Ask an Expert with Dr. Eric Brown
Rethinking bacterial testing: How host-mimicking conditions are transforming antibiotic research

By Kyla Krajcovic

Dr. Eric Brown
Biochemistry & Biomedical Sciences
McMaster University, Canada

Since the beginning of antibiotics, scientists have cultured bacteria on nutrient-rich media, such as Mueller-Hinton broth or nutrient-dense agar formulations.[1] The problem? We don't need antibiotics to work in broth, we need them to work in the human body. Inside the host, bacteria often face nutrient scarcity, and their physiology and behaviour change dramatically in response. This means that how bacteria respond to antibiotics during laboratory testing can differ significantly from how they behave during an active infection. This mismatch may have caused researchers to overlook key survival mechanisms that only emerge in nutrient-limited environments – mechanisms that could be targeted by new drugs.[1]

According to the WHO, only 12 new antibiotics were approved between 2017 and 2022, 10 of which belonged to existing classes which were already associated with antimicrobial resistance.[2] This stagnation in antibiotic discovery may be due in part to the use of non-physiological testing conditions. For example, the type II fatty acid synthesis pathway was used as a drug target – until Poulsen et. al. showed that some gram-positive bacteria bypass it by scavenging fatty acids from their host.[3] Nutrient-limited environments within the host can induce adaptive bacterial behaviours that are hidden under nutrient-rich lab conditions and overlooking this can lead researchers to pursue ineffective drug targets.

Dr. Eric Brown and his team at McMaster University have been working on this problem for the last decade. A professor of biochemistry and an expert in bacterial physiology, Dr. Brown leads a lab focused on understanding how bacteria behave under nutrient scarcity – conditions that more closely resemble those faced during an infection.

Simulating Infectious Conditions

In the body, nitrogen is limited in the large intestine[4], methionine is scarce in the nasal cavity[5], and host defences actively sequester metal ions like iron and zinc away from invading bacteria[6]. To simulate these stresses in the lab, Brown's team uses nutrient-limited media containing only glucose, ammonium chloride, and essential salts and phosphates, which are starkly different from traditional nutrient-rich broths.[7] In this scarce environment, *E. coli* was shown to require 119 additional genes for survival compared to nutrient-rich conditions.[7] Many of these genes are involved in biosynthesis of amino acids, vitamins and nucleotides, or nutrient scavenging from the environment.[7] The Brown Lab has been studying these newfound pathways as potential drug targets – ones which may be missed under standard testing conditions.

What Antibiotic Research is Missing

For decades, antibiotic development has focused on targeting essential bacterial processes: protein translation, DNA replication, and cell wall synthesis. Now, the Brown Lab is shifting this perspective by studying genes that only become essential under infection-like, nutrient-limited stress. Which nutrients are absolutely required for bacterial survival in the host? Which vitamins or amino acids? Do these vulnerabilities

https://doi.org/10.29173/hsi499
Received 28 March 2025
Accepted 2 July 2025

vary by pathogen or by site of infection? These are the questions driving the Brown Lab's search for the next generation of antibiotics.[8,9]

The Biotin Block

One major discovery from Dr. Brown's lab is a molecule called MAC13772 that inhibits biotin synthesis in bacteria, ultimately leading to bacterial death by forming a biotin blockade. However, this compound only works in nutrient-limited media, because rich media contains biotin, allowing the bacteria to grow and survive without need for the synthesis.[10] This may explain why this compound has been previously overlooked: mouse models, commonly used in early testing have significantly higher biotin than humans. Bacteria colonizing mice therefore do not need to synthesize their own biotin for survival – it is readily available the host.[10] For this reason, the drug appeared ineffective in mice even though it may have worked in humans. To combat this, the Brown Lab implemented streptavidin – a molecule that tightly binds biotin – to lower the available biotin levels in the test mice.[10] With these new mice, the biotin synthesis pathway once again became necessary for bacterial survival. This underlines the importance of validating antibiotic targets in models that better reflect human biology.

Siderophores: A Trojan Horse Strategy

In a recent study, the Brown lab explored how *Klebsiella pneumoniae* – a multi-drug resistant bacterium – responds to antibiotic compounds in human blood. Surprisingly, some antibiotics seemed to promote bacterial growth rather than inhibit it.[11] In human blood, *K. pneumoniae* is starved for iron. To survive, it sends out siderophores, which are small, iron-scavenging molecules that sequester iron from the environment. The team discovered that the antibiotic compound they were testing was acting as a siderophore itself and promoting bacterial proliferation.[11] Turning this challenge into an opportunity, the Brown Lab chemically linked a beta-lactam antibiotic to a siderophore, creating a 'Trojan horse' compound. This conjugate was able to exploit the bacteria's iron uptake mechanisms: when the siderophore delivered iron to the bacteria, it also smuggled the antibiotic into the cell, where it would inhibit cell wall synthesis and effectively kill the pathogen.[11] This strategy underlies the mechanism of cefiderocol, a novel antibiotic being investigated in Dr. Brown's lab. Cefiderocol has been approved for treating complicated urinary tract infections and hospital-acquired pneumonia caused by gram-negative bacteria. However, real-world data is still being collected to inform its safety and effectiveness across various patient populations.[12]

Building Bacterial Signatures

Dr. Brown's lab also uses omics techniques like metabolomics, gene-chemical interaction mapping, and promoter activity tracking to uncover how new antibiotic compounds work, especially under nutrient-limited conditions.[13] A promoter is a regulatory DNA sequence that helps to initiate transcription. Tracking promoter activity allows researchers to assess gene expression levels, revealing which genes are upregulated or downregulated in response to a drug.[13] Since few antibiotics have been tested in this way, Dr. Brown's team has had to do the groundwork: systematically deleting individual bacterial genes, then exposing the bacteria to antibiotic compounds while under nutrient stress. If deleting a gene makes the bacteria more vulnerable to a drug – or, conversely, makes the drug less effective – it reveals that gene's role in the drug's mechanism of action.[14] By gathering information about these responses across a bacterial genome, the team builds a repertoire of unique chemical-genetic "signatures" for each compound.[13] These signatures can then be compared to those of new antibiotics to help predict how these drugs will work.[1]

Extensive testing has generated mountains of data in the Brown Lab. For this reason, their team uses AI to analyze chemical patterns and signatures that would otherwise be very difficult to detect. Over time, such computational approaches have become essential in the lab.

Innovation Without Incentive

Another team at McMaster, led by Dr. Gerard Wright, has recently discovered a new class of antibiotics, the first in nearly 40 years.[15] Unfortunately, discoveries like this are incredibly rare. Why? Because for most patients, our current antibiotics still work. Outside of critically ill ICU patients with a multi-drug-resistant infection, most patients are reliably able to recover with standard antibiotic options.[9] From a business perspective, this makes antibiotic development a hard sell: pharmaceutical companies are expected to invest millions into a drug that will only be used by a small number of patients, and even then, for short durations to avoid resistance[16].

It is not a profitable business model. In addition to poor investment, labs like Dr. Brown's face practical hurdles as well. His team uses human plasma to grow bacteria under infection-like conditions, an approach that is expensive, time consuming and difficult to scale.[9] Incentives, policies and funding must shift to encourage innovative antibiotic discovery.[16]

The Joy of the Discovery

When asked about a career moment he's most proud of, Dr. Brown recalled a memory from his early days as a student working in a lab at the University of Guelph. He described the thrill of looking at something under the microscope that no one had ever seen before and realizing he could build a career around that feeling.

Today, what brings him the most pride isn't a particular discovery or award, but the people he gets to work with. Watching his students grow – coming into the lab as wide-eyed students, unsure of their path, and leaving with confidence and clarity is what he finds most meaningful. Recently, Dr. Brown celebrated his 25th anniversary at McMaster University – a milestone that brought together former students and reminded him how meaningful mentorship can be.

Dr. Brown's work serves as a reminder that even in a stalled field like antibiotic development, a shift in perspective can reveal new possibilities – sometimes, simply by changing the plate. By recreating the nutrient limitations bacteria face within the human body, his lab is reframing old questions in more clinically relevant ways and moving closer to the answers that we really need.

Kyla Krajcovic
2nd year medical student
University of Limerick, Ireland

References

1. Zlitni S, Ferruccio LF, Brown ED. Metabolic suppression identifies new antibacterial inhibitors under nutrient limitation. Nat Chem Biol 2013;9(12):796–804.
2. Lack of innovation set to undermine antibiotic performance and health gains [Internet]. [cited 2025 May 3];Available from: https://www.who.int/news/item/22-06-2022-22-06-2022-lack-of-innovation-set-to-undermine-antibiotic-performance-and-health-gains
3. Poulsen BE, Yang R, Clatworthy AE, White T, Osmulski SJ, Li L, et al. Defining the core essential genome of Pseudomonas aeruginosa. Proc Natl Acad Sci 2019;116(20):10072–80.
4. Reese AT, Pereira FC, Schintlmeister A, Berry D, Wagner M, Hale LP, et al. Microbial nitrogen limitation in the mammalian large intestine. Nat Microbiol 2018;3(12):1441–50.
5. Krismer B, Liebeke M, Janek D, Nega M, Rautenberg M, Hornig G, et al. Nutrient Limitation Governs Staphylococcus aureus Metabolism and Niche Adaptation in the Human Nose. PLoS Pathog 2014;10(1):e1003862.
6. Hennigar SR, McClung JP. Nutritional Immunity. Am J Lifestyle Med 2016;10(3):170–3.
7. El Zahed SS, Brown ED. Chemical-Chemical Combinations Map Uncharted Interactions in Escherichia coli under Nutrient Stress. iScience 2018;2:168–81.
8. Publications [Internet]. Brown Lab2023 [cited 2025 May 3];Available from: http://www.brownlab.ca/publications
9. Farha MA, Tu MM, Brown ED. Important challenges to finding new leads for new antibiotics. Curr Opin Microbiol 2025;83:102562.
10. Carfrae LA, MacNair CR, Brown CM, Tsai CN, Weber BS, Zlitni S, et al. Mimicking the human environment in mice reveals that inhibiting biotin biosynthesis is effective against antibiotic-resistant pathogens. Nat Microbiol 2020;5(1):93–101.
11. Weber BS, Ritchie NE, Hilker S, Chan DCK, Peukert C, Deisinger JP, et al. High-Throughput Discovery of Synthetic Siderophores for Trojan Horse Antibiotics. ACS Infect Dis 2024;10(11):3821–41.
12. Towards investigation, development and introduction of cefiderocol in children. Product brief [Internet]. [cited 2025 May 5];Available from: https://www.who.int/publications/i/item/9789240084599
13. Klobucar K, Jardine E, Farha MA, MacKinnon MR, Fragis M, Nkonge B, et al. Genetic and Chemical Screening Reveals Targets and Compounds to Potentiate Gram-Positive Antibiotics against Gram-Negative Bacteria. ACS Infect Dis 2022;8(10):2187–97.
14. Klobucar K, French S, Côté JP, Howes JR, Brown ED. Genetic and Chemical-Genetic Interactions Map Biogenesis and Permeability Determinants of the Outer Membrane of Escherichia coli. mBio 2020;11(2):10.1128/mbio.00161-20.
15. McMaster researchers discover new class of antibiotics [Internet]. Bright. World [cited 2025 May 6];Available from: https://brighterworld.mcmaster.ca/articles/mcmaster-researchers-discover-new-class-of-antibiotics-gerry-wright-lariocidin-hamilton-soil/
16. A new class of antibiotics is cause for cautious celebration — but the economics must be fixed. Nature 2024;625(7993):7–7.