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Perspective

The CARB-X Portfolio of Nontraditional Antibacterial Products

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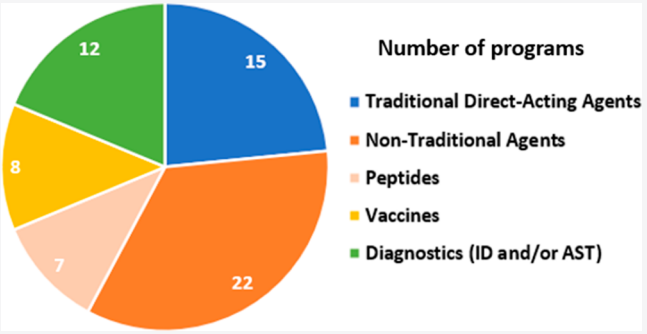
ABSTRACT: The growing prevalence of antibiotic-resistant bacterial pathogens and the lack of new medicines to treat the infections they cause remain a significant global threat. In recent years, this ongoing unmet need has encouraged more research groups to focus on the discovery and development of nontraditional antibacterial agents, ranging from anti-virulence strategies to bacteriophage and ways to modulate the microbiome. The Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) is a global nonprofit public−private partnership dedicated to accelerating antibacterial-related research. Importantly, the CARB-X portfolio supports a wide variety of novel and innovative nontraditional programs to help the global antibacterial research ecosystem understand the potential that these modalities can play in the management or prevention of serious infections. We describe here the breadth of the CARB-X pipeline of novel nontraditional products.



KEYWORDS: *antibacterial, nontraditional, CARB-X, research and development, therapeutics, preventatives*

As the world struggles with the constant rise in the level of antibacterial resistance, a limited pipeline of new agents remain that can be used to treat or prevent these life-threatening infections.[1,2] Of further concern is the fact that many of the products in clinical development represent incremental improvement of existing drug classes. In 2016, in response to this crisis, the Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X; www.carb-x.org) was formed to provide financial and scientific support to groups developing novel therapeutic, preventative, and diagnostic products to address the largely drug-resistant bacterial pathogens listed on the WHO Global Priority Pathogens List and the CDC Threat Assessments list.[3,4] CARB-X is headquartered at Boston University and is funded by three governments and two philanthropic organizations: (1) the Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response (ASPR) in the US Department of Health and Human Services; (2) the Wellcome Trust, a global charity based in the UK working to improve health globally; (3) Germany's Federal Ministry of Education and Research (BMBF); (4) the UK Department of Health and Social Care's Global Antimicrobial Resistance Innovation Fund (GAMRIF); (5) the Bill & Melinda Gates Foundation. In addition, CARB-X receives in-kind support from National Institute of Allergy and Infectious Diseases (NIAID), part of the US National Institutes of Health (NIH).[5]

In 2019, CARB-X held a specific funding call that was aimed at nontraditional modalities and innovative ways to address the growing threat of antibiotic resistance.[6,7] The past decade or so of scientific innovation and understanding has seen the maturation of technologies in multiple areas of drug discovery, including CRISPR, immunotherapy and harnessing of the innate immune system, and the link between the microbiome and a range of diseases. These approaches are also being explored in the field of antibacterial drug discovery, and CARB-X supports these novel approaches through the development stages of Hit-to-Lead to First-in-Human testing (Phase 1) with the mandate to progress forward a diverse group of medicines with the potential to transform patient care. This Perspective summarizes the wide range of nontraditional products that are currently supported by CARB-X funding.

NONTRADITIONAL ANTIBACTERIAL AGENTS SUPPORTED BY CARB-X

Nontraditional antibacterial agents cover a range of products with different modalities, including anti-virulence approaches,

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bacteriophage, and live biotherapeutic products.[8,9] Indeed, approximately 34% of the active CARB-X portfolio is composed of nontraditional products (Table 1) for both the treatment and prevention of serious bacterial infections. Although it is difficult to classify the 22 nontraditional products without knowing precisely how they would ultimately bring the most clinical benefit to patients, broadly speaking, we envisage our nontraditional portfolio as containing 8 therapeutic products that could be used as stand-alone therapy, 8 "adjunctive" products that would likely require the coadministration of another direct-acting antibacterial agent, and 6 preventative products that would be used to reduce the likelihood of developing a serious infection. In addition, the CARB-X portfolio contains seven programs that can be classified as direct-acting peptides, all of which are <50 amino acids in size (Table 1). There has been debate as to whether peptide agents truly constitute nontraditional agents and which characteristics should be used to classify them into either group. In this Perspective, the direct-acting peptide programs will be kept in their own category, but in recognition of the uncertainty of their classification, we have also included a brief summary of them below.

Table 1. Categorization of the CARB-X Pipeline

product class	number	percentage of portfolio
traditional direct-acting agents	15	23
nontraditional agents	22	34
peptides	7	11
vaccines	8	13
diagnostics (identification and/or susceptibility)	12	19
total programs	64	100

The nontraditional programs can be further divided on the basis of product type or target pathogen(s) (Figure 1), and below, we have briefly described the programs grouped by modality.

Bacteriophage. Although bacteriophage therapy has a long history (with infrequent use in humans outside the former Soviet Union), several recent high-profile compassionate use cases[10−12] have resulted in an increased focus on this modality. The current challenge in the field is that most clinical studies using bacteriophage have been performed under emergency-use approval from the regulatory authorities, resulting in numerous differences in important variables such as indications, pathogens, treatment regimens (dose and duration), and concomitant antibiotic therapy. Systematic evaluation of these variables in robust randomized clinical trials will be needed to understand how to leverage the best clinical value from bacteriophage therapy. A further challenge to clinical development is that, given the natural specificity of bacteriophages for their bacterial targets, most bacteriophage products will have a narrow spectrum of activity, which will necessitate careful screening of patients with a rapid diagnostic assay.

The CARB-X portfolio currently supports four phage projects with two focused on the prevention of infections and two on the treatment of infections in specific patient populations. Eligo Bioscience (https://eligo.bio/) is developing a cocktail of nonreplicative CRISPR-engineered bacteriophages that will be used to precisely target specific strains of *Escherichia coli* and *Klebsiella pneumoniae* in the gastrointestinal tract based on their carriage of extended-spectrum-β-lactamase and carbapenemase genes. The selective removal of these drug-resistant strains from the gut microbiome of transplant patients should prevent the onset of potentially lethal post-transplant infections. In a similar fashion, SNIPR Biome (https://www.sniprbiome.com/) aims to use engineered bacteriophages to precisely deliver CRISPR-guided vectors to, and subsequently eliminate, pathogenic *E. coli* from the gut microbiome in hematological cancer patients undergoing chemotherapy that are at an elevated risk of developing a neutropenic fever due to infection. These products would both preserve the overall microbiome and aim to selectively decolonize potential pathogens leading to improved outcomes in these vulnerable patient populations. This strategy supports the growing evidence of the significant clinical benefits of maintaining an ideal microbiota and not creating gut dysbiosis through the use of broad-spectrum agents when treating cancer.[13,14] CARB-X also supports other programs that modify the microbiome through live biotherapeutic products (LBPs), and these are discussed below.

There are two programs in the CARB-X portfolio that are exploring the therapeutic use of bacteriophage products. Locus Biosciences (https://www.locus-bio.com/) has successfully completed the first clinical trial of a CRISPR-engineered bacteriophage (crPhages).[15] This Phase 1b study was performed in patients with a history of urinary tract infections (UTIs) caused by *E. coli* colonization, having indwelling catheters, or requiring intermittent catheterization: a cocktail of crPhages targeting this species was employed as the therapeutic agent. The program supported by CARB-X aims to develop a similar cocktail of crPhages that targets *K. pneumoniae*, focused also on patients with a history of UTIs. If successful, these products

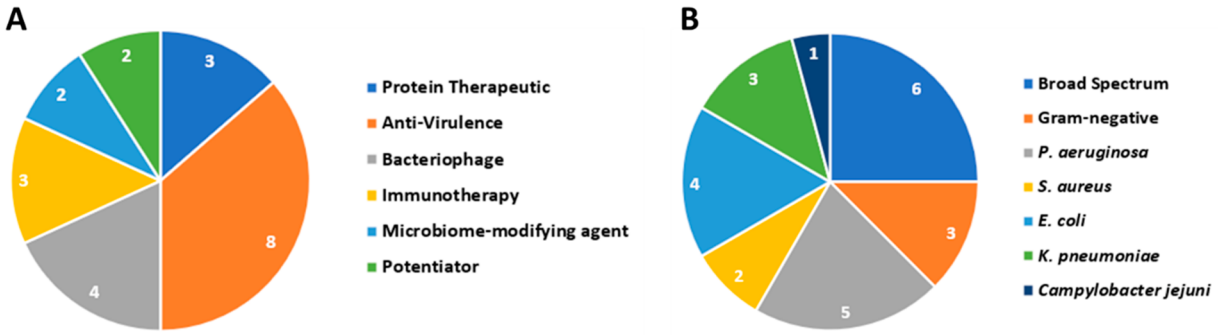

Figure 1. CARB-X nontraditional portfolio defined by (A) product type and (B) pathogen spectrum. Note that one product targets *Escherichia coli* and *Klebsiella pneumoniae*, while another targets *E. coli* and *Campylobacter jejuni* (see below). The 7 direct-acting peptide programs in the portfolio are not included in this figure.

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could be combined to treat patients with complicated UTIs caused by these Enterobacteriaceae species. Phico Therapeutics (https://www.phicotx.co.uk/) is supported to develop SASP-ject technology against *Pseudomonas aeruginosa*. The bacteriophage is used as a precise delivery vehicle to introduce a gene encoding a small acid-soluble spore protein (SASPs) that when subsequently produced can bind to and inactivate DNA, thereby preventing bacterial replication.[16]

Microbiome Modifying. There is growing evidence that manipulation of the gut microbiome to provide an improved microbial flora composition can decrease the risk of infection.[17] Two companies in the CARB-X portfolio are developing products aimed at reprogramming the intestinal microbiome to prevent the onset of infections in vulnerable patients. Vedanta Biosciences (https://www.vedantabio.com/) and Seres Therapeutics (https://www.serestherapeutics.com/) are developing rationally designed live bacterial consortia to prevent gut colonization by carbapenem-resistant and extended-spectrum-β-lactamase producing Enterobacteriaceae and vancomycin-resistant Enterococci and subsequent bloodstream infections in highly susceptible patients including hematopoietic stem cell and solid organ transplant patient populations.

Anti-virulence. Many nontraditional agents focus on interrupting or modulating the pathogenic cascade of a specific bacterial species with the intention of using these agents in combination with traditional direct-acting molecules. The disarmament of the bacterial pathogens is expected to prevent the progression of severe disease and tissue damage and aid in the treatment of recalcitrant infections, thus improving patient outcomes.

Given the distinct pathogenic mechanisms of different species, many anti-virulence strategies are inherently narrow in spectrum. There are eight programs in the CARB-X portfolio classified as anti-virulence. Three of these programs are directed against *P. aeruginosa* lung infections. Two programs, those by Antabio (https://antabio.com/) and Helmholtz Institute for Pharmaceutical Research (https://www.helmholtz-hips.de/en/), are focused on identifying and progressing inhibitors of LasB, the *P. aeruginosa* elastase enzyme. LasB is an extracellular metalloprotease that induces significant tissue injury through the degradation of host cell extracellular matrix proteins, such as elastin, collagen, and laminin.[18] Inhibition of this enzyme is proposed to result in less lung damage, and when the anti-virulence agent is administered adjunctively with other antibiotics, better clinical outcomes are anticipated for cystic fibrosis patients where *P. aeruginosa* is a significant pathogen. The third *P. aeruginosa* program, from Microbiotix (https://www.microbiotix.com/), focuses on inhibiting the type III secretion system (T3SS) with small-molecule inhibitors. The T3SS is a key virulence factor that is responsible for the translocation of specific effector proteins into the eukaryotic host cell.[19,20] The T3SS has been shown in a wide range of models to significantly increase the severity of disease, especially in lung infections, and antibodies against a key component of the T3SS, shown to be protective in animal models, have progressed clinically.[21]

There are two anti-virulence programs that target *Staphylococcus aureus*. The first, being pursued by BioVersys (https://bioversys.com/), is targeting a key protein involved in regulating biofilm production and a wide repertoire of pathogenicity factors. The accessory gene regulator (*agr*) system plays a critical role in the pathogenesis of *S. aureus*, and the precise interruption of its function is anticipated to help disarm the key weapons of this bacterium.[22] The second anti-*S. aureus* program is aimed at developing small molecule inhibitors of α-hemolysin and is being carried out at the Helmholtz Centre for Infection Research (https://www.helmholtz-hzi.de/en). α-Hemolysin is the major cytotoxin produced by *S. aureus*; it heptamerizes to form a pore in eukaryotic cell membranes, leading to cell lysis.[23] The level of α-hemolysin expressed by *S. aureus* isolates has been shown to correlate with the severity of disease. Antibodies directed against this toxin are in late stage clinical development[24] for intervention in *S. aureus* pneumonia, but this novel small molecule approach may offer both better lung penetration and a lower cost-of-goods.

The FimH mannose-specific adhesin located at the tip of the Type I pilus of *E. coli* isolates is a critical factor for the adherence of this species to urinary tract epithelial cells.[25] A small molecule mannose-mimic that binds this fimbrial adhesin can prevent the adherence of *E. coli* to the eukaryotic cells, thus preventing urinary tract infections.[26] GlaxoSmithKline (https://www.gsk.com/en-gb/) is evaluating the safety, tolerability, and pharmacokinetics of a FimH antagonist (GSK3882347) in a First-in-Human (FIH) study.

The last two anti-virulence programs in the CARB-X portfolio have a broader spectrum of activity and target the disruption of bacterial biofilms that make many species recalcitrant to treatment. The programs from Trellis Bioscience (http://www.trellisbio.com/) and Clarametyx (https://clarametyx.com/) are exploring antibodies that bind to DNABII binding proteins that help form the backbone of bacterial biofilms.[27] Specific binding to DNABII can result in the loss of integrity of the bacterial biofilm, precipitating its collapse and rendering the hitherto protected cells susceptible to attack with standard antibacterial agents. Many difficult-to-treat infections involve the successful establishment of biofilms, and adjunct therapy to help destroy these structures should result in superior clinical outcomes. In the near term, the Trellis program is focused on prosthetic joint infections and is actively enrolling an FIH study, while the Clarametyx program is focused on respiratory infections.

Protein Therapeutics. The CARB-X portfolio contains three programs that can be broadly classified as protein-based therapeutics. Contrafect (https://www.contrafect.com/) is developing an engineered lysin therapeutic (CF-370) for the treatment of *P. aeruginosa* infections. Lysins are enzymes produced by natural bacteriophage that are used to lyse the host bacterial cell at the end of the phage lifecycle. These direct-acting proteins can kill bacterial cells extremely rapidly by cleaving the peptidoglycan, an essential structure required for the integrity of the Gram-negative bacterial cell wall.[28] CARB-X is also supporting GangaGen Technologies (https://gangagen.com/) to develop engineered bacteriocins that are active against *K. pneumoniae*, or klebicins.[29] The klebicins selectively bind to receptors on the cell surface and subsequently enter and kill the bacteria through several distinct molecular mechanisms. The precise removal of pathogenic *K. pneumoniae* isolates would leave the microbiome intact but precisely eliminate the causative agent of an increasing number of serious infections. Amicrobe (https://www.amicrobe.com/) is developing an engineered synthetic protein that is being developed as a local antibacterial agent. Amicidin-β is over 100 amino acids in length and is produced with a block architecture that offers broad-spectrum microbiological and antibiofilm activity and unique physicochemical properties that can enhance tissue cleaning during surgical debridement. Amicidin-β is being developed both as a solution for the treatment of orthopedic and hardware-

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associated infections during surgery and as a novel formulation for the treatment of other surface-based infections.

Potentiators. Potentiators are molecules that, when used in combination with a known antibacterial agent, can improve or restore the activity of the agent.[30] The most common examples of potentiators are represented in the class of β-lactamase inhibitors, which restore the activity the β-lactam drugs by preventing their hydrolysis. ETX0282, a product in development by Entasis Therapeutics that recently graduated from the CARB-X portfolio following a successful FIH study, is a novel, oral β-lactamase inhibitor that aims to restore the activity of cefpodoxime against multidrug-resistant Enterobacteriaceae.[31] Other examples of potentiation include molecules that improve the cellular penetration of antibacterial agents whose entry is normally retarded. In this arena, CARB-X is supporting University of Queensland (https://imb.uq.edu.au/superbugs) to optimize engineered octapeptin cyclic peptides for their ability to restore and/or enhance the activity of other antibiotics against multidrug resistant Gram-negative pathogens. Of particular interest is to evaluate partner drugs that are readily available in low- and middle-income countries (LMICs) to help prolong their usefulness in these countries that suffer from a high rate of infections caused by MDR bacteria. Additional examples of potentiation include inhibitors of multiprotein efflux pump systems, which are often responsible for the first-step erosion of drug susceptibility in Gram-negative pathogens as their overexpression can result in less drug being available to interact with intracellular targets. Taxis Pharmaceuticals (https://www.taxispharma.com/) is developing a novel class of efflux-pump inhibitors that, when used in combination with antibacterial agents that are impacted by significant efflux, can restore clinically relevant activity. *P. aeruginosa* contains a plethora of diverse efflux pumps, and the Taxis compounds have demonstrated activity against this species in combination with a variety of drug classes.

Immunotherapy. There are three nontraditional immunotherapeutics in the CARB-X portfolio. First, Centauri Therapeutics (http://www.centauritherapeutics.com/) is leveraging its modular Alphamer platform to develop novel molecules that can harness pre-existing antibodies present in the host immune system, target specific Gram-negative bacteria, and promote bacterial cell-specific killing and clearance via the immune system. These molecules typically consist of a galactose-α-1,3-galactosyl-β-1,4-*N*-acetyl-glucosamine epitope linked through a noncleavable linker to a binding recognition element specific for the bacterial pathogen. Their targets, anti-Gal antibodies, are the most abundant antibodies in humans, accounting for approximately 1% of all immunoglobulins.[32] Second, Lumen Bioscience (https://www.lumen.bio/) is developing a food-grade immunoprophylactic biologic composed of therapeutic antibodies that can bind and inactivate the key toxins responsible for diarrheal disease secreted by *E. coli* and *Campylobacter jejuni*. This product will be produced using spirulina expression, which will lower the cost of goods and provide benefits to citizens of low- and middle-income countries.[33] Third, Cellics Therapeutics (https://cellics.com/) is advancing a macrophage nanosponge.[34] These nanosponges, manufactured by coating inert particles with macrophage membranes, are being evaluated for adjunctive therapy in the treatment of bacterial sepsis. The nanosponges act as decoys and can bind and neutralize a range of molecules including bacterial toxins and host inflammatory cytokines that significantly contribute to the sepsis cascade.

Direct-Acting Peptides. As mentioned above, the current CARB-X portfolio contains seven programs, representing approximately 11% of the total portfolio, that are developing direct-acting small to midsize peptide therapeutics. Figure 2

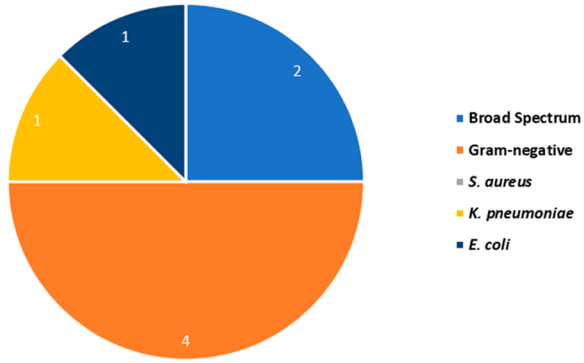

Figure 2. CARB-X portfolio of direct-acting peptides defined by the pathogen spectrum. Note: one program is focused on Enterobacteriaceae, limited in this graphic to *E. coli* and *K. pneumoniae*.

shows the distribution of pathogen coverage of these peptides. Whether these molecules should be classified as traditional or nontraditional is often debated, and so, they are included here as a separate group. MicuRx (https://www.micurx.com) has designed and synthesized a softdrug of polymyxin (MRX-8), aiming to shield the cyclic peptide as a means to enhance the therapeutic index of this last-resort class for treating infections caused by multidrug-resistant Gram-negative pathogens. The potential for this strategy to succeed will be tested presently, as the program is actively enrolling healthy human subjects in both single-ascending and multiple-ascending dose FIH studies. Assuming a successful outcome and given the promising preclinical efficacy of MRX-8 in both urinary tract and lung infection models, we anticipate MicuRx will pursue clinical Proof-of-Concept in complicated UTIs while performing additional Phase 1 studies to determine the extent to which a lower-respiratory tract indication is possible. Peptilogics (https://www.peptilogics.com/) is progressing an engineered protein-based therapeutic that possesses broad-spectrum activity against the critical ESKAPE pathogens. The PLG0206 molecule[35] is active against both biofilm and planktonic bacteria, is being developed for the local treatment of prosthetic joint and surgical site infections, and is one of only two products in the CARB-X portfolio to have been granted an Orphan Drug Designation by the FDA. Polyphor Ltd. (https://www.polyphor.com) is utilizing its macrocycle discovery platform to deliver novel synthetic antimicrobial peptides in two programs targeting the essential outer-membrane surface protein BamA and the LptC−LptA interaction. The novelties in both chemistry and in target offer the promise of avoiding cross-resistance to current therapies, including colistin, and might offer a safety advantage with regard to nephrotoxicity, which remains a challenge for the cationic peptide class of molecules. As the BamA program, exemplified by POL7306,[36] has a broader Gram-negative spectrum that includes activity against non-fermenters, the intended Target Product Profile includes lower-respiratory tract infections. With a spectrum that covers the Enterobacteriaceae, including multidrug-resistant isolates, the LptC−LptA program is focused on complicated urinary tract infections. A second program from Contrafect is focused on down-selection of phage-derived lytic peptides, called Amurins.

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With a broad spectrum of coverage of multidrug-resistant Gram-negatives, including the nonfermenters, these novel peptides are being developed to target lower-respiratory tract infections. In addition to exploring the extent to which the octapeptins can operate as potentiators, the University of Queensland is also evaluating the class for its standalone antibacterial activity, which includes activity against colistin-resistant isolates. The aspirational Target Product Profile includes lower-respiratory tract infections. Finally, Lytica Therapeutics (https://www.lyticatherapeutics.com) is exploiting Stapled Antimicrobial Peptide (StAMP) technology to identify novel agents that lyse bacterial cells. The potential advantages of StAMPs include robustness to proteolytic degradation, activity against colistin-resistant isolates, and a better safety profile than previously achieved for antimicrobial peptides.

STAGE OF DEVELOPMENT ASSESSMENT AND CHALLENGES

CARB-X nontraditional portfolio contains programs from Hit-to-Lead to Phase 1, specifically, a First-in-Human (FIH) study, the full scope supported by CARB-X (Figure 3). Importantly,

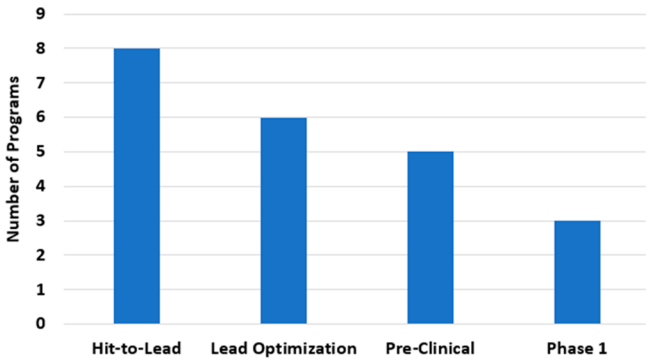

Figure 3. CARB-X nontraditional portfolio separated by the current phase of development.

the three contemporary programs achieving IND-effective (or the foreign regulatory-body equivalent) while under CARB-X support represent a cross-section of the portfolio: anti-virulence, immunotherapy, and microbiome-modifying. We also recognize success in the form of graduates, primed for advanced development, from this portfolio: the microbiome-modifying program of Vedanta, now in Phase 2 studies for recurrent *Clostroides difficile*, was supported through FIH under CARB-X and now is advancing under and Advanced R&D contract with BARDA.

The progression of many of these approaches brings new challenges to antibacterial research and development. Many of the programs require the development of novel *in vitro* assays to assess the potency of products that lack intrinsic antibacterial activity, and underscoring what defines success in these assays that encourage progression to later stages of discovery/development is not straightforward. Proof-of-concept of the efficacious benefit in animal models can also pose challenges. For example, simple colony-forming unit (CFU) end points may not be as instructive when assessing anti-virulence strategies; the demonstration of an enhancement due to harnessing of the immune system is complex, and using these products in combination with a direct-acting antibacterial agent often requires large experimental group sizes and optimization of dose and regimen to demonstrate the additional benefit. For antibody-based products in particular, there are questions around humanization as well as permeation or access to the site of infection.

The narrow spectrum of some of these agents is likely to provide an advantage in reducing collateral damage on the microbiome and could introduce a new focus on precision medicine as well as benefit improved antibiotic stewardship.[37] However, there are substantial challenges that many of these nontraditional products are likely to face during clinical development as they progress toward regulatory approval, for that path has not been well established. Some of these challenges include trial design to demonstrate the appropriate clinical benefit and dose selection and the possible need for superiority trials. Indeed, particularly where many of the decolonization programs are concerned, the high-risk nature of the target population embraces risk at levels not often considered in the early development of new antibiotics. These challenges have been discussed in detail elsewhere,[30] but CARB-X seeks to mitigate these risks with early engagement with regulatory authorities across the portfolio and has begun these conversations as we onboarded our bacteriophage portfolio. Finally, the narrow spectrum of several nontraditional agents is likely to also require the appropriate development and deployment of rapid and sensitive diagnostics to ensure that the appropriate patients are identified. The third pillar of the CARB-X portfolio, in addition to therapeutic and preventative agents, is a rapid diagnostic platform. Approximately 19% of the current CARB-X portfolio (Table 1) is dedicated to the development and validation of diagnostics systems that can be used for bacterial identification and/or antimicrobial susceptibility determination.

CONCLUSIONS AND PERSPECTIVES

The CARB-X and its funders have demonstrated a strong commitment to innovative, nontraditional agents and have provided significant financial and scientific support to help progress these novel products. Much of this portfolio has been built in the last year at CARB-X, and a significant portion of it is in the early stages of research and development. By taking a portfolio view to several of the modalities within the broad bucket of nontraditional agents, we aim not only to advance individual projects to human clinical trials but also to reduce the many challenges we and others recognize through the support of initiatives that benefit many programs; we intend to publish lessons from these studies to help in elevating the general understanding within the antibacterial research and development ecosystem. As the need for new antibacterial agents continues to grow against the backdrop of increasing levels of resistance, it remains vital to progress novel preclinical programs to replenish the clinical pipeline with truly innovative approaches. While challenges exist in the path to regulatory approval and registration, none should be insurmountable, and such products would usher in a new way to treat these serious life-threatening infections.

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Notes

The authors declare no competing financial interest.

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