The State of Innovation in Antibacterial Therapeutics

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Where breakthroughs begin

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Introduction

Antibacterial drug discovery and development during the 20th century yielded one of the greatest armaments available to physicians, with more than 90% of all antibacterial drugs discovered during this period. Since the introduction of numerous antibiotic classes during the 1940s, deaths from bacterial infections have been reduced significantly. However, this next century is already presenting challenges that may render these older antibiotics obsolete. The COVID pandemic, which exposed multiple preparedness shortcomings, has elevated attention to the increasing threat of drug-resistant strains of bacteria. More than 1.2 million people worldwide are dying each year from antibiotic resistant infections and it is estimated this number will grow to 10 million per year by 2050.[1,2] In the U.S. alone, more than 2.8 million antibiotic-resistant infections occur each year, with more than 35,000 people dying as a result.[3] The CDC, EMEA, WHO, IDSA, Pew, and other groups have issued warnings to public policymakers for more than a decade about the reduced effectiveness of last century's antibiotic arsenal (see **Appendix A1** for the most recent pathogen threat list from CDC and WHO).

Millions of currently treatable infections could become life threatening as the prior innovative advantage over bacteria wanes in the coming decades. Hospital acquired bacterial infections alone affect 1.7 million patients per year in the U.S., with almost 100,000 dying as a result.[4] A significant portion of these patients acquire *Clostridioides difficile*, in large part due to taking 20th century antibiotics that harm beneficial bacteria in the human gut.[5] Skin infections affect 14 million patients in the U.S. each year and are responsible for more than 3 million visits to emergency departments.[6] The American Lung Association reports that *Streptococcus pneumoniae* infects the lungs of 900,000 Americans each year, with other bacteria responsible for even more pneumonia cases.[7] There is also a rising threat from bacterial sexually transmitted diseases. According to a 2019 report by the U.S. CDC, Gonorrhea and Syphilis are at their highest case levels since 1991, and Chlamydia has reached 1.7 million cases.[8] Prior to Covid, the leading cause of death from infectious disease globally was *Mycobacterium tuberculosis* infection (TB), with 1.6 million deaths resulting from more than 10 million cases per year.

This report investigates recent investment into antibacterial innovation and the current clinical pipeline that will help bridge the gaps in the antibacterial armamentarium. In our previously published research, drug development investment for many common chronic diseases was found to be declining and low relative to total healthcare burden on society. In that survey of under-funded disease areas, antibiotic investment was also found to be low and not trending upward with the rest of the industry. Unfortunately, over the same time period the total number of infections has risen along with more reports of antibiotic resistance. The lack of investor interest can be seen by contrasting venture investment into antibiotics vs. cancer over the last decade (**Figure 9**). Oncology companies raised close to $7 billion in 2020 (*up* 900% from 2011), whereas antibiotic companies raised just $0.16 billion (*less* than what they raised 10 years prior). This prompted the ongoing investigation to expand our analysis beyond privately funded companies. Specifically, we investigate funding industry-wide and determine to what extent this weakness in funding has impacted innovative antibacterial drug candidates.

1 Murray, C. et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lance*t, accessed online January 20, 2022 at https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)02724-0/fulltext

2 Report to the Secretary-General of the United Nations. (2019). NO TIME TO WAIT: SECURING THE FUTURE FROM DRUG-RESISTANT INFECTIONS. United Nations interagency Coordination Group on Antimicrobial Resistance. Accessed October 2021 from https://www.who.int/publications/i/item/no-time-to-wait-securing-the-future-from-drug-resistant-infections (see Appendix A1 for a list of specific strains and threat levels.)

3 CDC AR Threats Report (2019), accessed October 2021 from: https://www.cdc.gov/drugresistance/biggest-threats.html

4 Hague, M., et al. (2018). Health care-associated infections – an overview. *Infect Drug Resist*, 11, 2321–2333

5 Center for Disease Control and Prevention (2015). Nearly half a million Americans suffer from C. difficile infections in single year. Retrieved from https://www.cdc.gov/hai/dpks/deadly-diarrhea/dpk-deadly-diarrhea.html

6 Hersh, A., et al. (2008). National Trends in Ambulatory Visits and Antibiotic Prescribing for Skin and Soft-Tissue Infections. *Arch Intern Med*, 168(14), 1585-159. and company press release (https://melinta.com/melinta-therapeutics-announces-commercial-availability-of-kimyrsa/)

7 American Lung Association. Accessed October 2021 Retrieved from: https://www.lung.org/lung-health-diseases/lung-disease-look-up/pneumonia/what-causes-pneumonia. Other bacteria include Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Legionella pneumophila,

8 Center for Disease Control and Prevention (2019). New CDC Report: STDs Continue to Rise in the U.S. Retrieved from: https://www.cdc.gov/nchhstp/newsroom/2019/2018-STD-surveillance-report-press-release.html

We begin the report with an historical perspective on what antibacterial innovation over the past century has yielded. Categorizing all FDA approved antibacterials according to strategic approach and molecular target allowed us to assess the current pipeline for novel targets. To investigate funding gaps beyond venture investment, we also analyzed the last 10 years of clinical trial starts – an industry-wide proxy for investment and overall interest, that includes large and small public biopharma companies. In addition, we assess public market investment into antibacterial-focused emerging biotech companies.

Key Takeaways for the State of Antibacterial Therapeutic Innovation

- **Approved Antibacterial Drugs**: Although there have been 164 FDA-approved direct-acting antibacterial new chemical entities (NCEs) since the early 1900s (**Figure 1)**, only one new molecular target NCE has been approved over the last 35 years, illustrating a need to broaden the antibacterial discovery engine (**Figure 2**). There have been 11 indirect-acting NCEs approved, including seven drugs that work to extend the activity of existing drugs and four monoclonal antibodies specific for exotoxins.

- **Pipeline**: The breadth and novelty of the antibacterial clinical-stage pipeline is insufficient to meet the ongoing threat of wide-spread infection from drug-resistant strains. The clinical pipeline contains 54 direct-acting novel chemical or biochemical entities and 10 microbial entities (**Figure 3**). However, of the non-microbial candidates, 61% have targets for which marketed drugs already exist. More than 38% of candidate programs are indicated for *C. difficile* and TB, leaving only 44 drugs for other pathogenic bacteria. Only 10 of these 44 candidates have a novel target. There have been 14 indirect-acting NCEs in the clinical pipeline, including nine that work to extend the activity of existing drugs and five monoclonal antibodies specific for exotoxins.

- **Emerging Company Contribution to Innovation**: Small companies discovered 81% of the antibacterial therapeutics being tested in the clinic. Large biopharmaceutical companies discovered 12%, and small non-profit organizations discovered 7%, of the antibacterial therapeutics being tested in the clinic (**Figure 4**).

- **Clinical Trial Success Rates**: The calculated success rate from Phase I to FDA approval during the period 2011-2020 was 16.3% for an antibacterial new chemical entity (NCE). This is more than twice the overall industry success rate of 7.9%. For novel targeting NCEs, the success rate was 13%, with only three novel target drug programs transitioning at Phase III during this ten-year period (**Figure 8**).

- **Emerging Company Investment**: Venture capital funding of U.S. antibacterial-focused biopharma over the last decade was $1.6 billion compared to oncology's $26.5 billion (**Figure 9**). IPOs for 12 U.S. antibacterial-focused biopharma brought in just $0.7 billion over this period, compared to 109 U.S. oncology companies raising $12 billion (**Figure 10**). This equates to almost 17x less funding of antibacterial vs. oncology companies during this time.

- **Clinical Trial Initiations**: Clinical trial initiations for antibacterial NCEs declined 33% when comparing the five-year period 2016-2020 vs. 2011-2015 (from 55 down to 27 starts, **Figure 11**).

- **Potential solutions to strengthen the antibacterial pipeline**: 1) Early-stage investment (push mechanisms for research), 2) regulatory incentives, 3) late-stage investment (push mechanisms for development), 4) market-based mechanisms (pull incentives), and 5) reimbursement reform.

Definitions

Antibacterials defined herein are direct-acting therapeutics that stop bacterial growth (bacteriostatic) or kill bacteria (bactericidal) or indirect-acting therapeutics that either enhance the effectiveness of direct-acting drugs or inhibit exotoxins secreted by bacteria. This definition for antibacterial therapeutics includes small molecules, proteins, nucleic acids, bacteriophage, and live bacteria. The microbial consortia and single strain bacterial medicines ("microbiome therapeutics") that can prevent target organism growth are included.

We have included mycobacteria in the definition of bacteria for this report. Of the more than 60 species that fall under the *Mycobacterium* genus, *M. tuberculosis* drug resistant strains are considered one of the biggest threats in the world today. (The reason Mycobacteria are excluded in some antibacterial pipeline reports is the highly differentiated molecular structures outside the inner cell membrane compared to gram-negative and gram-positive bacteria. Although all three types of bacteria have a peptidoglycan layer beyond the inner membrane, mycobacteria have a thick layer of lipophilic mycolic acids.[9] These structural differences are important in drug design and optimal drug penetration but can limit the breadth of infections that the drug can be used for).

The term NCE (New Chemical Entity) is used throughout this report and includes biologics, polymers and synthetic peptides in addition to small chemical entities. Live bacteria products are included in the broader group of "new antibacterial therapeutics."

[9] Brown, L., et. al. (2015). Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. *Nature Reviews Microbiology*, 13, 620–630

FDA Approved Antibacterial Therapies

For this report, we investigated every drug marketed in the U.S. since the first antibacterial drug (Salvasaran) debuted in the early 1910s.[10] Since that time, there have been 164 FDA approved direct-acting novel chemical entities. Multiple resources were used to compile this list of FDA approved NCEs: the EvaluatePharma database, the NIH NCATS Insight Drugs database, FDA websites, medical textbooks, and prior reviews on the topic.[11] There are 28 direct-acting NCEs approved outside the U.S. that are excluded from this analysis. It should be noted that all but one of these ex-US antibiotics work by the same strategic approaches as those approved in the U.S.[12] There have been 11 FDA approved indirect-acting antibacterial agents approved. These approved drugs work either as combinations to extend the half-life of *existing* antibiotics (*e.g.,* lactamase inhibitors), or as antibodies against exotoxins.

There are nine established antibiotic strategies for fighting bacterial infections through direct-acting antibacterials (**Figure 1**). The first two use the disruption of either the bacterial cell membrane or the synthesis of their outer cell wall. Both are key features for maintaining unicellular structural integrity and are core approaches that comprise 43% of the approved antibacterial NCEs. Three of the nine approaches target the central dogma of cellular life (the ability of a cell to use DNA to make RNA, and RNA to make proteins). Of these three, the approach with the most approved NCEs is protein synthesis (27%), of which the ribosome itself is most targeted. Three other strategies target enzymes of important metabolic pathways (fatty acids, folic acid, and energy production). Lastly, there are prodrugs that, once inside bacteria cells, are converted to reactive species (such as free radicals) and damage multiple targets (such as DNA, ribosomes, membranes, and various enzymes). This group has been assigned to the "macromolecular damage" strategy.

As can be seen in **Figure 3**, only one novel target has had an NCE approval in the last three decades. The ATP synthase inhibitor bedaquiline was FDA approved in 2012 for TB infections. Prior to this there had not been a single novel target NCE approval since 1987, when the isoleucyl-tRNA synthetase inhibitor, and natural product, mupirocin, was approved. For non-TB drugs, that implies 36 years of no NCE approvals for a new target based on this antibacterial definition.[13] Peak discovery and development of novel targets occurred between the 1940s and 1960s period, when 15 new targets received NCE approvals. This compares to only three new targets for the subsequent three decades.

Innovation has not been limited to new targets, however. There have been significant contributions in the form of NCEs for validated targets (*i.e.,* FDA approval) that offer extended strength against resistant strains, better patient experience (*e.g.*, ease of use, limited side effects, oral delivery, and better efficacy). Looking through that lens at the last 30 years we found 53 NCE approvals for established targets (**Figure 3**).

Below, we describe how previously approved drugs work on the specific target families that were outlined in **Figure 1**. Following this introduction to established antimicrobial strategies, we review the current clinical pipeline's new antibacterial strategies. All pipeline therapeutics reviewed herein for Phase I through NDA/BLA filing are new, with no prior indication approval. However, as some pipeline therapeutics continue to work through similar target and mechanism as last century's drugs, we further separate the pipeline by novelty status of targets.

[10] Zaffiri, L., et. al. (2012). History of antibiotics. From salvarsan to cephalosporins. *J Invest Surg*, 25(2), 67-77.

[11] EvaluatePharma: https://www.evaluate.com, NCATS: https://drugs.ncats.io/, Peterson, J. (1996). Medical Microbiology. 4th edition, and Waller, D., Sampson, T. (2014). Medical Pharmacology and Therapeutics, Fourth Edition. Publisher: Saunders

[12] One exception is fusidic acid, isolated from *Fusidium coccineum* in the 1960s and unique among the translation inhibitors in that it binds to GTP-hydrolyzing elongation factor G (EF-G). U.S. development of the drug was suspended.

[13] There have a been four approvals for antibody against exotoxins (Anthrax, and C. diff), but these stop severity and do not stop growth or kill bacteria. Gene knockouts for most exotoxins have been shown to not inhibit bacterial growth.

HOW FDA APPROVED ANTIBACTERIALS WORK

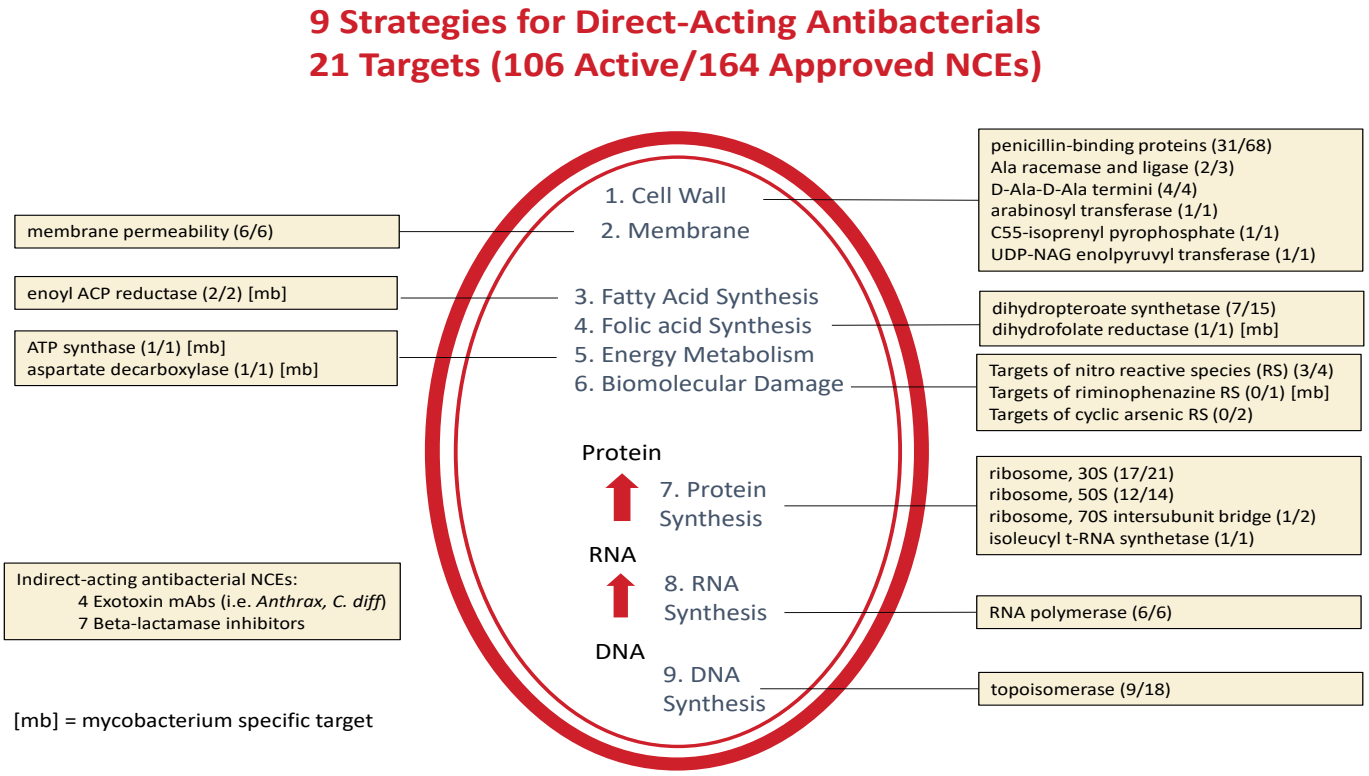

Figure 1. Diagram of currently employed drug targeting strategies to kill or prevent bacteria growth. Of the 164 NCEs approved, 106 remain actively marketed. Shown in parenthesis after the target name is the number of active NCEs, followed by the total number of NCEs approved.

TIMELINE OF FDA APPROVED DIRECT-ACTING ANTIBACTERIAL NCES 1900-2019

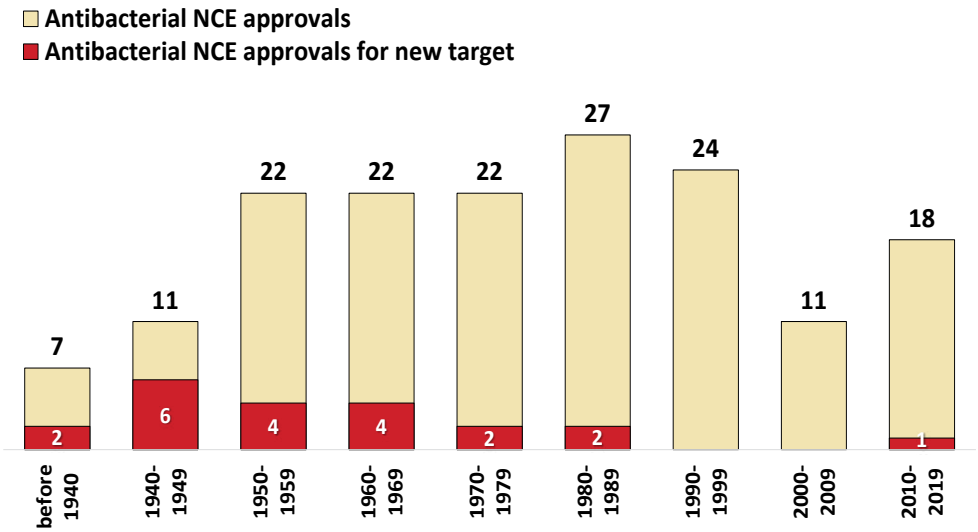

Figure 2. Timeline of 164 NCE FDA approvals for antibacterials by decade approved. The 21 new target approvals are also shown by decade first NCE was approved.

Strategy #1. Drugs that Target Cell Wall Synthesis

The antibacterial approach with the most FDA drug approvals is inhibition of the bacterial cell wall. Multiple classes of enzymes and ancillary molecules are required for cell wall synthesis and maintenance. As they are not found in human cells, these proteins and prokaryotic-specific molecules make for good drug targets. Another advantage these targets have for therapeutic intervention is their location outside the cytoplasmic membrane.[14] The following are six cell wall target families that have FDA approved drugs: penicillin binding protein (PBP), alanine racemase, arabinosyl transferase, C55-isoprenyl pyrophosphate, D-Ala-D-Ala terminus of NAG-NAM-peptide, and UDP-NAG enolpyruvyl transferase. The disadvantage of these approved treatments, which is a common theme for the majority of antibacterials reviewed below, is that multiple modes of resistance have developed due to widespread use over the last 80 years.

Penicillin binding protein (PBP) antibacterials: Of the 21 targets and target families listed in **Figure 1**, the penicillin binding protein (PBP) target family has received the most NCE approvals, with a total of 68 NCEs approved by the FDA since their introduction in the 1940s. Less than half of these remain on the market today. The 31 actively sold in the U.S. are classic beta-lactam structures that fall into four main chemical classes: penicillins, cephalosporins, carbapenems, and monobactams. These antibacterials have been used against a broad range of aerobic and anaerobic gram-positive and gram-negative organisms.

However, resistance to beta-lactam antibacterials has evolved based on two premises: beta-lactamase production and mutational evasion of the PBP genes. Lactamases cleave the beta-lactam ring inactivating the antibacterial drug. In other resistant bacteria, mutations evolve lower affinity PBPs to the beta-lactam drugs making them less specific and less inhibitory.[15] As bacteria have multiple PBPs (*E. coli* has more than 10, for example), they can compensate for therapeutic stress applied to one subtype.[16]

Not shown in **Figures 2** and **3**, are the beta-lactamase inhibitors. On their own they do not have antibacterial activity, thus do not meet the definition of direct-acting antibacterials in this report but have immense value in increasing the longevity of decades old beta-lactam drugs facing resistance. Seven innovative beta-lactamase inhibitors have been approved for use in combination with beta-lactam drugs for resistant bacteria.

Alanine racemase/ligase antibacterials: One of the early building blocks of the cell wall synthesis process is D-alanine, the stereoisomer of the more common amino acid, L-alanine. D-alanine is converted from L-alanine by alanine racemase. Two of the D-alanine amino acids are then linked via peptide bond by alanine ligase before being used in the pentapeptide-sugar-lipid components of cell wall synthesis. Two alanine racemase/ligase inhibitors were approved in the 1950s and 1960s, and one, Cycloserine, remains in use today. Cycloserine is a structural analog of D-alanine that fits into the active site of both enzyme targets needed for peptidoglycan synthesis. Cycloserine is indicated for the treatment of tuberculosis (TB) and urinary tract infections (UTIs). Resistance to cycloserine has developed through mutations in multiple genes.[17]

[14] Liu, Y., et al. (2016). The Membrane Steps of Bacterial Cell Wall Synthesis as Antibiotic Targets. *Antibiotics* 5(3), 28
[15] Zapun, A. et al. (2008) Penicillin-binding proteins and b-lactam resistance. *FEMS,* 32, p.361-385
[16] Sauvage, E. et al. (2008). The penicillin-binding proteins: structure and role in peptidoglycan Biosynthesis. *FEMS*, 32, p.234-258
[17] Chen, J. et al. (2017). Identification of novel mutations associated with cycloserine resistance in Mycobacterium tuberculosis. *Antimicrob Chemother*.

D-Ala-D-Ala terminus antibacterials: There are four drugs approved that target the D-alanine-D-alanine (D-Ala-D-Ala) terminus of the cell wall NAG-NAM-peptide chain, thus preventing transpeptidase elongation and cross-linking of the peptidoglycan cell wall. The most well-known drug within this target family is vancomycin, a cyclic glycopeptide approved in 1958 for gram-positive infections. Unfortunately, vancomycin resistant strains have developed through gene transfer of five genes involved in circumventing the requirement for D-Ala (for some strains, this means replacement by D-Lac).[18] Vancomycin-resistant *enterococci* (VRE) and Vancomycin-resistant *S. aureus* (VRSA) are represented in the CDC and WHO top priority pathogen lists (**Appendix A1**). Three other glycopeptide drugs with a similar mechanism have been approved in the last 15 years.

Arabinosyl transferase antibacterials: The drug ethambutol is the only approved drug that inhibits arabinosyl transferase. It is specific to mycobacteria as it inhibits polymerization of arabinogalactan, a saccharide polymer attached to mycolic acids in mycobacteria.[19] Ethambutol has been used for the treatment of pulmonary tuberculosis (TB). Over production of the transferase gene can lead to resistance.[20]

C55-isoprenyl pyrophosphate antibacterials: In 1948 bacitracin was approved for use as an injectable antibiotic to treat staphylococcus infections.[21] Its mechanism of action involves binding to C55-isoprenyl pyrophosphate. No other drugs in this class have been approved. Resistance can develop through gene acquisition of efflux and transporter pumps.[22]

UDP-NAG enolpyruvyl transferase antibacterials: In the 1970s, fosfomycin was approved for the treatment of bladder infections. Fosfomycin inhibits UDP-NAG enolpyruvyl transferase (sometimes referred to by its gene name, MurA), which is part of the initial steps in the cytoplasm required for the building blocks of cell wall synthesis (specifically, UDP-N-acetylmuramyl-pentapeptide).[23] Resistance can develop through mutations that inactivate the nonessential glycerophosphate transporter rendering bacteria resistant to fosfomycin.[24]

[18] Cong, Y., et al. (2020). Vancomycin resistant Staphylococcus aureus infections. J Adv Res. p.169–176
[19] Alderwick, L. et al. (2015). The Mycobacterial Cell Wall – Peptidoglycan and Arabinogalactan. *Cold Spring Harb Perspect Med*.
[20] Belanger, A.E. et al. (1996). The embAB genes of Mycobacterium avium encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. *Proc Natl Acad Sci*.
[21] The original approval for bacitracin was for a systemic injectable in 1948. In 2020 the U.S. FDA sent a request to manufacturers to withdraw this injectable form from the market. Bacitracin remains commonly used as a topical treatment for dermal infections.
[22] Ma, J., et al. (2019). Bacitracin resistance and enhanced virulence of Streptococcus suis via a novel efflux pump. BMC Vet Res. P.15: 377
[23] Liu, Y., Breukink, E. (2016). The Membrane Steps of Bacterial Cell Wall Synthesis as Antibiotic Targets. Antibiotics 2016, 5(3), 28. Retrieved from: https://www.mdpi.com/2079-6382/5/3/28/htm
[24] Castañeda-García A, Blázquez J, Rodríguez-Rojas A (2013). Molecular Mechanisms and Clinical Impact of Acquired and Intrinsic Fosfomycin Resistance. *Antibiotics*, 2(2). 217–36.

Strategy #2. Drugs that Disrupt the Cell Membrane

The cell membrane is not as rigid as the cell wall and can become porous, making it a good target for gram-negative species of bacteria, which are reliant on a two-membrane architecture and less dependent on a thick cell wall. Examples of chemical classes that function in this manner are linear amphipathic peptides, polymyxin cyclic peptides, lipopeptides, and amphipathic small molecules.[25] The first approved peptide antibacterial (1942) was the linear amphipathic peptide Gramicidin, which is so disruptive to membranes it was only used as a topical formulation to prevent skin infections. The same restrictions apply to the antiseptic chlorhexidine, a cationic polyguanide compound, as its membrane disruption is not selective for microbes and can disrupt fragile blood cells.[26] Three marketed polymyxins were approved in the 1950s-1960s: Polymyxin B, Polymyxin E, and a prodrug of Polymyxin E.[27] They are used for bacterial conjunctivitis and skin infections and are specific to gram negative bacteria. The single lipopeptide approved is the natural *Streptomyces* compound daptomycin (FDA approved in 2003 and marketed as Cubicin). Another approved treatment under this strategy is Daptomycin which aggregates with phosphatidylglycerol components of the cell membrane causing pores or holes. This causes ion gradient dependent systems, such as respiration, to cease operating functionally.[28] Daptomycin is differentiated from others in this group in that, in addition to skin infections, it can be used for blood infection caused by gram-positive *S. aureus*.[29]

Strategy #3. Drugs that inhibit fatty acid synthesis

Bacteria rely heavily on multi-enzyme complexes for the synthesis of fatty acid substrates that will be further processed as components on the cell membrane, cell wall, and for mycobacteria, the mycolic acid lipid layer. Fatty acid synthesis in bacteria involves at least seven enzymes that are distinct from those used by mammalian cells and thus make for great antibacterial targets. One of these enzymes has been successfully drugged: **enoyl-ACP reductase.** The trans-2-enoyl-ACP reductase has multiple isoforms that that can handle longer chain fatty acids in different organisms including: FabI (*E.coli* and other gram-negative), FabK (*Streptococcus pneumoniae*), and InhA (*Mycoplasma*).[30] InhA, which can bind longer chain acyl groups, is the target of isoniazid and its derivative, ethionamide (both marketed since the 1950s and 1960s, respectively). The widely marketed compound Triclosan inhibits FabI in numerous non-mycobacterial strains. However, resistance to these drugs can be gained through single point mutations in the reductase enzyme's active site.[31,32]

Strategy #4. Drugs that inhibit folic acid synthesis

Although some categorize the folate pathway as part of nucleic acid synthesis, there are other metabolic functions (*e.g.* amino acid synthesis) where folate (tetrahydrofolate, THF) plays a role. Thus, we have separated folic acid synthesis into its own strategy. There are two enzymes in the THF pathway targeted by approved drugs, **dihydropteroate synthetase** and **dihydrofolate reductase**. As humans get folate from diet, these enzymes are unique to bacteria making them great antibiotic targets. The first enzyme, dihydropteroate synthetase, ligates a phosphorylated pteridine and para-aminobenzoate (PABA) to

[25] Waller, D., Sampson, T. (2014). Medical Pharmacology and Therapeutics, Fourth Edition. Publisher: Saunders

[26] Liu, J. et al. (2018). Cytotoxicity evaluation of chlorhexidine gluconate on human fibroblasts, myoblasts, and osteoblasts. *J Bone Jt Infect*, 3(4): 165–172. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6098817/

[27] Bergen PJ, Li J, Rayner CR, Nation RL (2006). Colistin methane sulfonate is an inactive prodrug of colistin against Pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy* 50 (6): 1953–8.

[28] Pogliano, J., et al. (2012). Daptomycin-Mediated Reorganization of Membrane Architecture Causes Mislocalization of Essential Cell Division Proteins. *Journal of Bacteriology,* 194, 17 (2012). *To an extent, all the above membrane disrupting drugs could impact energy production through cellular respiration, but as there are multiple functionalities being hit, we have placed within membrane disruption more broadly*.

[29] FDA prescribing information for Cubicin. Retrieved from https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/021572s059lbl.pdf

[30] Rana, P., et al. (2020). FabI (enoyl acyl carrier protein reductase) - A potential broad spectrum therapeutic target and its inhibitors. *European Journal of Medicinal Chemistry*, 208. Retrieved from: https://www.sciencedirect.com/science/article/abs/pii/S0223523420307297

[31] Dessen, A. et al. (1995). Crystal structure and function of the isoniazid target of Mycobacterium tuberculosis. *Science,* 267(5204):1638-41. Retrieved from: https://pubmed.ncbi.nlm.nih.gov/7886450/

[32] Bernardes-Génisson, V. et al. (2013). Isoniazid: an update on the multiple mechanisms for a singular action. *Curr Med Chem,* 20(35), p4370-85. Retrieved from: https://pubmed.ncbi.nlm.nih.gov/23931278/

make dihydropteroate (DHP), which is then converted to DHF (by DHF synthase). DHF is then combined with glutamate by dihydrofolate reductase to form THF. There have been 15 FDA NCE approvals for the dihydropteroate synthetase enzyme (the first being in the 1930s) and only two for dihydrofolate reductase (approved in the 1940s and 1960s). Dihydropteroate synthetase inhibitors are dominated by the sulfonamide and sulfone chemical class antibacterials that work by mimicking the PABA substrate in the reaction. 4-Aminosalicylic acid and trimethroprim are the two drugs that inhibit dihydrofolate reductase.

Strategy #5. Drugs that inhibit energy metabolism and respiration

Although there are two drugs in this category, both used to treat TB, they are quite different. The first represents the only new target for an antibacterial in more than 30 years: **ATP synthase**. Bedaquiline was approved by the FDA in 2012 and marked the first TB drug approved in 40 years.[33] The second drug in this group is Pyrazinamide, approved in 1971. By tagging aspartate decarboxylase for degradation, mycobacterium cannot make the beta-alanine needed to generate pantothenate, the main component of coenzyme A (CoA). CoA is a critical enzyme cofactor for metabolic pathways.[34] Aspartate decarboxylase is a differentiated target from humans as human do not make their own pantothenic acid (the essential vitamin B5).

Strategy #6. Drugs that cause broad damage to macromolecules

There are a few antibacterial NCEs marketed in the U.S. that do not fit into the single target paradigm as described above. They exert pleiotropic cellular dysfunction, in large part due to the derivatives and byproducts (*i.e.* reactive oxygen species) generated once the drug is inside the bacterial cell. For example, nitrofurans and nitroimidazoles have a nitro group that can be reduced by nitroreductases to activate reactive secondary compounds and free radicals.[35] These reactive species then target multiple sites in the cell, causing direct damage to DNA, ribosomes, various proteins, membranes and energy respiration systems exhibiting potent antimycobacterial activity.[36,37] Nitrofurantoin and Metronidazole, approved in the 1950s and 1960s, respectively, are two of the three active drugs representing the nitro reactive species in **Figure 1**. For both of these drugs, prior studies have shown DNA modification as well as destruction of energy metabolism.

[33] Mahajan, R. Bedaquiline. (2013). First FDA-approved tuberculosis drug in 40 years. *Int J Appl Basic Med Res*, 3(1), 1-2
[34] Gopal, P et al. (2020). Pyrazinamide triggers degradation of its target aspartate decarboxylase. *Nature Communications*, 11, 1661
[35] Moreno, S. et al. (1984). Distinct reduction of nitrofurans and metronidazole to free radical metabolites by Tritrichomonas foetus hydrogenosomal and cytosolic enzymes. *Journal of Biological Chemistry*, 259, p.8252–8259
[36] Zuma, H. (2019). An update on derivatisation and repurposing of clinical nitrofuran drugs. *European Journal of Pharmaceutical Sciences*, 140, 105092. Retrieved from: https://pubmed.ncbi.nlm.nih.gov/31634556/
[37] Le VVH, Rakonjac J (2021) Nitrofurans: Revival of an "old" drug class in the fight against antibiotic resistance. *PLoS Pathog,* 17(7): e1009663. Retrieved from: https://doi.org/10.1371/journal.ppat.1009663

The third marketed drug generating nitro reactive species is the nitroimidazole Pretonamid, approved in 2019 under the Limited Population Drug Pathway (LPAD pathway) for TB. It was previously believed to be specific to mycolic acid synthesis pathway, but more evidence suggests a broader targeting of the activated species explaining its ability to work on rapidly growing and latent mycobacterial cells.[38,39,40] The structurally similar compound Delamanid (OPC-67683) is approved outside the U.S. but currently at Phase III in the U.S. (See **Appendix A4** for more detail on the categorization of these compounds.)

Clofazimine, approved in the U.S. for leprosy (*Mycobacterium leprae* infection) in the 1980s, does not have a nitro group but is believed to be reduced by bacterial oxidoreductases and oxidized by oxygen to form unstable intermediates that damage cell membranes and guanine bases of DNA (particularly mycobacteria and gram-positive bacteria).[41,42,43] Although Clofazimine has been withdrawn from the U.S. market (as indicated in **Figure 1**), it can still be obtained through the National Hansen's Disease (Leprosy) Program.

The third target group using this strategy of damaging a wide array of biomolecules contains two drugs no longer on the market: Arsphenamine (Salvarsan) and neoarsphenamine (NeoSalvarsan). These were the first chemical agents used specifically to kill bacteria, primarily *Treponema pallidum* (for Syphilis). Their use began in the early 1910s, but both were discontinued due to toxicity and the introduction of penicillin in the 1940s, respectively. The arsphenamines contain a cyclic arsenic structure responsible for their antibacterial properties. It is possible that that they bind thiol groups of enzymes involved in energy metabolism and other important cellular functions.[44] Other arsenic compounds used to fight parasitic infections use a similar strategy. For example, the arsenic containing antiparasitic Melarsoprol irreversibly binds to thiol groups on pyruvate kinase, a key enzyme in energy metabolism.[45]

Strategy #7. Drugs that Inhibit Protein Synthesis

There are numerous differences in prokaryotic vs. eukaryotic cell translation that allow for specific targeting of drugs to block protein production. There are also multiple proteins and RNA macromolecules to choose from along the four-step process of translation: aminoacyl-tRNA assembly, initiation, elongation, and termination. The second largest group of FDA approved NCEs (after inhibitors of penicillin binding proteins) target the bacterial **ribosome** at either the 30S or 50S subunit (21 and 16 NCE approvals, respectively), most working at the early initiation stage of translation . One other ribosomal binding drug is differentiated from the others as it targets the bridge between the 30S and 50S subunits. The outlier in the group acts at the early tRNA synthesis stage. Drug that targets these translational components of bacteria are described below.

38 Thakare, R., Dasgupta, A., Chopra, S. (2020). Pretomanid for the treatment of pulmonary tuberculosis. *Drugs* Today, 56(10):655-668.
39 Baptista, R., Fazakerley, D.M., Beckmann, M. et al. (2018). Untargeted metabolomics reveals a new mode of action of pretomanid (PA-824). *Sci Rep,* 8, 5084.
40 FDA Press Release, (2019). FDA approves new drug for treatment-resistant forms of tuberculosis that affects the lungs. Accessed Nov 2021: https://www.fda.gov/news-events/press-announcements/fda-approves-new-drug-treatment-resistant-forms-tuberculosis-affects-lungs
41 Gopal, M. et al. (2013). Systematic review of clofazimine for the treatment of drug-resistant tuberculosis. *INT J TUBERC LUNG DIS*, 17(8) 1001–1007.
42 Yano, T., et al. (2011). Reduction of Clofazimine by Mycobacterial Type 2 NADH: Quinone Oxidoreductase. *J Biol Chem,* 286(12): 10276–10287.
43 Cholo, M. et al. (2012). Clofazimine: current status and future prospects. *Journal of Antimicrobial Chemotherapy*. 67(2), 290–298
44 Shen, S., et al. (2013). Arsenic Binding to Proteins. *Chem Rev.* 113(10), 7769–7792 and Yarnell, A. (2005). Salvasaran. C&EN, 83, 25
45 Sharma, S., et al. (2017). Approaches to Design and Synthesis of Antiparasitic Drugs. *Pharmacochemistry Library*, 25, 1-577

Half of the **30S ribosome** targeting NCEs are tetracycline derivatives and the other half are aminoglycoside entities. Both chemical classes were introduced starting in the late 1940s. The tetracyclines inhibit binding of aminoacyl-tRNA to the ribosome. They are used to treat gram-positive and gram-negative infections. Resistance has developed through efflux transport (pumping antibacterials back out of the cell) and through mutations of the ribosome. The aminoglycosides work in a similar manner at the 30S ribosome aminoacyl-tRNA site but may also have a membrane disrupting mechanism. They are more active against gram-negative than gram-positive infections and do not work as well against anaerobic bacteria. Resistance to aminoglycoside drugs has developed through bacterial enzymatic modification of the drug itself.

50S ribosome inhibitors include the macrolides (*e.g.,* azithromycin), amphenicols (*e.g.,* chloramphenicol), oxazolidinones (*e.g.*, linezolid), pleuromutilins (*e.g.*, retapamulin), streptogramins, (*e.g.,* quinupristin/dalfopristin combo therapy), and lincosamides (*e.g.*, lincomycin). The first four chemical classes arrived on the market in the U.S. during the 1940s and 1950s, while the lincosamides were approved in the 1960s. Since the adoption of these antibacterials in the middle of the 20th century, various forms of resistance have developed. Like the aminoglycosides, the macrolides, streptogramins, and amphenicols can be modified to an inactive form or they can be exported through efflux mechanisms. Lincosamide and oxazolidinone resistance stems from ribosomal mutation.

Two structurally similar NCEs, the cyclic peptides viomycin and capreomycin bind between the two large subunits of the bacterial ribosome. The binding location suggests that these drug works by locking the tRNA in a pre-translocation state.[46] Although viomycin was approved in 1953 for TB, it was discontinued in the U.S. after the 1971 introduction of capreomycin.

The non-ribosomal binding target in this group is **isoleucyl t-RNA synthetase**. Mupirocin binds this target and depletes the cell of functioning Ile-tRNA. It was approved by the FDA in 1987 as a topical cream for *Staphylococcus aureus* (G+) or *Streptococcus pyogenes* (G+).

Strategy #8. Drugs that Inhibit RNA Synthesis

Rifamycins inhibit RNA synthesis directly through **RNA polymerase**. Six rifamycin class NCEs were approved between 1971-2018 to make up this target family and all six remain on the market (**Figure 1**). Rifamycins are active in gram-positive bacteria, gram-negative bacteria, and mycobacteria in part due to how selective they are compared to human RNA polymerase. Clinical use range is broad and includes diseases caused by *M. tuberculosis*, *M. avium*, *Brucella spp.* (G-), (Brucellosis/Mediterranean fever) *Legionella pneumophila* (G-), (Legionnaires' disease), *S. aureus*, *N. meningitis*, *H. pylori*, and traveler's diarrhea caused by gram-negative bacteria.[47] Chronic use has been shown to generate resistant bacteria through specific changes in the RNA polymerase beta subunit gene.[48]

[46] Zhang, L., et al. (2020.) The structural basis for inhibition of ribosomal translocation by viomycin. *Proceedings of the National Academy of Sciences*, 117 (19) 10271-10277.
[47] Waller, D., Sampson, T. (2014). Medical Pharmacology and Therapeutics, Fourth Edition. Publisher: Saunders
[48] Goldstein, B. (2014). Resistance to rifampicin: a review. *J Antibiot (Tokyo).* 67(9):625-30.

Strategy #9. Drugs that Inhibit DNA Synthesis

The quinolones and fluoroquinolones make up the majority of marketed NCEs that target DNA synthesis machinery. They work by binding to **topoisomerase** enzymes, primarily DNA gyrase (a topoisomerase II enzyme) and topoisomerase IV, that unwind DNA during replication, introduce negative supercoils, and remove knots found in the circular chromosome. The first generation of quinolones initiated through nalidixic acid's approval in 1964 but it has since been discontinued. Subsequent generations of quinolones were approved throughout the 1980s (*e.g.,* ciprofloxacin, 1987), 1990s (*e.g.,* levofloxacin, 1996), 2000s (*e.g.,* Gemifloxacin, 2003), and even as recently as 2017 (delafloxacin). Of the 17 FDA approved quinolone NCEs, eight remain on the market today. As with other NCEs described above, resistance can come from either mutations of the topoisomerase or through efflux pumps.

Only one non-quinolone drug in this target family has been approved: the aminocoumarin DNA Gyrase B inhibitor, novobiocin, isolated from *Streptomyces*. However, this drug was withdrawn from the U.S. market in the 1980s after more than 30 years on the market.[49]

For indirect-acting antibacterials, there are five targets. Beta-lactamase is a target for six FDA approved NCEs that work only in combination of existing PBM-targeting lactam drugs. A seventh drug also extends the activity of lactam drugs, but targets a human kidney enzyme, dihydropeptidase. The remaining three targets are exotoxins: *Bacillus anthracis* protective antigen (two approved mAbs), *C. difficile* Toxin B (one approved mAb), and *Clostridium botulinum* nerve toxin serotypes A-G (one approved polyclonal antibody mix for serotypes A, B, C, D, E, F, G). These secreted factors from bacteria cause damage to the immune system and can be fatal, making anti-toxin therapeutics a high priority. One advantage they have over the antibacterials described above is that they place little selective pressure on the pathogen itself and thus are less likely to result in drug resistance strains.

[49] Lowe, D. (2021). Novobiocin Returns? But Not as an Antibiotic. *Science*.

Clinical Pipeline for Antibacterials

The total clinical pipeline for new antibacterial therapeutics consists of 64 unique new antibacterial therapeutics, with 31 (48%) having novel targets. The number of drug ***programs*** (drug-indication pathways) for these 64 therapies is 72. Only eight drugs have a secondary indication pathway program and none of the therapeutic entities have more than two.

The antibacterial therapeutics pipeline is broken out by phase for total indication pathways and for individual entities in **Figure 3**. As is the case with most disease area pipelines, the largest number of programs reside in Phase II. For antibacterial indication programs, there are 31 programs in Phase II vs. 25 programs for Phase I and 15 programs for Phase III.

For the 64 new antibacterial therapeutic entities illustrated by Phase in **Figure 3**, we counted the most advanced phase for each unique drug candidate. There are more new antibacterial therapeutic entities in Phase II (16) vs. those for established targets (13). In Phase I and III, there are slightly fewer drug candidates with novel targets vs. those with targets previously approved. In Phase I, there are 10 novel target programs vs. 13 established target programs. In Phase III, there are 5 novel target programs vs. 6 established target programs. Only one drug candidate with an approved target was at the FDA NDA filing stage as of October 2021.[50]

CLINICAL-STAGE DRUG PIPELINE FOR DIRECT-ACTING ANTIBACTERIALS BY PHASE

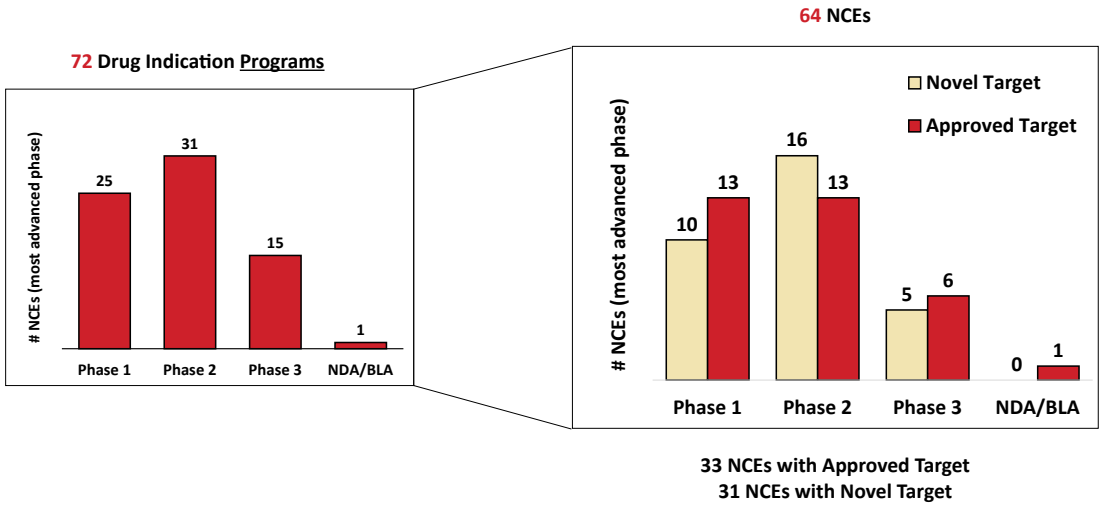

Figure 3. The clinical pipeline for direct-acting antibacterials by Phase (as of October 2021), based on Biomedtracker's classification methodology by Phase of development as well as company website information. Five programs listed as "Ex-U.S." in the Biomedtracker database are included here by Phase based on independent research of company websites. (The "Ex-U.S." listing in Biomedtracker database implies the companies have not yet intended to seek FDA approval.)

[50] For a comparison of our pipeline to recent reports from the WHO and the PEW Charitable Trust, see **Appendix A4**.

Clinical Pipeline by Originating Company

More than 60 companies and non-profit research institutes are developing the 64 clinical-stage drug candidates to meet the growing need for differentiated antibacterials. As shown in **Figure 4,** the number of new antibiotic therapies originating from small companies accounted for 80% of the drug discoveries, 8% originated from non-profit institutes and universities, and 12% originated from large companies.

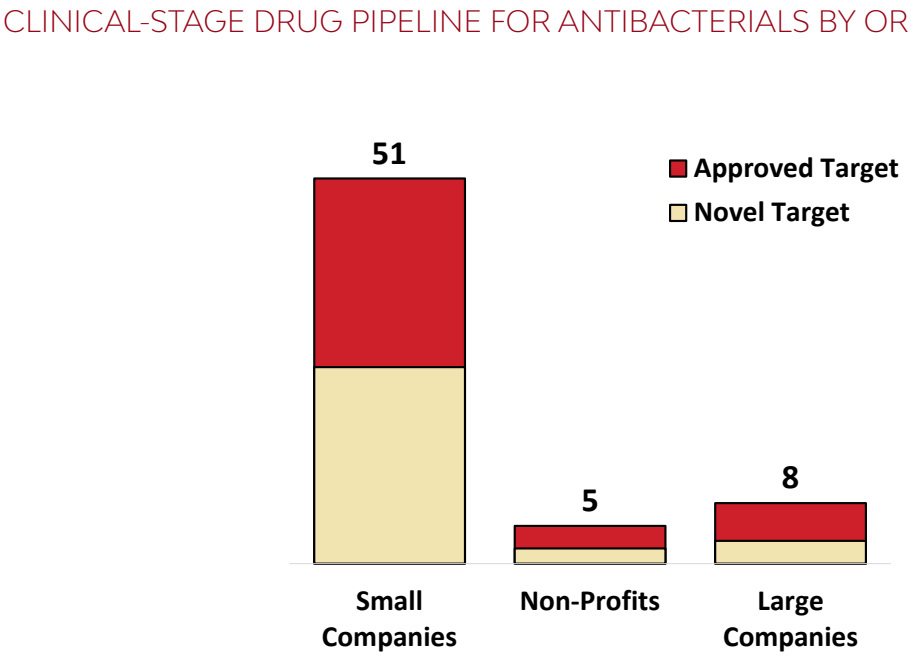

Figure 4. Antibacterial clinical pipeline NCEs by discovering entity. Non-Profits includes independent R&D institutes, and universities. Small companies are defined as less than $1 billion in revenue.

Clinical Pipeline by Modality and Delivery

The direct-acting antibacterial therapeutic clinical pipeline modalities and modes of delivery can be found in **Figure 5**. There are 47 small molecule NCEs (73%) and 17 biologics (27%) in the clinical pipeline. Traditional *systemic* small molecule "antibiotics" account for 97% (45 of 47) and topical small molecules account for 3% (2 of 47) of the small molecule pipeline NCEs. Of the 17 biologics, there are 7 systemic biologics (injectables for proteins or bacteriophage) and 10 live bacterial products that are orally delivered to the gut. **Figure 5** also separates the modality and delivery characteristics of the pipeline. There are three times as many small molecules for approved targets than for novel targets, whereas all 17 biologics are for novel targets.

CLINICAL-STAGE DRUG PIPELINE FOR ANTIBACTERIALS BY MODALITY AND DELIVERY SYSTEM

Modality/Delivery for NCEs

NCE type	Total	%
Systemic small molecules	45	70%
Topical small molecules	2	3%
Protein injectibles	5	8%
Phage injectibles	2	3%
Live oral/gut biotherapeutics	10	16%
Total	64	100%

Pipeline by Modality

NCE type	Target	Total	%
Small Molecule	Approved	33	52%
Biologic (bacterial)	Approved	0	0%
Biologic (protein)	Approved	0	0%
Biologic (phage)	Approved	0	0%
Small Molecule	Novel	14	22%
Biologic (bacterial)	Novel	10	16%
Biologic (protein)	Novel	5	8%
Biologic (phage)	Novel	2	3%
Total		64	100%

Pipeline by Delivery

NCE type	Target	Total	%
Systemic	Approved	31	48%
GI	Approved	0	0%
Topical	Approved	2	3%
Systemic	Novel	21	33%
GI	Novel	10	16%
Topical	Novel	0	0%
Total		64	100%

Figure 5. Antibacterial clinical pipeline NCEs by modality and delivery. * GI, gastrointestinal for microbiome therapies. Systemic includes inhaled therapies (n=2).

Clinical Pipeline by Strategy and Target

Figure 6 shows the direct-acting antibacterial clinical pipeline by therapeutic strategy and target family. There are currently 13 different strategic approaches being used across the 64 therapeutics in the clinical pipeline. Within these 13 strategies, 28 targets are being pursued. With live bacterial products grouped as a single target family (gut microbiome), there are 18 total novel targets and 10 targets with prior approval history. Each of these target families, and the drug candidates targeting them, are described below.

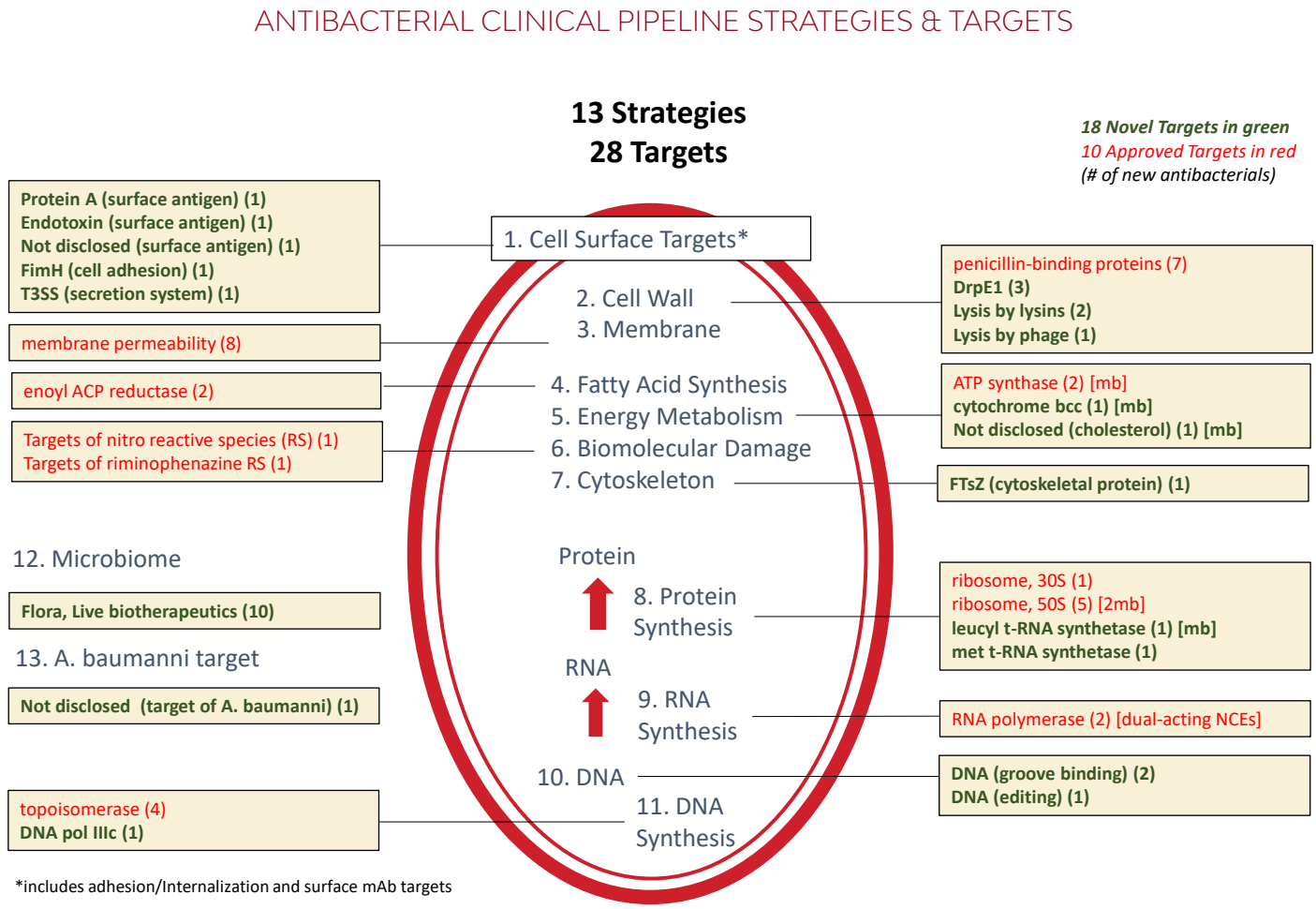

Figure 6. The clinical pipeline for 64 new antibacterial therapeutics by strategy and target. The 31 new antibacterial therapeutics with novel targets are shown in green and the 33 new antibacterial therapeutics with approved targets are shown in red. Flora refers to microbiome approaches to re-establish healthy bacteria or introduce bacteria that can kill disease-causing bacteria. See text for more information on the three undisclosed targets.

Drug Candidates with Novel Targets in the Clinical Pipeline (31 therapeutic candidates for 18 novel targets)

1. **Cell wall synthesis: DrpE1 (3)**. There are three drugs in the clinic that target decaprenyl-phosphoryl-beta-D-ribofuranose oxidoreductase (DrpE1). This enzyme holds a key role in synthesizing glycolipid precursor units of mycobacterium cell walls.[51] All three drugs targeting DRpE1 are in Phase II and indicated for *M. tuberculosis*.

2. **Cell wall lysis by lysins (2)**. There are two biotherapeutics in the clinic that utilize enzymatic lysis to kill bacteria. One is a purified phage lysin for *S. aureus* (in Phase III) and the other a purified lysin specific for *C. difficile* (in Phase I).

3. **Cell wall lysis by bacteriophage (1)**. One phage therapy has started human testing for drug resistant *Pseudomonas aeruginosa*.[52] This approach utilizes the bacteriophage life cycle process of progeny release that releases lysins to break the outer wall and membrane of the bacteria.

4. **Cell division: Cytoskeleton (1).** The bacterial protein product of the FtsZ gene is akin to tubulin in eukaryotic cells, with a similar role in cell division. There is one Phase I drug targeting FtsZ in the pipeline and it has the QIDP designation.

5. **Surface target: Secretion Systems (1)**. Type III Secretion System (T3SS) of pathogenic gram-negative bacteria is a multiprotein molecular syringe that can inject adhesion and internalization factors into human cells.[53] Certain T3SS inhibitors have demonstrated a reduction in growth and are thus included in this analysis of antibacterials (as separate from anti-virulence factors that would only lesson the toxicity of the secreted factor). Russia's Ftortiazinon is being studied for use in Complicated Urinary Tract Infections (cUTI) caused by *P. aeruginosa*.[54]

6. **Surface target: Endotoxin (1).** Endotoxin is a lipopolysaccharide residing on the outer cell surface of gram-negative bacteria. Within the structure of endotoxin is the O-polysaccharide moiety that has been targeted by mAbs. One of the mAbs is in Phase II for *P. aeruginosa* infection.

7. **Surface target: Protein A (1).** Protein A is a unique cell surface protein in that interacts with constant regions of human antibodies. While this has been known for more than 40 years and exploited for mAb purification, protein A is now the target itself for *S. aureus* infection. One antibody specific to *S. aureus* protein a is in Phase I/II.

8. **Surface target: Unknown for mAb (1).** One unknown surface protein target (and likely multiple targets) is the antigen of a pooled polyclonal cocktail from survivors of *C. difficile* infection. This product is currently in Phase II.

[51] Degiacomi, G. (2020). Promiscuous Targets for Antitubercular Drug Discovery. *Appl. Sci.*, 10(2), 623. DrpE1 uses FAD to oxidize decaprenylphosphoribose into decaprenylphosphoarabinose, the arabinose donor subunits used for polymers lipoarabinomannan and arabinogalactan.

[52] Adaptive Phage Therapeutics press release. (2021). Adaptive Phage Therapeutics Highlights the Publication of a Case Study of Investigational Phage Therapy for an Antibiotic-Resistant Bacterial Infection in a Child. Retrieved from: https://www.aphage.com/adaptive-phage-therapeutics-highlights-the-publication-of-a-case-study-of-investigational-phage-therapy-for-an-antibiotic-resistant-bacterial-infection-in-a-child/

[53] Hotinger, J., Pendergrass, H., May, A. (2021). Molecular Targets and Strategies for Inhibition of the Bacterial Type III Secretion System (T3SS); Inhibitors Directly Binding to T3SS Components. *Biomolecules,* 11(2), 316.

[54] NIH/U.S. National Library of Medicine. (2021). Safety and Efficacy Study of Ftortiazinon in the Treatment of Patients with Complicated Urinary Tract Infections Caused by P. Aeruginosa.

9. **Surface target: FimH (1).** Bacteria have protein-based filament rods that allow them to attach to host cells. Of the many subunits that comprise these rods protruding from the bacteria cell surface, is FimH (a fimbria-mannose attachment protein. A fimbira is a long appendage protruding from the bacteria). FimH has been shown to bind N-linked glycans on urinary epithelial cells.[55] The antibody in Phase I is being tested to treat and prevent UTIs without inducing antibiotic resistance.

10. **Energy metabolism: cytochrome cc (1)**. As described above, the ATP synthase inhibitor Bedaquiline (SIRTURO™) was approved in 2012 for pulmonary multi-drug resistant tuberculosis. This small molecule drug represents the first novel target approval in more than 30 years, as shown in **Figure 2**. Another target within the respiratory chain upstream of ATP synthase is cytochrome cc, an enzyme akin to the cytochrome bc1 complex (complex III) found in human mitochondria and respiring bacteria.[56,57] There is one NCE in the clinical pipeline targeting mycobacterium cytochrome cc.

11. **Metabolism: Cholesterol catabolism (1).** A small molecule drug targeting cholesterol catabolism is in Phase I. Certain bacteria, such as *M. tuberculosis*, can survive on cholesterol and this candidate would effectively prevent this optional lipid energy source.

12. **Translation: Leucyl-tRNA synthetase (1).** There is one drug candidate for leucyl-tRNA synthetase in Phase II for *M. tuberculosis*. There is currently one other FDA approved tRNA synthetase inhibitor, Mupirocin, but it is specific for isoleucyl-tRNA synthetase.

13. **Translation: Methionine tRNA synthetase (1).** The second tRNA synthetase in the pipeline is the methionine-tRNA synthetase in Phase II to treat *C. difficile*.

14. **DNA synthesis: DNA polymerase (1)**. The prokaryotic replisome involves at least 10 enzymes working in a coordinated fashion to synthesize DNA. One of these enzymes, topoisomerase, was discussed above under the DNA synthesis strategy. **DNA polymerase III**, the primary enzyme in the DNA synthesis process, would be a new target for this category. Currently, there is one DNA polymerase IIIc inhibitor in Phase II for *C. difficile*.

15. **DNA damage: Cas-3 editing (1)**. One therapy in the pipeline utilizes engineered bacteriophage with CRISPR-Cas3 to target *E. coli* for destruction. The therapy has completed Phase 1b for urinary tract infections.

16. **DNA: direct binding (2).** There are two DNA minor groove binding NCEs being developed to treat *C. difficile infections*, with one in Phase III and the other in Phase II.

17. **Gut and reproductive tract flora (10).** Although not a specific *molecular* target, this group of live biotherapeutics target microbiome and bile acid restoration shown to exert growth pressure on *C. difficile*.[58,59] Nine of the ten live biotherapeutic treatments are indicated for *C. difficile*, with one indicated for bacterial vaginosis. The majority of these gut microbiome therapies are microbial consortium, with two containing lactobacillus, two containing non-toxic strains of *C. difficile*, and others containing more complex mixtures of bacterial species or proprietary clonal variants. A number of programs are progressing successfully with three now in Phase III, four in Phase II, and three in Phase I.

[55] Sauer, M., et al. (2016). Catch-bond mechanism of the bacterial adhesin FimH. *Nature Communications*, 7, Article number: 10738.
[56] Sone, N., et al. (2001). A novel hydrophobic diheme c-type cytochrome. *Biochim Biophys Acta*, 1503(3), 279-90.
[57] Lu, P., et al. (2018). The anti-mycobacterial activity of the cytochrome bcc inhibitor Q203 can be enhanced by small-molecule inhibition of cytochrome bd. *Nature Scientific Reports*, 8, Article number: 2625.
[58] Mullish, B., Allegretti, J. (2021). The contribution of bile acid metabolism to the pathogenesis of Clostridioides difficile infection. *Therapeutic Advances in Gastroenterology*, 14.
[59] Taur, Y., Pamer, E. (2014). Fixing the microbiota to treat Clostridium difficile infections. *Nat Med*, 20(3), 246-247.

18. **Unknown target for Acinetobacter baumannii (1).** The third NCE for an unknown target is a macrocyclic peptide that is specific for *A. baumannii* (G-), the highest priority pathogen threat according to both CDC and WHO. This gram-negative bacterium is associated with hospital-acquired infections that target the urinary tract, lungs (pneumonia), blood, or in wounds.[60,61] Carbapenem-resistant *Acinetobacter* caused an estimated 8,500 infections in hospitalized patients in the United States in 2019.[62]

Drug Candidates with Approved Targets in the Clinical Pipeline

1. **Penicillin-binding proteins (7 NCEs).** As described above, the penicillin binding protein (PBP) target family has a long history in drug development, with 68 FDA approved NCEs since the 1940s. Development of non-lactam containing drugs was once the hope for innovative targeting for a new repertoire of PBP inhibitors, but this has not panned out as seen in the seven NCEs in the current clinical pipeline.[63] Six of the seven NCEs are modifications of beta-lactam structures of the past. Some have advanced properties, such as cell entry advantages or less susceptibility to beta lactamase degradation. The unique NCE in this group is not a classic beta lactam drug and in fact has dual activity, inhibiting not only PBPs but also lactamases. Of the seven NCEs targeting PBPs, three have approvals outside the U.S. The most advanced drug in the pipeline, and only NDA-stage drug found in our analysis, is a beta lactam drug approved ex-U.S.

2. **Cell membrane permeability (9 NCEs).** The polymyxins and peptide mechanisms for pore formation in bacterial membranes were described above and both serve as a starting point for NCEs that work using this strategy, albeit with unique properties and perhaps slightly different mechanisms. Three new polymyxins are in early stages of clinical testing for general indications. Three synthetic amphipathic peptides are in the clinic, two in Phase I and one in Phase II. Two small chemicals are also in this category of membrane disruptors, with one in Phase IIb for *Staphylococcal* infection and one in Phase IIb for joint infections.

3. **FA synthesis: Enoyl ACP reductase (1):** There are two NCEs in the clinical pipeline that target the staphylococcal FabI enzyme. One is in two Phase II trials, one for skin infections (QIDP) and one for joint infections. The other NCE is in Phase I for skin infections with specificity for drug resistant *S. aureus* (G+).

4. **Energy Metabolism: ATP Synthase (2).** Nearly 10 years after the landmark approval in 2012 of the first ATP synthase inhibitor, not a single other drug in this target family has been approved. There have been failures, but two new inhibitors are in clinical testing. Both are in Phase I for TB and both are analogues of Bedaquiline, belonging to the Diarylquinoline chemical class described above.

5. **Macromolecular damage: Nitro group Reactive Species (1)**. The Otsuka drug Delamanid has been approved outside the U.S. but remains in Phase III for U.S. development according to the Otsuka website. Delamanid (OPC-67683) has a very similar structure and works similarly to Pretomanid as described above.

[60] CDC. (2021). Retrieved from: https://www.cdc.gov/hai/organisms/acinetobacter.html
[61] Howard, A., et al. (2012). Acinetobacter baumannii. *Virulence*, 3(3), 243–250.
[62] CDC AR Threats Report, (2019), accessed October 2021.
[63] Zervosen, A., et al. (2012). Development of New Drugs for an Old Target — The Penicillin Binding Proteins. *Molecules*, 17, p.12478—505.

6. **Macromolecular damage: Riminophenazine reactive species (1).** There is an analog of clofazimine in Phase II for TB. As described above, this drug likely works as a redox cycling agent that destabilizes membranes and DNA.[64]

7. **Translation: 30S Ribosome (1).** Bipyrocycline, a broad-spectrum antibiotic sharing structural elements of tetracycline, is in Phase I developed for Community Acquired Pneumonia (CAP). A clinical development program has demonstrated antibacterial activity on gram-positive and gram-negative bacteria, including *Acinetobacter baumannii* (G-).

8. **Translation: 50S Ribosome (5 NCEs).** There are five 50S ribosome inhibitors in the clinic. Three of the compounds are oxazolidinone compounds, two are in development to treat tuberculosis, and one for treating treat gram positive skin infections. The fourth compound is a ketolide antimicrobial which is a semi-synthetic derivative of erythromycin A. The fifth compound is aminoglycoside antimicrobial produced from *Streptomyces tenebrarius* that was previously approved for veterinary use.

9. **RNA polymerase (2 NCEs).** Both of these NCEs are from TenNor Therapeutics and are hybrids of rifamycin (the primary class of RNA polymerase inhibitors) and another class of antibiotics, either nitroimidazole class that has macromolecular damaging effects, or the fluoroquinolone with DNA gyrase/topoisomerase activity. Both are indicated for skin infections.

10. **DNA Synthesis: Topoisomerase (4).** There are four topoisomerase inhibitors in the clinic, with two in Phase III. Both Phase III candidates are indicated for urinary tract infections. The other two candidates are in Phase II. One is a fluoroquinolone antibiotic indicated for urinary tract infections. The other Phase II candidate is a narrow spectrum antibacterial with structural elements of oxazolidinones and quinolones, indicated for the treatment of *C. difficile*.

The pipeline for indirect-acting antibacterial drug candidates contain nine beta-lactamase inhibitors and four exotoxin inhibitors. All four exotoxin targets are novel (no prior FDA approval): *Staphylococcus aureus* alpha toxin (one mAb in Phase I and one mAb in Phase III), staphyloxanthin (Phase II mAb), Shiga toxins 1/2 (Phase II mAb), and unbound Endotoxin (Phase I mAb).

[64] Bvumbi, M. (2020). Activity of Riminophenazines against Mycobacterium tuberculosis: A Review of Studies that Might be Contenders for Use as Antituberculosis Agents. *Chemmedchem*, 15(23), 2207-2219.

Clinical Pipeline by Indication Area

Figure 7 shows the 64 clinical pipeline therapeutics by their indication specific drug programs, of which there are 72 currently in development. Nearly half of the products are targeted toward the specific strains *M. tuberculosis, C. difficile*, *Staphylococcus*, or *Pseudomonas*, while others are geared toward eight types of infection (*e.g.,* skin, joint, abdominal, blood, urinary, CAP, HAP, or general infection). As a percentage of the total pipeline, 38% of programs target *C. difficile* and *M. tuberculosis*, and 32% are for urinary tract infections (UTI) and skin infections.

Some of the antibacterials with organ specific or general indication categories found in **Figure 7** have been shown to have specific activity against resistant strains and priority pathogens. For example, six of the eight skin infection programs have activity against *S. aureus* (MRSA). However, each candidate under these broad indication pathways has its own unique spectrum of resistant strain efficacy making categorization difficult. For drug developers, narrow indication claims for clinical trials has potentially limiting implications as will be discussed below. Those programs that are designed for specific organisms tend to be aligned with novelty of target as shown by the orange bars in **Figure 7**.

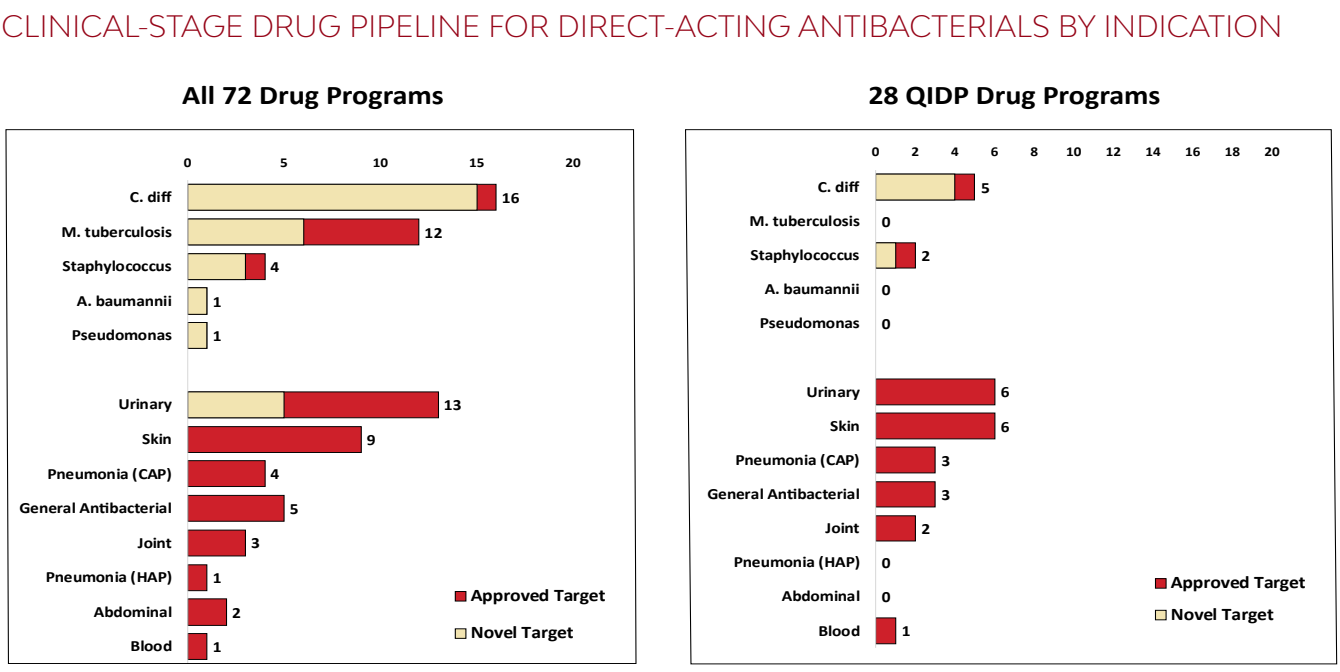

Figure 7. Clinical pipeline programs for direct-acting antibacterials by indication (as of October 2021, 72 programs for 64 NCEs). NCE program indications that are species specific are listed at the top and organ specific programs below. Lung infections are captured by Pneumonia separated into HAP and CAP.

There are 28 antibacterial programs in development that have the FDA Qualified Infectious Disease Program (QIDP) designation.[65] Of these 28 QIDP designated products, only five are for novel targets. The novel target therapeutics with QIDP designation are only designed to treat *C. difficile* and *S. aureus* specific indications. It is important to note that QIDP designations are currently limited to small molecules.

As presented in **Figure 7**, 33 of 72 programs are indicated for specific strains. All four strains are listed as priority pathogens in the most recent lists from the U.S. CDC (2019) and the WHO (2017), presented in **Appendix A1**.[66] Combined, the lists include 11 other resistant bacterial strains and one family of bacteria.[67] Drug development for bacterial infections has a precedent for being organ specific (*e.g.*, skin, ear, etc.) and not species specific. This precedent makes it hard to uncover the exact species targeted within the organ-specific indication. For example, a Phase I drug for "skin infection" might work on *Staphylococcus* (G+), and *Klebsiella* (G-), or only one and not the other. CAP and HAP include strains that can lead to pneumonia, but the specific strains targeted are often not listed in the trial protocol. In many cases, the answer is not known outside of lab testing. As trial enrollment would require genetic analysis of each strain, this makes data on some resistant organisms hard to obtain even at the clinical phase. Potential activity against threat pathogens can be estimated based on preclinical research, but clinical validation of efficacy can be difficult to obtain for every bacterial strain. The PEW and WHO pipeline analysis, referenced in **Appendix A4**, provides an assessment of pipeline products that could potentially target threat pathogens.

Preclinical Pipeline for Antibiotics

According to a 2020 review of the preclinical-stage pipeline for threat pathogens, 407 antibacterial projects are ongoing.[68] A third of these are novel in terms of new class, new target, or new mechanism of action, and many of them are pathogen-specific approaches. Similar to what is reported above for the clinical pipeline, small companies represent 81% of the preclinical pipeline. Large companies represent 3% of preclinical R&D with academic and non-profit institutions making 15% of the difference.

[65] The QIDP designation comes from the Generating Antibiotic Incentives Now (GAIN) Act, Title VIII within the Food and Drug Administration Safety and Innovation Act (FDASIA). According to an FDA guidance document, this designation offers incentives for the development of antibacterial and antifungal drugs for human use to treat serious or life-threatening infections. The primary incentive is a 5-year exclusivity extension for certain applications of drug products that have been designated as a QIDP and approved under section 505 of the FD&C Act. This 5-year exclusivity extension is added to any exclusivity for which the application qualifies upon approval - https://www.fda.gov/files/drugs/published/Qualified-Infectious-Disease-Product-Designation-Questions-and-Answers.pdf

[66] CDC AR Threats Report. (2019). accessed October 2021. Retrieved from: https://www.cdc.gov/drugresistance/biggest-threats.html and WHO 2020 Antibacterial agents in Clinical Development

[67] The family of bacteria is Enterobacteriaceae and it is listed twice (for CRE and ESBL) in Appendix A1. The family Enterobacteriaceae includes individual members like Salmonella, Escherichia coli, Klebsiella, and Shigella, even though they are listed separately, with different threat levels, on those lists.

[68] Theuretzbacher, U., Outterson, K., Engel, A., Karlen, A. (2020). The Global Preclinical Antibacterial Pipeline. *Nature Reviews Microbiology*, 18, 275-285

Success Rates

The overall success rate for antibacterial NCEs was found to be higher than that for the industry overall. Our previously published results showed success rates of 7.9% across all disease areas to bring a drug from IND to FDA approval (2011-2020, n=12,728 transitions).[69] Using the same dataset, and selecting only antibacterial NCEs, we calculate the success rate to be 16.3% (n=182 transitions). The primary differentiator is Phase II, with a 48.0% (n=75) success rate for antibacterial NCEs, well above the 28.9% (n=4,933) success rate for the entire industry at Phase II.

The broad infectious disease area had a success rate overall of 13.2% (n=1,170), implying that antibacterial NCEs have higher success rates than the non-antibacterial group, which includes antiviral, antifungal, antiparasitic and vaccines.

Refining the dataset further to include only NCEs with novel targets, the Phase I to approval success rate drops to 13% (n=47). However, the low number of transitions could make this unreliable in predicting future outcomes. In particular, the Phase III data shows only one success and one failure and only one NCE for a new target (ATP synthase) over the ten-year period analyzed. NCEs for old targets were more successful as a group and were found to have a higher success rate from Phase I to approval, 19.2% (n=135).

CLINICAL DEVELOPMENT SUCCESS RATES FOR DIRECT-ACTING ANTIBACTERIAL DRUGS 2011-2020

2011-2020	Phase I to II	Phase II to III	Phase III to NDA/BLA	NDA/BLA to Approval	Phase I to Approval
All Diseases	52.0%	28.9%	57.8%	90.6%	7.9%
N	4,414	4,933	1,928	1,453	12,728
All Infectious Dis.	57.8%	38.4%	64.0%	92.9%	13.2%
N	403	414	197	156	1,170
ABX NCEs	73.1%	48.0%	56.3%	82.6%	16.3%
N	52	75	32	23	182

Breakdown of ABX NCEs:	Phase I to II	Phase II to III	Phase III to NDA/BLA	NDA/BLA to Approval	Phase I to Approval
w/Novel Target	78.3%	33.3%	50.0%	100.0%	13.0%
N	23	21	2	1	47
w/Old Target	69.0%	53.7%	56.7%	81.8%	17.2%
N	29	54	30	22	135

Figure 8. Clinical success rates for infectious disease indications and direct-acting antibiotics compared to success rates for all disease areas combined, January 2011 through January 2020. Data is based on drug program transitions listed in the Informa Biomedtracker and Pharmapremia databases. (The full dataset for the industry is available at www.bio.org/iareports)

[69] Thomas, D., et al. (2021) BIO Industry Analysis, Informa Pharma Intelligence, QLS Life Sciences. Clinical Development Success Rates and Contributing Factors 2011–2020. (Available at www.bio.org/iareports)

There was also difference by modality. Biologic NCEs, with only 45 total transitions, had a lower Phase I to approval success rate of 12.8% vs. the small molecule NCE success rate of 18.6% (n=161 transitions). This differs from our finding in the broader industry where biologics, in particular mAbs, tend to have higher success rates.

We also investigated QIDP designated drug success rates for Phase III. NCE programs with QIDP had 69.6% (n=23) vs. 37.5% (n=16) without QIDP. Phase I and II success rate analysis has the disadvantage of selection bias as the designation can be given at various phases. This creates a cherry-picking effect resulting in very high success rates.

Suspended programs for novel targets do not overlap significantly with the current pipeline candidate targets. Only one Phase III novel drug program was suspended during the period analyzed.[70] For Phase II programs, 14 of 21 failed to reach Phase III and seven progressed to Phase III. Among the suspended targets were UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC), polypeptide deformylase, Leucyl-tRNA synthetase, mAbs for *Staphylococcus* and *Pseudomonas* targets, and a few microbiome programs. A similar mix of targets was seen in Phase I, albeit for only six suspended novel drug programs (LpxC, *Staphylococcus* mAbs, and a few microbiome therapies). No biologics transitioned from Phase III to BLA.

[70] Patel, M., Kaufman, D. (2015). Anti-lipoteichoic acid monoclonal antibody (pagibaximab) studies for the prevention of staphylococcal bloodstream infections in preterm infants. *Expert Opin Biol Ther*, 15(4), 595-600. (This antibody program was suspended during the 10-year period of analysis)

Investment into Emerging Antibacterial Companies

Venture Capital

Over the past 10 years (2011-2020) 22 antibacterial companies started their R&D journey by raising their first major financing round, called a series A-1 round. However, these financing rounds only account for only 2% of the funding provided to start-up biopharmaceutical companies created over this period. This low percentage shows a stark contrast in the investment of antibacterial companies versus other diseases. The disparity can also be seen in the amount invested in all antibacterial companies vs. other diseases. As shown in **Figure 9**, antibacterial companies received 17-fold less money than oncology companies for the decade. The biggest disparity was seen in 2020 when oncology companies raised 44x more money than antibacterial companies. The acceleration seen in oncology venture investment, and other areas of medicine such as rare disease, was missed by the antibacterial entrepreneurs in the U.S. Venture funding for antibacterial companies increased by 29% for the two five-year windows, 2011-2015 vs. 2016-2020, but 275% for oncology companies and the 175% for all companies in the industry.

2011-2020 VENTURE INVESTMENT INTO U.S. COMPANIES
WITH LEAD NOVEL DRUG PROGRAMS IN ONCOLOGY VS. ANTIBACTERIALS

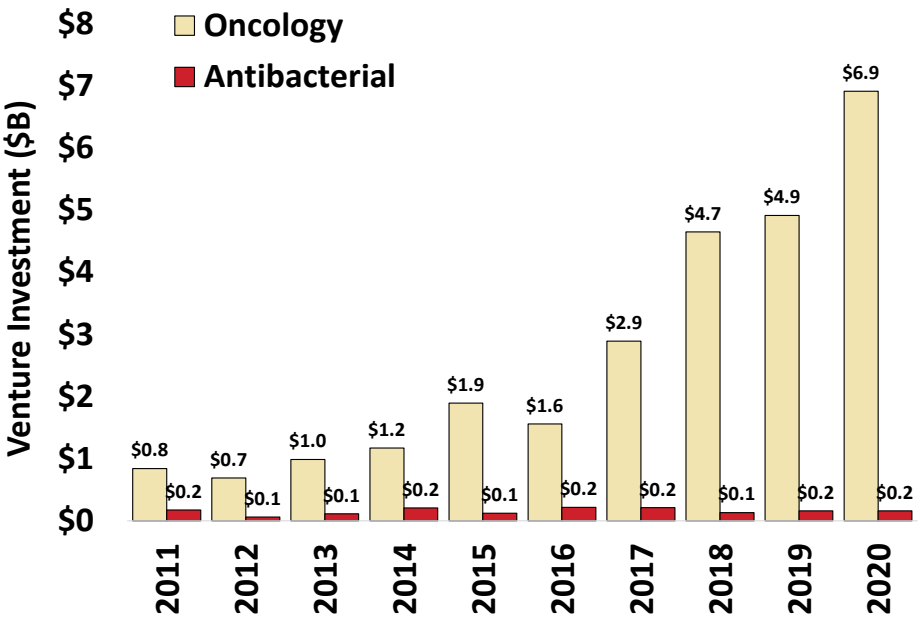

Figure 9. Venture funding of companies with lead products in antibacterials vs. oncology, 2011-2020. Venture investment into oncology is 17x more than the funding received for novel antibacterials companies during this time period.

IPOs

Similar to the trends shown in venture capital, investment into newly public U.S. based antibiotics companies has been sparse to non-existent in recent years. Over the last 10 years there were 12 antibacterial company IPOs raising $769 million versus 109 oncology companies raising $12 billion (2011-2020). An alarming trend is the decrease in antibacterial companies going public in the last five years (only 3). In contrast, during this same time period most other diseases like oncology have shown a large increase in companies going public (71 companies) versus the previous five years (38 companies). Of the 12 antibiotics companies that have gone public in the past 10 years, only five are still active today. Four of the companies were acquired (most at fire-sale valuation due to clinical or market failure), two went through the reverse merger process due to trial failures, and one company that reached FDA approval simply went extinct post-bankruptcy.

2011-2020 IPO INVESTMENT INTO U.S. COMPANIES
WITH LEAD NOVEL DRUG PROGRAMS IN ONCOLOGY VS. ANTIBACTERIALS

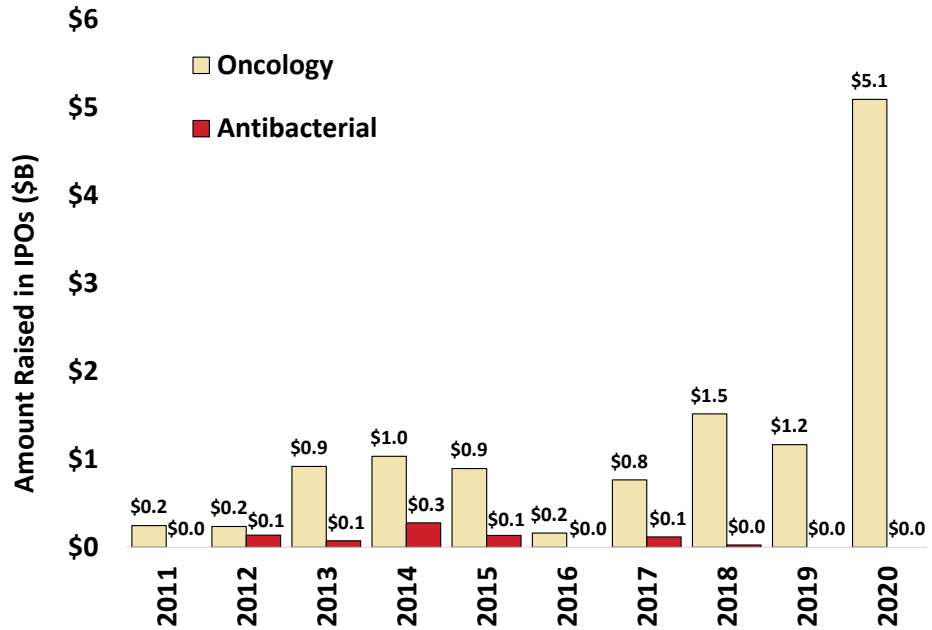

Figure 10. IPO funding of U.S. companies with lead products in antibacterials vs. oncology, 2011-2020.

Clinical Trial Initiations for Novel Antibiotics

To assess investment at the clinical level across the industry, we quantified the number of clinical programs started each year for the last decade. From a starting list of 602 antibacterial trial starts in the TrialTrove database over the 10-year period 2011-2020, we found 92 that met our criteria for true antibacterial NCE trial starts (removing nonintervention trials, reformulations, combinations of older drugs and other duplicate trials per Phase). Further analysis by novelty of target shows that there were 43 trial starts for NCEs with novel targets.

As shown in **Figure 11,** clinical trial starts for antibacterial NCEs has declined over the last 10 years, with a peak of 14 in 2016. Comparing the five-year periods 2011-2015 and 2016-2020 shows a decline of 33% (from 55 to 27). For Phase I initiations, the drop was steeper at 46%.

For drug candidates with novel targets, there was a 28% drop (from 25 to 18) when comparing the five-year periods. Small company sponsored programs led the decline, dropping 45% from 22 (2011-2015) to 12 (2016-2020). For Phase I starts by small companies, the decline was 60%.

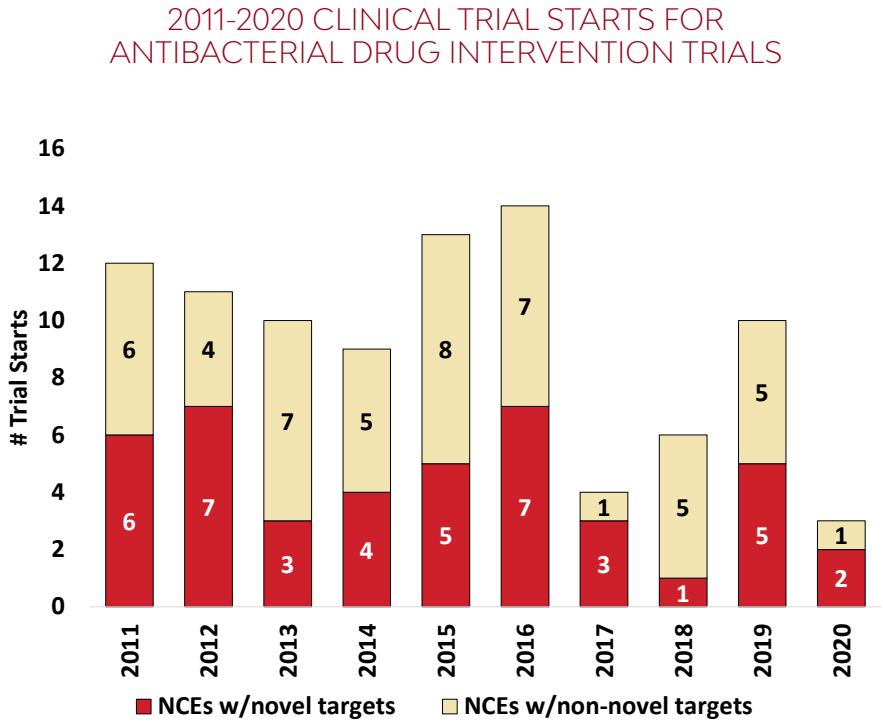

Figure 11. Clinical trial starts for antibacterial NCEs, 2011-2020. TrialTrove data accessed October 2021. Trials were individually assessed for NCE intervention trials only and trial Phase cohorts de-duplicated. A total of 92 NCE intervention trials for were initiated during this time period, with 43 for NCEs with novel targets.

Discussion

Despite the availability of 106 unique direct-acting antibacterial therapeutic entities on the market in the U.S. and 28 unique antibacterials outside the U.S., there remains a need for alternatives to currently available drugs that will circumvent bacterial resistance to current medicines. More than 82% of all antibiotic approvals occurred prior to the year 2000. The majority of the drugs remaining on the market are facing eventual loss in efficacy due to resistance developed by bacterial strains encountering these treatments in the population. For the next generation of antibacterials to fill this gap, there needs to be a well-funded and appropriately rewarded biotech ecosystem for translational science to reach the clinic and beyond.

The majority of antibacterial innovation stems from small, emerging biotechnology companies, as indicated by 80% of the current clinical pipeline candidates originating in these companies. However, funding has been sparse for these antibacterial developers, and the ecosystem is fragile and failing. Over the last decade, antibacterial start-ups raised a total of only $2.3 billion in both venture capital *and* IPOs, well below other areas in medicine and not enough to compensate for the need of a broad ecosystem and diverse pipeline of candidates. By comparison, over this same decade oncology companies raised $38 billion in venture capital and IPOs, more than 16-fold over the amount invested in antibacterials. To further place the $2.3 billion for antibacterials into context, consider the following reference points from non-antibacterial companies: one single cell and gene therapy company was recently able to raise $1.3 billion in just under three years; and other individual small biotechs have recently raised $1 billion in a single year from follow on offerings.[71]

Our analysis of clinical trial initiations, a broader proxy for R&D investment trends for both large and small antibacterial-focused companies, reinforces the picture of an exodus from antibacterials. Phase I trial initiations declined 46% when compared to the previous five-year periods (2011-2015 vs. 2016-2020). Phase II and III trial initiations declined 33%. The same percentage drop was seen when segmenting by company size, suggesting the wane in large company interest is shared by small companies and their investors. This is particularly concerning as large companies have traditionally been a critical part of the antibiotic ecosystem, with their extensive manufacturing infrastructure and global distribution capacity.

There are only 44 drug programs in the current antibacterial pipeline, excluding the innovative products for TB and *C. difficile*. That is far below what is needed to address the 13 threat pathogens outside of TB and *C. difficile* listed by CDC and WHO (**Appendix A1**), based on historic success rates.[72] Consider that for COVID-19 alone, there were 1,030 drug and vaccines programs launched in 2020-2021, and more than 450 reached the clinic.[73] The magnitude of R&D for individual oncology indications also underscores the size of the funnel that is needed to generate just a handful of meaningful therapeutic options for patients. For breast cancer alone, there are 160 NCE drug programs in the clinic.[74] Unfortunately, the commitment to antibacterial R&D is narrow by comparison. In fact, for systemic small molecules (the traditional bar for effective, widely available antibiotics) there is only one Phase III drug in the clinic. Historic success rates for drug development have shown that the number of shots on goal must increase if we are to see life-saving therapies become available for patients. Furthermore, multiple products on the market are also needed to ensure real-world success.

[71] Sana Biotechnology, sold 23.5 million shares at $25 per share ($588 million) in February 2021 and raised more than $700 million in venture capital since its founding in 2018. During the last decade (2011 to 2020), U.S. antibacterial companies raised just $769 million in IPOs. Bluebird bio raised more than $1 billion in follow-on offerings in 2017.

[72] As a simple example calculation: Assuming at least two new drugs to rely on for each of the 13 pathogens and assuming a similar distribution of asset progression as found in the current pipeline (35% NCEs in Phase I, 45% in Phase II, and 20% in Phase III), the probabilities suggest a minimum of 153 candidates would be needed in the pipeline.

[73] BIO Covid pipeline tracker: https://www.bio.org/policy/human-health/vaccines-biodefense/coronavirus/pipeline-tracker

[74] Based on a search of the Biomedtracker database November 2021

Contrary to what might be expected for a heavily underinvested disease area, the success rate for new antibacterials was found to be above the industry average. New antibacterials were found to have a 16.3% success rate from Phase I to FDA approval, above the overall industry average of 7.9% for 2011-2020. New antibacterial therapies with novel targets had a success rate of 13.0%, above average and in line with the greater infectious disease area. Interestingly, oncology has an extremely low success rate near 5%, yet continues to receive record amounts of investment. This reinforces that there are other factors preventing investment into antibacterials, as higher rates of success have had little impact.

There are three main factors that have scared off investors from antibacterial development. First, large companies have been exiting from the space for some time, with very few listed as co-sponsors of small company pipeline candidates. Without a vested interest from large biopharmaceutical companies, licensing deals and M&A dry up, souring the incentive for early-stage investors such as venture capitalists. Second, the majority of recent examples of "successful" biotechs (those that have raised venture capital, obtained funding through public offerings, obtained FDA support via QIDP, and achieved FDA marketing approval) have been commercial failures. Investors point to these recent stories of antibacterial company bankruptcies and acquisitions at fire sale valuations as evidence to avoid investment in this segment of medicine.[75,76] The third factor is the lack of effective policy and regulatory solutions to address the unique characteristics of the antimicrobial marketplace.

The primary issue forcing big pharma out of the antibacterial sector and leaving small company innovators empty handed is that the traditional market dynamics do not exist for antimicrobials, and this has not been resolved through new policies. First, new antimicrobials will primarily be used as "last line" therapies for use in hospitals when other options are ineffective. These products are short duration therapies and will experience slow uptake since they are usually used sparingly to preserve effectiveness. Novel antimicrobials are also generally undervalued by reimbursement systems relative to the benefits they bring society. Finally, hospital bundled-payment reimbursement mechanisms can discourage use of novel antibacterials, even when they are the most appropriate treatment for a patient, contributing not only to market challenges but also patient access to novel products. Taken together, these challenges create a market with little to no return on investment for antibacterial medicines.

This market reality for antibiotics, due to appropriate stewardship implementation, cheap available generics, oscillations in waves of infectivity (potentially non-existent in pre-crisis periods), means that both drug market volumes and drug pricing are under unique pressures that turn investment return models negative.[77]

Solutions: From government and non-profit funding and regulatory incentives to market fixes

Ongoing conversations about policy solutions span the entire pathway of drug development and access: early-stage investment (push mechanisms for research), late-stage investment (push mechanisms for development), clear and efficient regulatory pathways (regulatory incentives for development), and, importantly, the post-approval market incentives (pull mechanisms). There is broad alignment across academia, industry, policymakers, and other stakeholders that all four modes of incentivizing innovation are needed to stabilize and sustain the antimicrobial ecosystem and address the ongoing fight against AMR. As illustrated in **Appendix A2**, these solutions, and the entities that drive them, are interconnected and dependent on each other.

[75] Plackett, B. (2020). No money for new drugs: Despite an overwhelming global need for pharmaceutical companies to develop more antibiotics, there's little financial incentive to encourage them to act. *Nature*, 586, S50.

[76] Recent examples include Achaogen, a company with an approved antibiotic to treat resistant Infections, that filed for bankruptcy in spring 2019, less than a year after FDA approval. A second company with four antibiotics on the market, Melinta Therapeutics, filed for bankruptcy protection in late 2019 and restructured.

[77] Klug, D., et al. (2021). There is no market for new antibiotics: this allows an open approach to research and development. *Wellcome Open Res.*, 6, 146.

Early-stage investment (push mechanisms for research). The early funding of new target discovery at the lab bench must be consistent and sizable. This starts with government funding and grants (*e.g.,* NIAID, BARDA, CARB-X etc.), and philanthropic foundations and institutes. However, the early-stage funding that needs to quickly follow these efforts must support new drug candidate discovery by funding chemists and biochemists to make the drug entities themselves. This is the phase where angel investors, entrepreneurs, and venture capitalists have traditionally converged to enable successful drug discovery. Small companies account for roughly 70% of drug candidates across the industry, and their funding is heavily reliant on this investment capital.[78] However, as noted above for antibacterials, these traditional sources of funding are not supplying the investment needed. To solve this deficiency in the ecosystem, in recent years other creative hybrid models for funding have been launched, leading to promising NCEs. For example, a global non-profit partnership, CARB-X, recently completed five years of funding to support early development of innovative products, totaling over $360 million across 92 projects in 12 countries.[79] However, as candidates emerge from this discovery stage, they will go nowhere unless the following three solutions are in place.

Regulatory incentives for development. In the last decade, multiple regulatory measures have been taken to change the efficiency and incentives for drug developers. For example, the Generating Antibiotic Incentives Now **(GAIN)** Act of 2012 brought forward the Qualified Infectious Disease Product (**QIDP**) designation to pipeline candidates. The QIDP designation provides eligibility for priority FDA review, fast-track designation, and an additional five years of market exclusivity.[80] There have been 17 NCE approvals with QIDP.[81] In 2016, the Limited Population Pathway for Antibacterial and Antifungal Drugs (**LPAD**) was introduced in the U.S. as part of the 21st Century Cures Act. Amikacin and Pretonamid were the first two drugs approved under this pathway in 2018 and 2019, respectively.[82] In 2017, the U.S. Center for Drug Evaluation and Research (CDER) issued updated FDA guidance on clinical trial design for antibacterials. All of these regulatory steps acknowledge the hurdles industry is facing in the antimicrobial arena and take steps to streamline development. However, for drug developers, technical issues with trial enrollment and running large, complex comparative effectiveness studies remain as late-stage obstacles.[83]

Late-stage investment (push mechanisms for development). Small companies that may emerge with a candidate from early-stage research often struggle to obtain funding for the complex and costly Phase II and III trails. Multi-entity funds, such as the recent AMR Action Fund, can serve to fill this gap.[84] Other public private partnerships, such as BARDA's Project BioShield program or BARDA's Broad Spectrum Antimicrobials program can also provide support to accelerate later stage research and development. The TB Alliance is a successful example of non-profit funding of clinical-stage development, with seven products currently in trials.

78 BIO's interactive clinical pipeline review available at https://www.bio.org/fda-approvals-clinical-development-pipeline
79 CARB-X investments. Retrieved from: https://carb-x.org/carb-x-news/carb-x-celebrates-five-years-of-progress-in-early-stage-product-development-against-antibiotic-resistant-bacteria/. Others examples can be found in Appendix A2 and A3.
80 Schneider, M., Harrison, N. R., Daniel, G. W. & McClellan, M. B. (2020) Delinking US Antibiotic Payments through a Subscription Model in Medicare. Retrieved from: https://healthpolicy.duke.edu/publications/delinking-us-antibiotic-payments-through-subscription-model-medicare
81 See the BIO FDA tracker's regulatory filter: https://www.bio.org/fda-approvals-clinical-development-pipeline.
82 FDA website, retrieved Nov 2021: https://www.fda.gov/drugs/development-resources/limited-population-pathway-antibacterial-and-antifungal-drugs-lpad-pathway
83 Bart, S., et al. (2021). Geographic Shifts in Antibacterial Drug Clinical Trial Enrollment: Implications for Generalizability. Clinical Infectious Diseases, 72, (8) p. 1422–1428
84 AMR Action Fund details accessed December 2021 at https://www.amractionfund.com/

Market-based mechanisms – Pull incentives and Reimbursement Reform. Policy reforms that are not in place at the moment – and are most critically needed – are solutions to address the most disabling pillar of the ecosystem: the marketplace. The investment issues and anemic pipeline presented in this report are a result of broken market dynamics that create a risk-benefit imbalance when it comes to developing and launching antibiotics. With average sales revenue for antibiotics well below the threshold for remaining commercially viable (due to both price and volume), and with similar drug development costs to other disease areas, the incentive to invest for comparable ROI – and in some cases any ROI – is missing. Unlike other areas of drug development with potential benefits commensurate with risk taken and value delivered to patients, the private sector pathway for antibacterials does not work. Unlike the obstacles seen with chronic diseases, such as the inability to stratify patient populations, lack of understanding of the pathophysiology, and the challenging and costly regulatory requirements and difficult reimbursement environment, the antibiotic developers are facing a more nuanced market issue – the market for these types of novel antibiotic products does not exist in the traditional pharmaceutical form.

To address the unique market challenges for antibacterials, a combination of two complementary post-approval incentives is necessary: a pull incentive to ensure sustainable investment into the AMR product pipeline, and reimbursement reform to stabilize the commercial marketplace and improve patient access.

First, new economic incentives that reward successful innovation at a level sufficient to attract further R&D may serve to pull participants back into the fray. Studies estimate that incentives in the range of $1-4 billion per successful launch of a new and innovative antibiotic are needed globally. Several types of "pull incentives" have been proposed and pilots are ongoing to address the broken marketplace for antibacterials.[85] One solution is an incentive, such as a market entry reward or subscription model, which rewards the successful approval of a novel AMR medicine that meets critical unmet medical needs. This mechanism could provide an important incentive for private investment in these products by providing a return on investment for AMR programs that is competitive with other areas of potential R&D investment. This could also be achieved through a subscription model that would allow reimbursement to be partially de-coupled from volume of sales to reduce the incentive to inappropriately use novel antibiotic medicines. The bipartisan Pioneering Antimicrobial Subscriptions to End Upsurging Resistance (PASTEUR) Act, introduced in both chambers of Congress in 2020 (and reintroduced in 2021), is one policy proposal currently under consideration which would establish such an incentive.

Reimbursement reform, if combined with appropriate valuations, could play a complementary role to stabilize the antibiotics market. Current Medicare in-hospital bundled-payment mechanisms may discourage the use of novel antimicrobial medicines – even in situations where they are more clinically appropriate – as hospitals will lose money using these novel products on these patients. This creates a barrier to patient access and contributes to the poor uptake of AMR medicines from companies struggling to remain commercially viable. One solution to this problem would be for qualifying antimicrobials to receive separate payment under in-hospital Medicare reimbursement. This would ensure hospitals are adequately reimbursed for novel antimicrobials, enabling doctors to prescribe the most appropriate antimicrobial based on clinical, not financial, considerations. This separate payment would ensure patient access when appropriate and help address the poor market uptake of AMR medicines. This reform could be achieved either through the Centers for Medicare and Medicaid Services (CMS) current rulemaking authorities, or a legislative proposal such as the Developing an Innovative Strategy for Antimicrobial Resistant Microorganisms (DISARM) Act, introduced in Congress in 2020 with bipartisan support. [86]

All innovation needs a functional, efficient ecosystem for it to succeed in generating life-saving products. The Biotechnology Innovation Organization (BIO) and member companies view innovation as the key to tackling the emerging threat of antibiotic resistance. Policies that stimulate greater investment in antibiotic R&D through a combination of both push and pull incentives, are necessary to achieve this goal.

[85] Outterson, K. Estimating. (2021). The Appropriate Size Of Global Pull Incentives For Antibacterial Medicines. *HEALTH AFFAIRS*, 40, (11) p.1758–1765

[86] Text for the Disarm Act (H.R. 4127) can be accessed at https://www.congress.gov/bill/117th-congress/house-bill/4127

Appendix

Appendix A1. CDC and WHO pathogen threat lists

Organism	CDC 2019	WHO 2017
Acinetobacter baumannii (G-) [CRAB]	Urgent	Critical
Enterobacteriaceae [family] (G-) [CRE]*	Urgent	Critical
Neisseria gonorrhoeae (G-) [CR, FR]	Urgent	High
Clostridioides difficile (C. diff) (G+)	Urgent	Not listed
Enterobacteriaceae [family] (G-) [ESBLE]*	Serious	Critical
Pseudomonas aeruginosa (G-) [CRPA]	Serious	Critical
Enterococcus faecium (G+) [VRE]	Serious	High
Staphylococcus aureus (G+) [MRSA,VRSA]	Serious	High
Campylobacter spp. (G-) [FR]	Serious	High
Salmonellae Typhi (G-) [FR]	Serious	High
Streptococcus pneumoniae (G+)	Serious	Medium
Shigella spp. (G-) [FR]	Serious	Medium
Mycobacterium Tuberculosis (TB) (G-/+)	Serious	Not listed
Streptococcus pyogenes (Group A) (G+) [ER]	Concerning	Not listed
Streptococcus agalactiae (Group B) (G+) [CR]	Concerning	Not listed
Helicobacter pylori (G-) [ClR]	Not listed	High
Haemophilus influenzae (G-) [AR]	Not listed	Medium

Figure A1. CDC and WHO list of antibiotic resistance bacterial threats. CR = carbapenem-resistant, VR= vancomycin-resistant, MR = methicillin-resistant, ClR = clarithromycin-resistant, FR = fluoroquinolone-resistant, AR = ampicillin-resistant. *M. tuberculosis* and *C. diffile* are covered in recent WHO reports, but not on the original pathogen list of 2017. *Family Enterobacteriaceae includes Escherichia coli, Klebsiella, Salmonella, and Shigella. The CDC list is available at https://www.cdc.gov/drugresistance/biggest-threats.html and the WHO list at https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed. (*Mycobacterium tuberculosis* and *Clostridioides difficile* are covered in recent WHO reports, but not on this threat list)

Appendix A2. Entities involved in Antibacterial Innovation

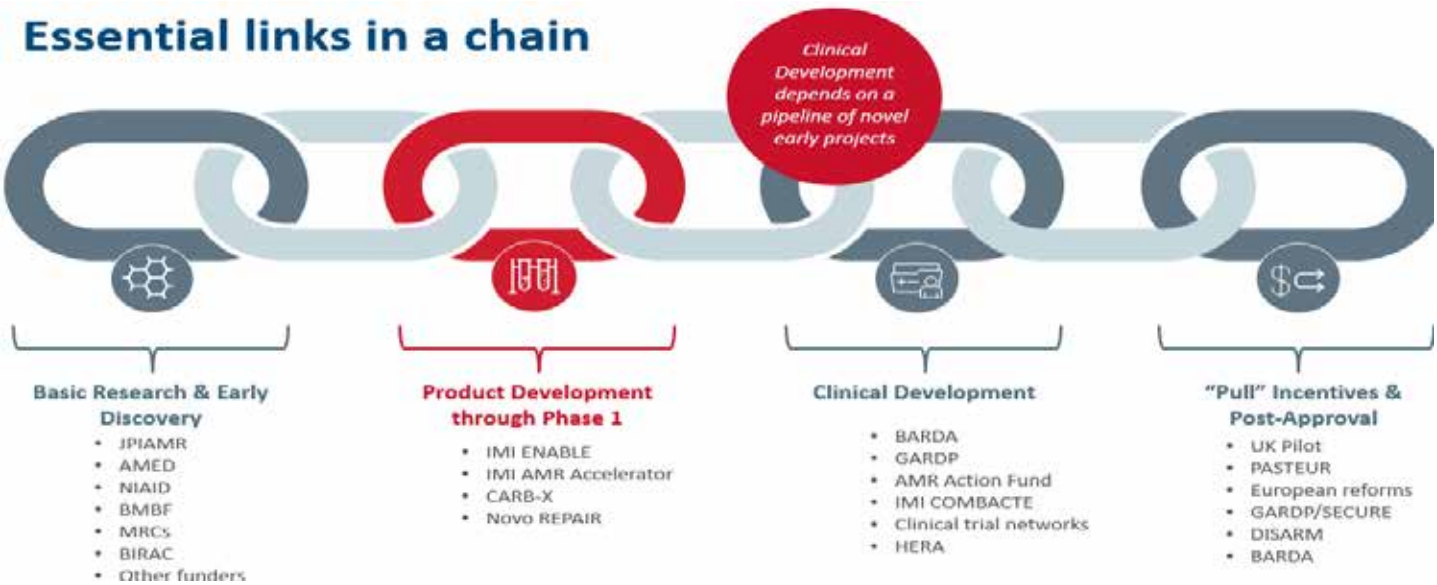

Figure A2. CARB-X diagram of interconnected entities along the R&D process for antibacterial development. Accessed January 2022 at https://carb-x.org/resources/presentations/

Appendix A3. Abbreviations for entities involved in antibacterial innovation

Abbr	Name	Launch Year	Description
Acronyms for Push Collaborations, Initiatives, and Funds			
Action Fund	AMR Action Fund	2020	Established by 23 pharmas for AMR trials in Phase II and III ($1B)
CARB-X	Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator	2016	Established by BARDA, Wellcome Trust, Germany's BMBF, UK's GAMRIF, BMGF, and Boston Uniiversity (with preclinical services by NIH NIAID)
REPAIR	Replenishing and Enabling the Pipeline for Anti-Infective Resistance Impact Fund	2018	Established by Novo Holdings for early-stage AMR drug start-ups ($165M)
GARDP	Global Antibiotic Research and Development Partnership	2016	Established by WHO and DNDi for new for AMR drug development (€100M, as of 2021)
ENABLE	European Gram-negative Antibacterial Engine	2014	Established by IMI for early-stage discovery and development of antibiotics against Gram-negative bacteria
JPIAR	Joint Programming Initiative on Antimicrobial Resistance	2013	International collaborative of 28 nations and the EC for AMR
Acronyms for Pull Initiatives/Incentives for Antimicrobial Innovation			
NHSEI pilot	NHS England and NHS Improvement pilot study for antibiotic subscription model	2019	NICE-sponsored antibiotic subscription pilot project
PASTEUR ACT	Pioneering Antimicrobial Subscriptions To End Up Surging Resistance Act of 2020	2020	U.S. Senate bill that includes a subscription contract for novel antibacterials
Acronyms for Non-Profits involved in Antimicrobial Innovation			
BMGF	Bill & Melinda Gates Foundation	2000	Non-profit, private philanthropic foundation
Pew	Pew Charitable Trusts	1948	Non-profit organization that seeks to improve public policy, inform the public
DNDi	Drugs for Neglected Diseases initiative	2003	Non-profit organization developing new treatments for neglected patients
TBA	TB allliance	2000	Non-profit product development partnership (PDP)
Wellcome	Wellcome Trust	1936	Non-profit foundation focused on health research based in London
IDSA	Infectious Diseases Society of America	1963	Medical association for infectious disease specialists
Acronyms for InterGovernment Entities involved in Antimicrobial Innovation			
WHO	World Health Organization	1948	United Nations coordinatation of health affairs related to antibacterials
IMI	Innovative Medicines Initiative	2012	Public-private partnership in Europe, runs New Drugs for Bad Bugs (ND4BB) and DRIVE-AB (pull incentive research)
Acronyms for IntraGovernment Entities involved in Antimicrobial Innovation			
GAMRIF	Global Antimicrobial Resistance Innovation Fund	2016	UK Government's Department of Health and Social Care program
DZIF	German Centre for Infection Research founded by Germany's Federal Ministry of Education and Research (BMBF)	2012	Germany's government funding source of antibiotic resistance and for antibacterials
BARDA	Biomedical Advanced Research and Development Authority	2006	U.S. Government public/private partnerships for antibacterial medical countermeasures
NIAID	National Institute of Allergy and Infectious Diseases	1955	U.S. Government funding to conduct and support research on antibacterials

Appendix A4. Methodology and Comparison with other Pipeline Reports

This report is restrictive in its definition of "antibiotics," focusing exclusively on antibacterials (i.e., does not include antifungal and anti-protozoan drugs), and including those that act by killing or directly inhibiting growth of any bacteria. We include drugs that have been approved ex-U.S. but remain in the pipeline under a U.S. regulatory pathway.

Two other recent reports for this field can be found at the WHO and PEW websites.[87,88] Differences between the WHO 2020 clinical pipeline report with this report are that WHO excludes topical treatments and does not separate beta-lactamases nor exotoxin mAbs as indirect-acting antibacterials. With those criteria, WHO reported 68 clinical therapies vs. the 78 antibacterials reported here (as 64 direct-acting + 14 indirect-acting antibacterials). Although 43 direct-acting drugs overlap in the reports, some recent updates to programs in this report (as of November 2021) led to some suspended drugs and new Phase I drugs.

The PEW analysis, which is updated annually (2014-2021), has numerous differences in methodology compared with this report. The PEW 2020 analysis of antibacterials (published in 2021) excludes TB and *H. pylori* drugs and excludes topical and inhaled products. With those criteria, PEW reported 69 clinical-stage therapies (43 traditional and 26 non-traditional therapies).

One difference from other reports includes the categorization of nitroimidazoles: (e.g., Pretonamid, approved in 2019, and Delamanid, approved ex- U.S. but in Phase III in the U.S.). They are commonly assigned to either fatty acid or mycolic acid synthesis pathway (as they block the generation of methoxy/keto mycolic acids), but more evidence suggests a broader targeting of the activated species explaining its ability to work on rapidly growing *and* latent mycobacterial cells.[89,90,91,92]

Antibacterial vaccines were not included. However, with the pipeline thin and slow to expand, could vaccines be applied solution to resistant bacterial threats? Unfortunately, the current clinical pipeline for bacterial vaccines contains only 11 new vaccines in the clinic for seven bacteria strains. There are also 32 second generation vaccines (for eight bacteria strains with a prior vaccine approval). This is not encouraging as the success rate for bacterial vaccines is lower than for antibacterials. From Phase I to FDA approval, bacterial vaccines had a 10% success rate for the period 2011-2020.

87 PEW report on 2020 pipeline, accessed December 2021: https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2014/antibiotics-currently-in-clinical-development (non-traditional pipeline: https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2017/nontraditional-products-for-bacterial-infections-in-clinical-development)

88 WHO report on 2020 pipeline, accessed December 2021: https://www.who.int/publications/i/item/9789240021303

89 Babtista, R., et al. (2018). Untargeted metabolomics reveals a new mode of action of pretomanid (PA-824). Nature Scientific Reports, 8, Article number: 5084.

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