Focused Antibiotics

Fighting infections. Sparing the rest.

Antibiotic resistance: A crack in the foundation of modern medicine

Antibiotics are the bedrock of modern medicine. Every year, 1 in 3 people worldwide rely on an antibiotic to treat or prevent bacterial infections, including billions of outpatient conditions like urinary tract infections (UTIs), ear infections, and lower respiratory tract infections.[i,ii] Antibiotics serve as essential protection for over 300 million major surgeries annually, reducing the risk of post-operative infection by an estimated 75-80%. For the more than 18 million cancer patients globally and 170,000 organ transplant recipients per year, antibiotics are necessary to treatment success and survival.[iii,iv] Historically, the impact of antibiotics has been profound – the introduction of penicillin reduced pneumonia deaths by over 70%, a shift that fundamentally altered human life expectancy.[v]

However, this foundation is under global threat as bacteria develop resistance to antibiotics. Today, 1 in 6 infections are resistant to standard first-line treatments.[vi] Resistance is increasing over time and moving beyond first-line antibiotics. Even in community settings, one type of extensive resistance (Extended Spectrum Beta-Lactamase (ESBL)) has increased four-fold over the past 20 years.[vii] For common outpatient conditions such as UTIs, resistance makes treatment failure 2.5 to 5 times more likely, and often adds an additional week to illness.[viii,ix] These resistant infections also impose a heavy burden on the healthcare system and economy, requiring three times as many clinic visits to achieve a cure, resulting in a 40–50% loss in work productivity, and increasing cost to treat by two- to four-fold.[x] Resistant bloodstream infections are two to three times more likely to result in death and hospitalized patients with resistant infections remain in the hospital for an average of three to five days longer than those with non-resistant infections.[xi,xii] In 2021 alone, resistant infections were linked to 1.14 million deaths – more than deaths from HIV/AIDS or malaria.[xiii] If left unaddressed, resistant infections are projected to kill more than 8 million people annually by 2050, with economic costs estimated to exceed $1 trillion USD every year by 2030.[xiv]

Animal and environmental exposure to antibiotics contribute to resistance, but human antibiotic consumption is the primary driver of antimicrobial resistance, with research showing a close correlation between the volume of human antibiotic use and the prevalence of resistant pathogens.[xv,xvi,xvii] Data and models of bacterial epidemiology suggest that human antibiotic use drives more than 90% of resistant infections, with smaller contributions from other factors such as antibiotic use in livestock (7.9%) and

environmental contamination (0.1%).[xviii] And resistance emerges at both individual and population levels. Individuals who take antibiotics are at higher risk for subsequent resistant infections. For example, after taking a common antibiotic like amoxicillin for any infection (e.g., ear infection) the risk of developing a different resistant infection (e.g., a UTI), increases two- to four-fold, and the risk of developing a resistant *E. coli* bloodstream infection increases 2.5 to three times, as compared to individuals who did not take an antibiotic.[xix,xx,xxi] While this increased risk is highest in the initial three to four months after taking the antibiotic, the risk can persist for a year or more.

Simultaneous with the growing threat of antibiotic resistance, we are witnessing a collapse in antibiotic development. Reserving new antibiotics to "last resort" has created a small, uncertain market. The estimated loss on investment to develop an antibiotic is $50 million USD compared to a $1.1 billion USD return for developing a new musculoskeletal drug.[xxii] As a result, most large innovative pharmaceutical companies have stopped developing new antibiotics. In 1990, 18 major pharmaceutical companies were actively producing antibiotics. Only 3 remain today.[xxiii] Between 2019 and 2023, investors have seen over $2.4 billion in losses from bankruptcies and liquidations, even for the companies that successfully brought new antibiotics to market.[xxiv]

Important initiatives from groups such as the Wellcome Trust, the Gates Foundation, and Novo Nordisk, CARB-X, and GARDP work to bolster development of new antibiotics, particularly for resistant Gram-negative bacteria. These initiatives are crucial. However, to date, bacterial resistance has outpaced our ability to develop new antibiotics. Bacterial defense mechanisms have surged, exemplified by a 300-fold increase in the different types of resistance-driving enzymes (beta-lactamases) since the 1940s, while the discovery and approval of new antibiotic classes have simultaneously declined approximately eight-fold.[xxv,xxvi] This widening gap highlights a fundamental challenge in modern medicine as antibiotic exposure continues to drive the development of resistant pathogens faster than new therapeutic options are developed. We need complimentary approaches that would extend the lifespan of antibiotics, slow the development of resistance, and make the antibiotic market more attractive.

What are the limits of current practice?

The growing antibiotic resistance threat is the result of a fundamental design problem. Approximately 85-90% of human infections are local,[xxvii] contained within the bladder, the middle ear, the skin, or the respiratory tract – yet nearly 100% of antibiotics are administered systemically, either through oral tablets or intravenous injections. This mismatch between systemic administration and local infection exposes the entire body and the bacteria living on it to antibiotics.

Systemic antibiotics exert significant off-target effects on the trillions of bacteria living in and on our bodies in communities known as our microbiomes, putting the bacteria under selection pressure to evolve resistance. Microbiomes are composed of a range of bacteria, the majority of which are non-harmful (even beneficial) and are not resistant to antibiotics. When these microbiomes are exposed to antibiotics, selection pressure results in the development of resistance. This evolutionary shift puts individuals at increased risk for developing resistant infections as the resistance genes move to harmful bacteria. While these off-target effects occur across all human microbiomes, the gut serves as the primary "resistance factory" for the most problematic bacteria. Seven out of ten of the most resistant, harmful (pathogenic) bacterial pathogens identified on the Priority Bacterial Pathogen List by the World Health Organization (WHO) are found normally or opportunistically in the gut.

In the gut, a single course of oral antibiotics can eliminate up to two-thirds of the non-harmful bacterial species.[xxviii,xxix] Without competition from non-harmful populations, a niche opens for potentially pathogenic bacteria to multiply, increasing in number by up to 5 times.[xxx,xxxi] Under the selection pressure of the antibiotic, the pathogenic bacteria that survive and multiply are those that are resistant to antibiotics, increasing the prevalence of antibiotic resistance genes (ARGs) in the gut 100- to 1,000-fold.[xxxii] This increased reservoir of resistance is not transient. Especially during the period of antibiotic stress, the bacteria swap genes, including ARGs, through a process called horizontal gene transfer.[xxxiii] This results in a prolonged increase in the amount of ARGs, both in pathogenic and non-pathogenic bacteria, lasting for months or even years following a single antibiotic exposure.

It is this colonization of the gut with resistant pathogens that leads to risk of future clinical resistant infections. It's not a small risk – individuals who are colonized with a resistant pathogen are about 20 times more likely to develop a resistant infection than individuals who are not colonized.[xxxiv,xxxv,xxxvi]

We need a novel approach. What if we could reformulate existing antibiotics to be focused – so that they treat the infection and spare the rest of our bacteria to avoid driving resistance?

Why now: Growing resistance meets new technologies

The acceleration of antibiotic resistance is forcing us to use existing antibiotics more strategically to slow the tide of resistance and reduce their resistance-driving, off-target impact on our microbiome. Our scientific understanding of the microbiome has also

evolved, leading to a better understanding of how alterations to this complex internal ecosystem contribute to infections, including resistant infections. For example, progress has been made towards identifying microbiome-based predictive markers for *C difficile*, such as high levels of *Enterococcus* and low *Bifidobacterium* species.[xxxvii] Simultaneously, our ability to document these microbiome alterations has been transformed by sophisticated diagnostic tools, such as shotgun metagenomics, long-read sequencing, and single-cell profiling that now allow us to broadly analyze the DNA in a sample, link resistance genes to specific bacterial species, and differentiate between pathogenic and non-pathogenic strains of the same species.

Therapeutic development in other disease areas, such as cancer, has shown that targeted treatment using nano-carriers or peptide carriers can result in reduced toxicity to human cells and improved treatment success.[xxxviii] For example, pegylated liposomal doxorubicin reduces heart damage by 80% by keeping the drug in the bloodstream, only allowing transfer into the tissues where blood vessels are leaky, which is the case in tumors.[xxxix] Some antimicrobials that are toxic to human cells have also already been reformulated. For example, liposomal amphotericin reduces kidney damage by 75% compared to standard formulations by keeping the drug in the bloodstream until it encounters a fungus.[xl]

What is the path forward?

Focused antibiotics can leverage these types of new and innovative technologies to avoid off-target effects on the trillions of bacteria normally residing in our microbiomes. Early research indicates that successful reformulation of existing antibiotics using such technologies are promising. Three approaches, in particular:

- *Approach 1: Release or activation of antibiotic at the site of infection alone.*
  An injectable lipid nano-formulation of an antibiotic designed to release the active antibiotic in the low-pH, high oxidative environment of an infection studied in a mouse pneumonia model resulted in 100% survival versus just 50% survival for the standard antibiotic. At the same time, this formulation caused significantly less disruption to the gut microbiome.[xli]
- *Approach 2: Minimize gut exposure to the active antibiotic.*
  It may be possible to use sugar-based nano-formulations to minimize the collateral damage caused by oral antibiotics by mirroring the pharmacological profile of drugs like nitrofurantoin. By attaching antibiotics to sugar molecules (glycosylation), these formulations are actively and extensively absorbed in the proximal small intestine, preventing significant concentrations of the drug from reaching the bacteria-dense colon. Preclinical research in a mouse pneumonia model demonstrates that glycosylation of an antibiotic maintains therapeutic efficacy—achieving bacterial clearance comparable to standard oral antibiotics—

while simultan1`eously preserving gut microbial richness. Early research has shown that such an approach holds promise for preventing the proliferation of ARGs within the gut, effectively decoupling the treatment of primary infections from the promotion of internal resistance reservoirs.[xlii]

- *Approach 3: Direct delivery of antibiotic to the site of infection.* Pediatric ear infections are the most common indication for antibiotic use in young children. Early research has shown that a single-dose, liposome-encapsulated, ciprofloxacin gel delivered directly to the ear achieved a 100% cure rate in a chinchilla model of acute otitis media. This localized approach eliminated pathogens and reached concentrations predicted to prevent the development of resistance at the site of infection.[xliii] In theory, such a direct delivery approach should avoid impacting the gut microbiome – though this remains to be demonstrated.

Importantly, microbiome-sparing antibiotics that drive less resistance and treat common infections could be positioned as first-line therapies due to their lower likelihood to drive resistance. This is in keeping with the WHO AWaRe paradigm.[xliv] If the price point of these new products can be cost-effective, the ability to position new antibiotics as first-line will also help address the primary disincentive to invest in antibiotic development – namely that new products, when developed, are intentionally held in reserve.

Program Goal

The goal of the Focused Antibiotics program is to reformulate existing antibiotics to spare the gut microbiome without loss of treatment efficacy.

Scaling these formulations as first-line therapeutics for the most common outpatient bacterial infections, Focused Antibiotics could reduce the global annual occurrence of antibiotic-driven resistant infections by as much as 40% — potentially avoiding over 100 million resistant infections even within the next decade.

Call for Abstracts and Proposals

To achieve this goal, the Focused Antibiotics program will work across 2 primary thrust areas. Thrust 1 seeks to reformulate existing antibiotics to avoid increasing antibiotic resistance in the bacteria that live in us, while maintaining the same or better treatment success. In parallel, work in this thrust aims to ensure that these antibiotics can be produced at scale and at an acceptable price to enable their use as potential new first-line therapeutics. Thrust 2 seeks to enable iterative design of these newly formulated, focused antibiotics by identifying a surrogate marker based on specific antibiotic-induced microbiome changes. This will require new analyses of human cohort data to

match gut microbiome predictors of subsequent resistant infections with better than 80% predictive accuracy.

Three types of infections: The program seeks to demonstrate success of the approach for three of the most common human infections: Ear infections, respiratory infections, and urinary tract infections (UTIs). Together, these infections account for more than half of all outpatient antibiotic prescriptions.

The program will prioritize *three reformulation approaches:*

1. Release or activation of the antibiotic at site of infection (e.g., using nano- or peptide-carriers)
2. Minimize gut exposure to the active antibiotic (e.g., using nano- or peptide carriers)
3. Direct delivery of the antibiotic to the site of infection

The Focused Antibiotics program is soliciting abstracts and proposals across these thrust areas, each contributing to the program goals and discussed in detail below. Individual teams are not expected to address all facets of the program. Progress will be achieved through collaborations among performers that integrate at the program level toward shared, measurable outcomes. Across selected projects, performance metrics will be harmonized to enable comparison, integration, and validation. Wellcome Leap will enable cross-collaboration within and between thrusts, which will be integral to the success of the program. Findings from Thrust 2 may be used by performers in Thrust 1 to iteratively develop their products via testing using *in vitro* models like MiPro. Proposals will be expected to articulate clear strategies for data sharing, integration, and coordination across projects.

Thrust Areas

Thrust 1: Demonstrate investigational focused formulations of existing antibiotics that do not impact the gut microbiome while maintaining or improving efficacy relative to standard-of-care antibiotics in pre-clinical *in vitro* and animal models.

Thrust 1 will support design, development, and demonstration of focused antibiotic formulations that maintain efficacy without gut microbiome impacts such as increasing resistant pathogen colonization, changes to microbial richness, or increases in antibiotic resistance genes. Work under this thrust will focus on the three approaches outlined above: 1) Release or activation of the antibiotic at the site of infection; 2) Minimize gut exposure to the active antibiotic; 3) Direct delivery of the antibiotic to the site of

infection. For approaches 1 and 2, we will prioritize strategies compatible with oral administration. Those not compatible with oral administration need to provide rigorous analysis that supports the potential to achieve broad clinical adoption after regulatory approval, including in lower-resource environments. We are interested in investigational products that are easy to administer for outpatients, do not have a burdensome dosing schedule (no more than twice daily), use scalable manufacturing processes (potentially capable of producing tens of millions of doses each year at scale), and whose predicted cost of goods will not be prohibitive so as to enable demonstration of cost-effectiveness when considering the impact on resistance development and other microbiome outcomes compared to standard of care. For novel technologies not yet capable of meeting these scalability criteria, an explanation of how the technology will plausibly be scalable in the next decade is needed.

Thrust 1 will be divided into two sub-thrusts, 1A and 1B. 1A focuses on designing innovative carriers that target the site of infection, are compatible with existing first-line antibiotics, have the potential for oral formulation, and may be used over various indications at different anatomic sites (e.g., the same carrier may be used for a UTI and acute otitis media (AOM)). Thrust 1B focuses on pre-clinical development of focused antibiotics, including both carrier and direct delivery strategies.

Applicants can apply to both Thrust 1A and Thrust 1B or to Thrust 1A or 1B alone. Performers in Thrusts 1A and 1B are expected to maintain frequent, extensive collaboration. Technical developments must be shared every 3 to 6 months to facilitate the seamless transition of carriers from discovery (1A) into pre-clinical development (1B). This iterative feedback loop also allows 1B performance data and carrier failures to directly inform 1A optimization and validation protocols. The objective of this iterative collaboration is to achieve an overall portfolio success rate of at least 3 of 4 investigational candidates designed and selected in 1A completing successful demonstration in 1B.

Performers in Thrusts 1A and 1B will be expected to share design strategies, results, and challenges as they emerge so as maximize innovative speed and to streamline development.

Thrust 1A: Create new carrier options, such as nanoparticles or peptides, that can be formulated with existing antibiotics to enable the development of focused antibiotics (e.g., accumulate at the site of infection, target receptors in sites of infection, or trigger antibiotic activation or release at the site of infection) with <5% exposure of active antibiotic in the gut and retention of antimicrobial activity

(e.g., as measured by Minimum Inhibitory Concentration (MIC) against relevant bacteria).

Despite promising preclinical data, significant gaps remain in developing carriers for targeted antibiotic delivery systems. Thrust 1A seeks discovery and design efforts to establish well-characterized carrier options, such as nanoparticles, peptide-drug conjugates, or hybrid systems, that enable focused delivery of antibiotics to the site of infection and eliminate impact on the gut microbiome. Carriers that work across a range of different syndromes (especially urinary tract infection (UTI), lower respiratory tract infection (LRTI), and/or acute otitis media (AOM)) will be prioritized.[xlv] [xlvi] [xlvii] [xlviii] [xlix] [l] Carriers must be designed for compatibility with first-line antibiotics against UTI, LRTI or AOM (e.g., amoxicillin, amoxicillin-clavulanate or fluoroquinolones for AOM and LRTI, TMP-SMX, nitrofurantoin, or pivmecillinam for UTI). If applicants wish to demonstrate the ability to pair with another antibiotic (e.g., not currently included in first-line), the applicant should include a justification for this choice, including cost considerations. We will prioritize approaches compatible with oral administration. To enable oral formulation, investigation of novel absorption enhancers (e.g., that will help larger molecules have improved bioavailability) are encouraged. Carriers may utilize passive, active, or stimuli-responsive targeting mechanisms, or combination approaches. Carriers may also be used to change an antibiotic's pharmacokinetics such that it avoids impact on the gut microbiome.

Thrust 1A performers must also address downstream feasibility as a priority. Applicants should articulate how their strategy will enable Thrust 1B performers to assess performance in model systems quickly and frequently. Proposers should address carrier architecture, linker chemistry (for conjugates), antibiotic payload compatibility, and release kinetics under infection-site conditions. We are particularly interested in applications incorporating machine learning or computational modeling to accelerate carrier design and reduce experimental burden.[li,lii]

Thrust 1A success criteria: Candidates must meet the following quantitative thresholds: (1) Stability in circulation: <10% premature antibiotic release or activation in plasma or relevant biological matrices over the intended circulation time at 37°C; (2) Controlled release at target site (for targeted or stimuli-release carriers): Demonstration of ≥80% antibiotic release at the site of infection with characterized release kinetics; (3) Antimicrobial activity preservation: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the carrier-antibiotic formulation equal to or lower than the unconjugated antibiotic against target pathogens in relevant physiologic conditions; (4) Batch reproducibility: Three independent batches demonstrating coefficient of variation 15% for particle size or molecular weight, 10% for

drug loading or drug-to-peptide ratio, and 20% for zeta potential or 5% for aggregation (these criteria enable follow-on animal-model testing, as needed).

Thrust 1B: Through pre-clinical development, including IND-enabling development, demonstrate that focused antibiotics do not cause gut microbiome changes (as compared to placebo) and have the same efficacy in an animal model as standard first-line treatment for the relevant infection.

Thrust 1B is focused on the pre-clinical development of carrier technologies (e.g., nano-carriers or peptide-drug conjugates) or direct delivery technologies paired with existing antibiotics to address UTI, AOM, and/or LRTI. Applicants proposing to use existing carrier technologies in this thrust to evaluate gut sparing effects must include data on the success criteria listed above in Thrust 1A. (N.B., If these data do not yet exist, applicants should apply under both Thrust 1A and 1B.) Applicants may propose local delivery technologies that are paired with innovations such as nano-carriers to improve local drug pharmacokinetics/pharmacodynamics (PK/PD) or penetration enhancers (e.g., otic preparation with a penetration enhancer to transit the tympanic membrane). The antibiotic used should be an existing antibiotic that is recommended as first-line for common outpatient infections for the indication(s) proposed (e.g., amoxicillin). First-line antibiotics have the benefit of clinician familiarity and affordability. A newer antibiotic, or an antibiotic not currently recommended as first-line, may also be used so long as there is a justification (including cost considerations and impact on eventual price) for using it to target common infections.

Pathogens studied should be appropriate to the target site infection and should include at minimum:

- UTI: *E coli* (including Extended-Spectrum Beta-Lactamase (ESBL) producers when relevant to the antibiotic being tested), *K pneumoniae, P mirabilis;*
- LRTI: *S pneumoniae* (including penicillin-resistant strains), *H influenzae* (including beta-lactamase positive strains), *M catarrhalis, S aureus* (MSSA); and
- AOM: *S pneumoniae* (including penicillin-resistant strains), *H influenzae* (including beta-lactamase positive strains), *M catarrhalis*.

Success criteria:

A. *Efficacy in* in vitro *models:* MIC and/or MBC similar to or superior to a standard first-line antibiotic against the target pathogen(s), with consideration of time-kill kinetics and/or other pharmacodynamic parameters as appropriate. If the drug is being used to target an infection which is often polymicrobial, additional studies of MIC/MBC in a microbial community model are encouraged. Where biofilms are

expected, plans should include the assessment of activity against relevant pathogens in biofilms and establishment of minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC) in an accepted model (e.g., Calgary Biofilm Device). The target concentration should at least be above the MBIC90 and ideally above the MBEC90.

B. *Efficacy in animal infection model:* Efficacy similar or superior to standard-of-care antibiotic in an appropriate model (e.g., murine ascending UTI model), with probability of target attainment ≥90% by Monte Carlo simulation.
C. *Low development of resistance at the site of infection:* The selected dose should demonstrate no amplification of resistant isolates in an animal infection model using a mixed inoculum of isolates with different MICs (including MICs above CLSI or EUCAST resistance thresholds) and no regrowth of resistant isolates (e.g. after 72-120 hours). Any regrowth or potentially resistant isolates should be tested using phenotypic and genomic methods to look for resistance mechanisms.
D. *Microbiome preservation:* Fecal antibiotic concentrations undetectable or at minimum below the MIC_{90} for key gut commensals, with no significant changes in alpha diversity or beta diversity ($p<0.05$), increased relative abundance of proteobacteria or *Enterococcus* spp (using an appropriate method such as ALDEx2 or ANCOM-II), or antibiotic resistance gene burden (e.g., <2-fold change) compared to placebo in an animal microbiome impact study. Use of an *in vitro* model such as MiPro is also encouraged once sufficient data are available to estimate the amount of active antibiotic that may be present in the gut (e.g., due to early release for carrier strategies).
E. *Safety profile:* Acceptable safety studies according to the technology being evaluated.
F. *Where a carrier strategy is being used, dual-labeled compartment analysis*: Demonstration of <10% premature release.

Thrust 2: Identify specific antibiotic-induced human gut microbiome changes that predict the development of subsequent resistant bacterial infection with >80% accuracy.

While it is well established that antibiotic-induced alterations to the gut microbiome (e.g., decreases in species diversity, increased likelihood of carrying resistant pathogens) facilitate the development of resistant infections, a significant knowledge gap persists regarding the specific microbial changes that drive this risk. Currently, robust contiguous human datasets establishing the biologic links between antibiotic use, gut microbiome changes, and subsequent resistant infections are lacking. Without identifying these

precise predictive factors, it remains challenging to refine focused antibiotic approaches or demonstrate the clinical value of microbiome-sparing therapies. Thrust 2 aims to bridge this gap. The primary objective is to identify specific antibiotic-induced gut microbiome changes that predict subsequent resistant bacterial infections with an accuracy exceeding 80%.

A surrogate marker that reflects specific antibiotic impact on the gut and accurately predicts subsequent resistant infections would provide an objective and quantitative method to demonstrate the clinical value of microbiome-sparing focused antibiotics. By measuring specific changes to the gut microbiome in the fecal microbiota, a readily accessible sample, it becomes possible to predict clinical outcomes and use these changes as a design tool to iteratively improve development of focused antibiotics. In addition, the data generated in Thrust 2 should be used to assess if *in vitro* models of the human gut microbiome, such as MiPro, can accurately reflect the most important antibiotic-induced changes to the gut microbiome and/or what refinements to these *in vitro* models are needed.[liii,liv] This thrust seeks research that establishes an end-to-end understanding of antibiotics, specific gut microbiome impact, and subsequent risk for development of resistant infections in humans.

Applicants to this thrust should use human cohort datasets and samples. Existing data should be used where possible, and where existing data are not sufficient, applicants can include prospective cohorts with serial microbiome sampling (before and after antibiotics) and assessments of selected clinical outcomes (e.g., resistant UTI). Microbiome assessments are expected to include measures of alpha and beta diversity, pathogen relative abundance (especially Enterobacterales and *Enterococcus* spp), and changes to antibiotic resistance genes. Applicants are encouraged to include microbiome functional testing through metabolomics with a clear rationale driving this area of investigation. Proposals that include the use of machine learning to integrate multi-dimensional biomarker data to improve performance are of particular interest.[lv] Applicants proposing machine learning models should describe the type of model that will be used and provide a justification for the selection.

Projects under Thrust 2 must meet the below success criteria:

A. *Microbiome surrogate marker:* Utilize existing human cohort data and establish new cohorts, as needed, to identify which specific antibiotic-induced changes to the gut microbiome predict subsequent resistant infection. Applicants should include a power analysis justifying total sample size. Achieve prediction accuracy >80% using validated statistical approaches, such as area under the receiver operating characteristic curve. Proposals should incorporate a gap analysis comparing the specific microbiome factors that are determined to be linked to

resistant infection risk with the capabilities of established *in vitro* models, such as MiPro.

B. *Protective factor identification:* Identify demographic factors, baseline microbiome characteristics, and other variables that alone or in combination reduce the likelihood of developing high-risk microbiome changes. Power studies to detect odds ratios of 0.5-0.67 (representing 33-50% risk reduction) with 80% statistical power at alpha=0.05. From this data identify potential modifiable protective factors that could inform intervention strategies.

C. *Population Impact Assessment:* To quantify the public health benefit of focused antibiotics, estimate the population attributable fraction (PAF) and associated 95% confidence intervals for individual-level antibiotic use in the development of resistant bacterial infections. Using both existing and new cohort data, model the extent to which focused antibiotics can achieve a >50% reduction in the PAF.

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