specifically, handling of bioterrorism agents along with some of the urgent and emerging AMR threats require special facilities, expertise, and may need to partner with established biosafety level (BSL)-2 and BSL-3 laboratories. Ensuring that computational teams build relevant infrastructure for knowledge storage and generation such as those mentioned earlier would set the stage for the PFK with modest investment. Prioritizing specific pathogens for phage-based treatment would allow pilot characterization and therapeutic design programs to be tested, and these too could be directed toward use and deposition in the appropriate open computational infrastructure. A community building effort around these programs could help refine and mature this vision and gain buy-in internationally. The Foundry "community" would become a hub interfacing with different facility ecosystems internationally including industry, public health organizations, hospitals, farmers, food manufacturers, and other government agencies (for example, US National strategic stockpile [see later discussion]).

Limitations of the framework

Our proposed PCL framework provides a pathway for assessing whether phages or a phage/antimicrobial cocktail formulation has been incrementally characterized in accordance to its end use. Like the classical TRL framework (Olechowski et al., 2020), our PCL framework helps in planning and making strategic, investment and management decisions; however, it does suffer from lack of clarity at level-interfaces

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

A call for global action

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

Some of the priority pathogens listed in Figure 1 have been shown to be untreatable with currently available therapies and urgently need a focused effort to develop alternative treatments (Tacconelli et al., 2018). This need is especially acute from a national biosecurity point of view, as we need to be better equipped to counter an incidence of natural or intentional release of MDR pathogens including antimicrobial resistant bioterrorism agents into our food, dairy and meat processing facilities, water supply, or healthcare facilities (Fauci, 2001; Weigel and Morse, 2009; National Academies of Sciences, Engineering, and Medicine, 2019). For example, although the US Strategic National Stockpile contains >$7 billion worth of emergency supplies (Board on Health Sciences Policy, Health and Medicine Division and National Academies of Sciences, Engineering, and Medicine, 2016) including antibiotics, vaccines, and medicines address any all-hazard mass casualty in any part of the USA, it may not have readily available antibiotic-alternative solutions that are scalable and rapidly deployable to address unseen national MDR emergencies (Weigel and Morse, 2009; PHEMCE SIP, 2015; Gerstein, 2020). As an adjuvant/alternative treatment to antibiotics, phage therapy has the capability to be scaled globally and deployed via "just-in-time" manufacturing (Cheng and Podolsky, 1996; PHEMCE Strategy, 2012) as new infections emerge. Although policy discussions around solving AMR (IDSA Report, 2011; Handfield et al., 2020) are beyond the scope of this article, our PCL framework and Phage Foundry approach presented here address the needed innovations to fill the knowledge and technological gaps to meet this grand goal. We believe a fully

realized Phage Foundry will provide a unifying platform for generating and sharing knowledge, technology, and phage reagents to the broader research community in public and private institutions in a fair and equitable manner. We envision the Phage Foundry will accelerate the biobased economy in the long run with innovations in phage-based AMR bacterial diagnostics, phage-based microbiome intervention strategies, phage-based vaccine discovery and development, biocontainment strategies for the bioproduction industry, development of next generation molecular biology reagents, phage-based biopesticides, and in addition enable phage-resistant starter culture engineering in food and dairy industry. The scale and scope of this endeavor including the research and development needed for countering top pathogens across diverse contexts and industries (shown in Figure 1) is huge but urgently needed. This grand goal will have to be supported with longer-term investments from diverse federal funding agencies with stronger public-private and philanthropic entity partnerships led with collaborative, multisectoral, and transdisciplinary teams across the world.

DECLARATION OF INTERESTS

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

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