

‘Challenges in the Discovery of Gram-Negative Antibacterials: The Entry & Efflux Problem’

Summary of proceedings

About the workshop

On Feb. 6 and 7, 2017, the National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID) and The Pew Charitable Trusts hosted a workshop, “Challenges in the Discovery of Gram-Negative Antibacterials: The Entry & Efflux Problem,” in Rockville, Maryland. The goal of the workshop was to identify concrete next steps and collaboration opportunities to determine the physicochemical properties that affect the permeation and accumulation of molecules in Gram-negative bacteria that could be used to better find and design new types of antibiotics. These meeting notes summarize the key points and recommended solutions identified during the discussions.

Introduction

The workshop was organized by NIH/NIAID and Pew. Over 60 people—representing academic institutions, biotechnology, and pharmaceutical companies, nonprofit organizations, and government agencies—attended the workshop in person and over 100 more followed the live webcast.

The workshop discussion focused on the following questions:

- What evidence do we have that physicochemical guidelines for more rational antibiotic drug design and optimization can be established?
- How can we determine structure permeation relationships to better find and design molecules that get into and stay inside of Gram-negative bacteria?
- What information and tools are needed to fill gaps in understanding?
- How can we encourage collaboration across disciplines to advance this work?

NIH/NIAID recently announced a [funding opportunity](#) on this topic focused on the development of innovative research tools to help scientists find and design new antibiotics that can defeat three of the most dangerous Gram-negative pathogens: carbapenem-resistant Enterobacteriaceae, multidrug-resistant (MDR) *Acinetobacter*, and MDR *Pseudomonas aeruginosa*. These bacteria cause difficult, sometimes impossible-to-treat, drug-resistant infections, due in part to their gantlet of natural defenses that few existing antibiotics can overcome.

The workshop consisted of six panel presentations followed by moderated discussion sessions designed to encourage participation and debate, and build upon [Pew's Scientific Roadmap for Antibiotic Discovery](#), which outlines a concrete approach to support this research—both a scientific plan and organizational structure—that would lay a foundation for the sustained and diversified discovery and development of new antibiotics and therapies over the coming decades.

Recommendations & next steps

The workshop brought together stakeholders from across sectors to share lessons learned and identify collaborative opportunities to overcome barriers to antibiotic discovery. The level of discussion and input not only from the speakers and panelists, but also the entire audience, demonstrated engagement and interest in actionable next steps.

The discussions resulted in a few key recommendations for the scientific community.

Participants identified a need to:

- 1) Create a collaborative information-sharing platform that enables the scientific community to better access, share, and use information.** Participants generally agreed that establishing a web-based software platform that organizes preclinical data, allows for public and private data exchange, builds on published studies, and incorporates prospective research findings would help scientists share information across sectors and disciplines to advance the discovery of antibiotics that target Gram-negative bacteria.

Importantly, data included on the platform should be curated and targeted—focused on information relevant to addressing key questions that have stalled new drug discovery (outlined above).

Success would likely require:

- Mechanisms to address the technical, social, and behavioral barriers to sharing data.

- An advisory committee to set data standards and establish best practices for sharing information.
- Leading experts to identify promising data sets that should be incorporated into the platform.

If established, a collaborative information-sharing platform could provide an impetus and nexus for inquiry and mentorship in a field that has experienced substantial downsizing over the past 20 years.

2) Establish an antibiotic discovery “society” or virtual consortium. A cohesive group of scientists interested in antibiotic innovation could represent the unique perspectives of the field, set milestones, and track progress toward common goals. Additionally, such a group could help diversify the community of researchers working to address key barriers to antibiotic discovery by reaching out to scientists across disciplines and supporting efforts to cultivate the next generation of antibiotic discovery scientists.

3) Build momentum by tackling key research priorities in a systematic way. While a number of ongoing research efforts support antibiotic discovery, important barriers remain unresolved. The establishment of issue-specific working groups to tackle these barriers (e.g., development of new technologies to measure the entry of molecules into Gram-negative bacteria and creation of a collaborative platform to share information and knowledge) with targeted goals and approaches could help drive progress. These working groups could help set priorities, and track research progress over time. Examples of working group topics may include but are not limited to:

- Assay development to measure compound entry, accumulation, and efflux avoidance in Gram-negative bacteria.
- Computational modeling to determine physicochemical guidelines for compound entry, accumulation, and efflux avoidance in Gram-negative bacteria.

A structured approach to build momentum could help the field as a whole better identify and work toward promising solutions for common challenges, fill gaps in information that impede antibiotic discovery, and drive progress toward shared goals.

Opening Session: Barriers to compound penetration and efflux avoidance

The workshop was opened by words of welcome by Emily Erbelding (NIH/NIAID) and Allan Coukell (Pew). Erbelding provided an overview of NIH/NIAID activities to support antibiotic discovery and outlined the goals of the workshop. Coukell then discussed Pew’s work to spur innovation of new antibiotics and therapies to treat patients with serious or life-threatening bacterial infections.

The opening remarks were followed by keynote presentations from Lynn Silver (LL Silver Consulting) and Hiroshi Nikaido (University of California, Berkeley), who provided an overview of the barriers to the discovery of Gram-negative antibacterials and discussed ongoing research progress and information gaps that need to be resolved. Keynote speakers were then joined by John Finn (formerly with Trius Therapeutics) and Wright Nichols (formerly with AstraZeneca) for a panel discussion. The session was moderated by Richard Lee (St. Jude Children's Research Hospital).

Key points

- Antibiotic activity depends on the ability of molecules to get into and stay inside of bacteria. Given the orthogonal sieving properties of the outer and cytoplasmic membranes in addition to the presence of efflux pumps, which actively expel toxic molecules from the cell, it is particularly difficult to find molecules that can successfully access drug targets located in the cytoplasm of Gram-negative bacteria.
- Additionally, “singlet” efflux pumps located in the cytoplasmic membrane may be a contributing factor to resistance and should be further studied.
- The permeability of the outer membrane of Gram-negative bacteria has a major impact on the susceptibility of pathogens to antibiotics. Small hydrophilic compounds, such as B-lactams, passively enter the cell through pore-forming membrane protein complexes (porins). Porins vary considerably in size and permeability across bacterial species.
- Molecules that are too large and hydrophobic to diffuse across porin channels (e.g. macrolides and rifamycins) cross the outer membrane through a lipid-mediated pathway. This process is slow in certain types of bacteria, so antibiotic activity may be more easily counteracted by the activity of multidrug efflux pumps.
- Nonfermenting bacteria, such as *P. aeruginosa* and *A. baumannii*, have nonspecific, low-permeability porins, which, coupled with efflux pumps and low permeability of the outer membrane, may account for high levels of resistance to a number of antibiotics.
- Early computational models can be built using available data to predict the permeation rates across the outer membrane for antibiotics, such as fluoroquinolones, tetracyclines, and aminoglycosides. However, a new generation of computational models is needed to better understand the chemical features that might predict the entry of molecules into the cytoplasm of Gram-negative bacteria.
- Participants discussed the limitations of existing compound collections for determining conditional guidelines for which types of molecules get into and stay inside of Gram-negative pathogens. Industry compound collections are generally optimized for human drug targets, such as G-protein-coupled receptors or kinases, and biased toward penetration of mammalian rather than Gram-negative bacterial cells.

- On the other hand, some participants made the point that existing compound collections could still be useful for measuring the entry and accumulation of molecules in bacteria (vs. activity).
- A number of sources of chemical matter were discussed, including natural products and semisynthetic compound collections.
- A few participants expressed skepticism that limited resources should go toward the creation of an “antibacterial compound collection” and highlighted that compound collections are helpful only if the field is better able to measure and manipulate the entry of molecules into and out of the cell.

Opening session references

1. Helen I. Zgurskaya, Cesar A. López, and Sandrasegaram Gnanakaran, “Permeability Barrier of Gram-Negative Cell Envelopes and Approaches to Bypass It,” *ACS Infectious Diseases* 1, no. 11 (2015): 512–22, <http://pubs.acs.org/doi/abs/10.1021/acsinfecdis.5b00097>.
2. Jeffrey B. Locke et al., “Structure-Activity Relationships of Diverse Oxazolidinones for Linezolid-Resistant *Staphylococcus aureus* Strains Possessing the *cfr* Methyltransferase Gene or Ribosomal Mutations,” *Antimicrobial Agents and Chemotherapy* 54, no. 12 (2010): 5337–43, <http://aac.asm.org/content/54/12/5337.full.pdf+html>.
3. Lynn L. Silver, “A Gestalt Approach to Gram-Negative Entry,” *Bioorganic & Medicinal Chemistry* 24, no. 24 (2016): 6379–89, <http://www.sciencedirect.com/science/article/pii/S0968089616304679>.
4. The Pew Charitable Trusts, “A Scientific Roadmap for Antibiotic Discovery” (2016), <http://www.pewtrusts.org/~media/assets/2016/05/ascientificroadmapforantibioticdiscovery.pdf>.
5. Wright W. Nichols, “Permeability of Bacteria to Antibacterial Agents,” *Antibiotic Discovery and Development* (2012): 849–79, https://link.springer.com/chapter/10.1007%2F978-1-4614-1400-1_26#page-1.

Session 2: Case studies—Finding ways to overcome barriers to compound penetration and efflux

Scientists from Achaogen, Melinta Therapeutics, and Entasis Therapeutics provided concrete examples of structure-activity relationship (SAR) studies to improve the entry of molecules into Gram-negative bacteria. Frederick Cohen (Achaogen) described efforts to improve the entry of molecules that target biotin carboxylase by optimizing physicochemical properties. The program builds on the observation that amine substitutions improve cellular entry and uses matched pairs of strains and conditions to assess the contribution of each barrier to entry. Erin Duffy (Melinta) described a computational clustering approach to assess the relationship

between chemical properties and efflux in order to convert a chemical series that targets Gram-positive bacteria into one that targets Gram-negative bacteria. Ruben Tommasi (Entasis) provided an overview of a new method, Titratable Outer Membrane Permeability Assay System (TOMAS), for generating SAR data based on entry through outer membrane porins. The session was moderated by Carl Balibar (Merck).

Key points

- Panelists presented a series of case studies to demonstrate how understanding the physicochemical features that affect the transit of molecules across the membrane can provide valuable insights into antibacterial medicinal chemistry and rational drug design.
 - Melinta has used crystallography and computational chemistry to generate new classes of antibiotics, including pyrrolocytosines, which have been rationally designed for high binding affinity and broad-spectrum activity against a range of Gram-negative bacteria. The company employed a computational “clustering” approach to find virtual molecules that might address problems with efflux, test molecules for activity, and improve activity against *P. aeruginosa* multidrug-resistant strains.
 - Achaogen has used medicinal chemistry to test the physicochemical properties of inhibitors of biotin carboxylase and LpxC, an essential enzyme for the formation of the bacterial outer membrane, and improve the activity of these molecules in Gram-negative pathogens.
 - Entasis’ sensitive and specific whole-cell approach in *E. coli*, TOMAS, defines the relationship between specific molecular features and permeation through an isolated porin. Entasis has incorporated this improved understanding of the physicochemical properties of molecules into the design of new antibacterials.
- Participants discussed the value of optimizing each Gram-negative antibiotic discovery program empirically. The challenge is determining whether useful knowledge could be applied across chemical series.
- While it may not be possible to generate a “Lipinski’s rule of five”^{*} for antibiotic discovery, it may be possible to increase the likelihood of success based on improved understanding of three-dimensional features of molecules.

^{*} Lipinski’s rule of five describes the physicochemical properties that correlate with a compound’s likelihood of being orally bioavailable. The rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- No more than five hydrogen bond donors.
- No more than 10 hydrogen bond acceptors.

- Participants discussed the lack of available assays that can quickly and accurately measure the cytoplasmic concentration of molecules, which would be needed to assess whether molecules get into and stay inside of Gram-negative bacteria. Panelists noted that because there is no good way to measure cytoplasmic concentration, they have relied on minimum inhibitory concentration or enzymatic activity.
- There may be technologies that the antibiotic discovery community has not yet tapped into. The challenge is how to engage other research communities and learn from other disciplines.
- Researchers working on different programs have independently carried out SAR analysis for distinct antibiotic classes. There may be common themes across programs, but lessons learned are not shared across companies and research institutions.
- Participants generally agreed that more high-quality data (chemical structures, biochemical data, and whole-cell activity data) are needed to carry out analyses to determine SAR for the entry and efflux of molecules for specific Gram-negative bacteria. Companies have published information, but it would be useful to put this information into a common database.
- Participants noted that access to published journal articles is helpful, but it is also important to have access to the underlying chemical structures.
- Additionally, data and assays should be standardized to enable comparison across studies and research programs.
- While some research groups may have computational power to generate useful models based on physicochemical data, such analyses may be prohibitively expensive for a small company to use on a routine basis.

Recommendations & next steps

- Given that large companies may not be willing or able to disclose chemical structures, participants discussed the need for a third-party nonprofit that could collect public and private data from across companies for SAR analysis. A third-party nonprofit could then share prospective learnings, to the benefit of ongoing and future antibiotic discovery programs.
- Examples of nonprofit consortia include the TB Alliance, the Bill and Melinda Gates Foundation partnership with GlaxoSmithKline's Tres Cantos Open Lab, the Innovative

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- A molecular mass less than 500 daltons.
 - A measure of lipophilicity (log P) not greater than 5.

The rule of five is based on a distribution of properties for several thousand drugs. Some categories of drug types—such as antibiotics, antifungals, vitamins, and cardiac glycosides—are exceptions to the rule.

Medicines Initiative's New Drugs for Bad Bugs (ND4BB) InfoCentre, and the Gates Foundation's Tuberculosis Drug Accelerator program.

- Rather than gathering any and all antibiotic discovery data, participants recommended a targeted effort to retrieve information on specific chemical series (e.g., seeking data across companies focused on inhibitors of particular drug targets, such as LpxC). Panelists noted that companies may be more willing to share data from discarded programs.

Session 2 references

1. Mathias Winterhalter and Matteo Ceccarelli, "Physical Methods to Quantify Small Antibiotic Molecules Uptake Into Gram-Negative Bacteria," *European Journal of Pharmaceutics and Biopharmaceutics* 95, Part A (2015): 63–67, <http://www.sciencedirect.com/science/article/pii/S0939641115002246?via%3Dihub>.
2. Ruben Tommasi et al., "ESKAPEing the Labyrinth of Antibacterial Discovery," *Nature Reviews Drug Discovery* 14, no. 8 (2015): 529–42, <http://www.nature.com/nrd/journal/v14/n8/abs/nrd4572.html>.

Session 3: Enabling technologies to measure compound permeability and accumulation

Panelists discussed examples of technologies that could be developed to measure the entry and accumulation of molecules in Gram-negative bacteria. Derek Tan (Memorial Sloan Kettering Cancer Center) discussed a platform to quantify small molecule accumulation in bacteria using mass spectrometry and principal component analysis, a statistical procedure to determine whether observed variables of structural and physicochemical properties are correlated. Kyu Rhee (Weill Cornell Medical College) discussed research findings using a liquid chromatography-mass spectrometry (LC-MS) mass balance assay to assess the entry of sulfonamides into *Mycobacterium tuberculosis* cells. Helen Zgurskaya (University of Oklahoma) discussed an LC-MS-based assay her lab is developing to measure the entry of molecules into Gram-negative bacteria by controlling the permeability of the outer membrane. The session was moderated by Alita Miller (Entasis).

Key points

- The holy grail of compound uptake assays would be:
 - Robust (sensitive, reproducible).
 - Involved with direct detection of compounds (without the need for pre-labeling).
 - Kinetic.
 - Qualitative.
 - Whole-cell based (including relevant strains).

- Capable of informing subcellular localization.
- High-throughput.
- Cost-effective.
- If even half of these features were achieved, such an assay would benefit the field.
- Advances in assay development to measure the penetration of molecules for tuberculosis could be applicable to Gram-negative bacteria. *M. tuberculosis* is a Gram-positive organism but has two membranes, similar to Gram-negative bacteria.
- Panelists discussed the advantages and disadvantages of MS-based analysis
 - Advantages:
 - Sensitive.
 - High-throughput.
 - Allows for native analysis.
 - Molecular-level resolution.
 - Can be linked to mechanism of action profile.
 - Disadvantages:
 - Ionizability.
 - Difficulty with endpoint measurements.
 - Relative quantitation.
 - Limitations in kinetic measurements.
- Assay conditions can complicate kinetic measurements of compound uptake and efflux. For example, transferring a sample from 37 degrees Centigrade to ice to room temperature permeabilizes the outer membrane of bacterial cells, leading to artificial effects on the data.
- It is important to be able to control permeability in order to accurately measure molecular kinetics. The use of mutant bacteria with pores in the outer membrane that allow molecules to freely pass through allows for the study of entry of molecules through the cytoplasmic membrane.
- Participants discussed complicating factors when measuring molecular kinetics, including the challenge of discerning whether a compound is binding to the outside of the bacterial cell or diffusing out of the cell, how to control for and measure efflux rates in kinetic measurements, establishing standard assay conditions to enable researchers to compare findings across studies, sample manipulation (e.g., centrifugation), and thermodynamic state (i.e., what determines final concentration in the cytoplasm is a combination of kinetics, efflux rates, and affinity to the drug target).

Recommendations & next steps

- The research community would benefit from a mechanism to evaluate methodologies and establish standards for assay conditions, such as growth media, cell densities, and cell extraction protocols to enable scientists to meaningfully compare assay data across studies.
- Bacterial strains, including efflux pump and porin isogenic mutants (bacteria with similar genomes) for *A. baumannii*, *Klebsiella pneumoniae*, and *P. aeruginosa*, should be standardized and made available to researchers so that research findings are more easily comparable across studies.
- An advisory committee or working group of leading experts could establish a common “gold standard toolkit” set of compounds and bacterial strains (wild type, knockout mutants) for researchers to test new assays.
- Once developed and validated, tools (quantitative assays) to quickly and accurately measure drug penetration and kinetics for Gram-negative bacteria that are independent of drug activity could then be used to carry out SAR analyses to understand what physicochemical properties might increase the penetration of a molecule into the bacterial cell, decrease efflux, or improve the way a molecule inhibits or binds to the drug target.

Session 3 references

1. Hossein Heidari Torkabadi et al., “Following Drug Uptake and Reactions Inside *Escherichia coli* Cells by Raman Microspectroscopy,” *Biochemistry* 53, no. 25 (2014): 4113–21, <http://pubs.acs.org/doi/abs/10.1021/bi500529c>.
2. Tony D. Davis, Christopher J. Gerry, and Derek S. Tan, “General Platform for Systematic Quantitative Evaluation of Small-Molecule Permeability in Bacteria.” *ACS Chemical Biology* 9, no. 11 (2014): 2535–44, <http://pubs.acs.org/doi/abs/10.1021/cb5003015>.

Session 4: Establishing physicochemical guidelines for compound entry and efflux

Panelists discussed concrete ideas for how to establish conditional guidelines for molecules that get into and stay inside of Gram-negative bacteria, the limitations and caveats for establishing such guidelines, and recommendations for advancing this line of work. Heinz Moser (Novartis) provided an overview of the particular challenges for Gram-negative antibiotic discovery and offered some recommendations for how to identify valuable chemical starting points to improve understanding of compound permeability and efflux. Lynn Silver outlined an example of how Collaborative Drug Discovery software could be used to identify physicochemical features that may be predictive for entry of molecules into Gram-negative bacteria. Mathias Winterhalter (Innovative Medicines Initiative/Jacobs University, Bremen) described approaches

that IMI Translocation is exploring to measure compound uptake in bacterial cells. The session was moderated by Troy Lister (Spero Therapeutics).

Key points

- Similar to drug discovery for other therapeutic areas, antibiotic discovery requires that researchers optimize multiple chemical and biological parameters in parallel.
- There are additional barriers for Gram-negative antibiotic discovery, including an additional outer membrane with a fundamentally different structure and permeability to the cytoplasmic membrane, evolutionarily optimized efflux pumps that expel molecules out of the cell, issues with drug resistance, and higher safety and toxicity standards given the need to safely administer antibiotics at higher doses than other types of drugs.
- Finding suitable chemical starting material that addresses all of these barriers is a challenge. It is important to realize that understanding the physicochemical properties of the types of molecules that get into and stay inside of Gram-negative bacteria is only a part of the solution.
- Participants generally agreed that physicochemical guidelines for molecules that get into and stay inside of Gram-negative bacteria would not be applicable across all types of molecules, but could be dependent upon key factors, such as route of entry into the cell, chemical class, mechanism of action, or bacterial species.
- IMI Translocation has focused on:
 - The study of the molecular and structural features of porins to better understand how molecules enter bacterial cells.
 - Microfluidics-based methods to measure the kinetics of molecules in single bacterial cells.
 - LC-MS-based methods to measure how molecules get into and stay inside of Gram-negative bacteria.
- One computational-based approach to understanding the physicochemical properties of molecules may be to compare the features of known antibiotics with drug targets located in the cytoplasm vs. known molecules that do not target Gram-negative bacteria. Further, differentiating molecules based on “route of entry” into the cell could lead to new insights and hypotheses on the types of molecules that should be tested in the laboratory.
- Participants emphasized that the development of a useful model for predicting what types of molecules get into and stay inside of Gram-negative bacteria requires having the right data set, asking the right questions, and annotating the results so that other researchers can evaluate the data and conclusions. They noted that data originating

from different sources may be difficult to compare, depending on how these data are collected and different parameters are calculated. Participants also highlighted the importance of considering more sophisticated measures of physicochemical and structural properties that move beyond two-dimensional descriptors, such as counting H-bond donors and rotational bonds.

Recommendations & next steps

- Coordinated multidisciplinary research efforts could help integrate experimental findings and computational modeling to better define suitable chemical starting points for antibacterial discovery and help researchers design new types of molecules that have a greater chance of success against Gram-negative bacteria.
- More engagement is needed from across disciplines, including computational chemists who could help develop more sophisticated statistical techniques to understand the relationship between permeability and physicochemical properties of molecules.
- An advisory committee or working group of leading experts could help to determine which physicochemical parameters might be most useful for establishing scaffold-dependent SAR for molecular entry and efflux in Gram-negative pathogens.
- If conditional guidelines for compound entry and efflux avoidance were deduced, researchers could then create compound collections that are biased toward cellular targets in Gram-negative bacteria. Coupled with techniques such virtual screening may enable researchers to better design compounds that have a higher likelihood of Gram-negative activity.
- In addition to informing new antibiotic discovery, technologies and guidelines could be used by researchers to re-examine previously studied compounds that may have been discarded due to poor permeability.

Session 4 references

1. Hiroshi Nikaido, and David G. Thanassi, "Penetration of Lipophilic Agents With Multiple Protonation Sites Into Bacterial Cells: Tetracyclines and Fluoroquinolones as Examples," *Antimicrobial Agents and Chemotherapy* 37, no. 7 (1993): 1393–99, https://www.researchgate.net/publication/14836669_Penetration_of_lipophilic_agents_with_multiple_protonation_sites_into_bacterial_cells_Tetracyclines_and_fluoroquinolones_as_examples.
2. Rosemarie O'Shea and Heinz E. Moser, "Physicochemical Properties of Antibacterial Compounds: Implications for Drug Discovery," *Journal of Medicinal Chemistry* 51, no. 10 (2008): 2871–78, <http://pubs.acs.org/doi/abs/10.1021/jm700967e>.

Session 5: Ongoing initiatives and funding opportunities

Panelists representing funding organizations and initiatives to advance antibiotic discovery provided overviews of their ongoing work and discussed ways they support efforts to improve the ability of scientists to find and design molecules that get into and stay inside of Gram-negative bacteria. The participants heard from Francesca Chiara (Wellcome Trust, Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator, or CARB-X), Jane Knisely (NIH/NIAID), David Pardoe (Medical Research Council Technology), Rob Stavenger (GlaxoSmithKline, IMI Translocation), and Jonathan Thomas (Outer Membrane Efflux Gram-Negative Assault, or OMEGA, Project). The session was moderated by Carolyn Shore (Pew).

Key points

Wellcome Trust

- Wellcome has supported drug-resistant infection research for more than a decade and will continue to support competitive projects in this space through existing funding mechanisms.
- The Wellcome Trust is implementing a recently approved strategic plan focused on drug-resistant infections as a priority area. The aim of this priority area is to safeguard our capacity to treat infectious diseases and address the rising threat of antimicrobial resistance (AMR) through coordinated actions. This is not a new funding call, and Wellcome will continue to support researchers in this field through its traditional funding schemes.
- Within the priority program on AMR, Wellcome is focusing on four areas to reduce the threat of drug resistance:
 - 1) **Effective global governance:** Wellcome is working with policymakers to support the development of a global framework to coordinate, monitor, and evaluate global efforts following the 2016 United Nations resolution on AMR.
 - 2) **Support for development of new treatments:** Wellcome has partnered with CARB-X, a global public-private partnership to stimulate the development of promising new antibacterial therapies and diagnostics over the next five years.
 - 3) **Faster clinical trials:** Wellcome is building global clinical trials networks to innovate and standardize protocols with the aim of reducing costs and accelerating drug approvals.
 - 4) **Epidemiology of drug-resistant infections:** Wellcome is creating a global portfolio of open research and data to guide national and global strategies to tackle drug resistance.

NIH/NIAID

NIAID, the lead NIH institute for research on AMR, provides a number of funding opportunities to help stimulate research in this important area, including small-business grant and contract funding announcements and targeted research initiatives, such as those issued under the NIAID Partnerships Program (e.g., RFA RFA-AI-16-081). The Partnerships Program aims to stimulate translational research and product development activities and encourages collaboration between academic and industry scientists on milestone-driven projects. NIAID also offers a broad suite of preclinical and clinical services to assist investigators in advancing products through the drug development pipeline, such as in vivo and in vitro testing, animal model development, and assay development to accelerate the identification of promising new drug candidates and enable Investigational New Drug submissions to the Food and Drug Administration.

Medical Research Council Technology (MRCT)

- MRCT (now known as LifeArc) is a public charity that partners with others to advance the translation of early-stage research. One of its goals is to “unblock the pipeline” by moving stalled projects along to the point where they are de-risked enough for pharmaceutical companies to re-enter development. The primary goal is to initiate projects to share outputs, not to develop new molecules.
- One partnership program launched by MRCT, the Dementia Consortium, started with one charity and two pharmaceutical companies and has since added three more pharmaceutical companies. Partners co-funded and selected projects for translation. Industry partners brought funding and technical expertise to the partnership.
- MRCT has launched three new initiatives related to antibiotic resistance:
 - Collaboration with the Centre for Drug Research and Development to look at essential genes in Gram-negative pathogens.
 - Pooling knowledge across academia and industry.
 - Studying structural space that allows penetration/accumulation and decreases efflux.

IMI Translocation

- IMI Translocation, a collaborative project of five companies, has built a valuable network of researchers exploring Gram-negative permeability. This network is at risk of dissipating as Translocation nears the end of its funding stream. It is unclear whether the void left by IMI Translocation will be filled by another organization or whether the network will fall apart.

OMEGA project

- The California Institute for Regenerative Medicine (CIRM) was created by the California Stem Cell Research and Cures Act (Proposition 71) to distribute state funds for stem cell research. The mission of CIRM is to accelerate stem cell treatments to patients with unmet medical needs. Jonathan Thomas of OMEGA would like to apply his experience with CIRM to address the problem of antibiotic resistance.
- The OMEGA project was conceived following a daylong summit at Northeastern University last summer that laid out a game plan for a five-year Manhattan Project-like effort to tackle two key priority areas: Gram-negative permeation and natural product drug discovery. The 15-plus scientific experts at that summit expressed interest in contributing to OMEGA if it moves forward.
- The OMEGA project is currently seeking to raise \$40 million for the first two to three years of work. The ultimate cost for the five-year project could exceed \$100 million.
- The nonprofit project would focus on pre-competitive research and could feature a “brick-and-mortar” institute component as well as grant opportunities to build a network of scientists from around the world.

Recommendations & next steps

- Targeted funding opportunities and collaborative efforts are needed to develop new technologies that can quickly and accurately measure the entry and accumulation of molecules in Gram-negative pathogens that are independent of cell-killing activity.
- There was general agreement that periodic meetings among funders would be a useful way to coordinate existing research opportunities and identify gaps in research funding.
- While beyond the scope of this workshop, participants noted that it would be helpful if funders could agree on a common set of materials for grant applications or other approaches to help streamline the application process.

Session 6: Information-sharing platform on compound penetration and efflux

In this session, panelists outlined how an information-sharing platform could add value to the field. Brad Sherborne (Merck) provided an industry perspective on some of the barriers to sharing data, noting that measures must be taken to encourage the use of an information-sharing platform. For example, scientific journals could require data-sharing as a prerequisite for publication or funders could link platform use to funding opportunities.

Philip Gribbon (Fraunhofer Institute for Molecular Biology and Applied Ecology, IMI Translocation) described the ND4BB InfoCentre, a data-sharing platform that was intended to span several IMI projects and include multiple types of data ranging from chemistry and biology data required for drug discovery to clinical trial data. He emphasized that due to the expansive

range and heterogeneous nature of these data, the standard data systems used by corporations were not applicable. Barry Bunin (Collaborative Drug Discovery) discussed the company's software platform, a drug discovery research informatics resource that hosts biological and chemical databases for companies and collaborative projects, including the NIH Blueprint Neurotherapeutics Network, which supports small molecule drug discovery and development, and the More Medicines for Tuberculosis consortium, which seeks to advance new tuberculosis drugs. The session was moderated by Pooja Kothari (Pew).

Key points

- Curating available data (published and unpublished) and making this information accessible to the antibiotic discovery research community would enable scientists to share lessons learned based on what has been done in the past and modify or improve upon previous experiments.
- A collaboration-oriented online database to synthesize published and unpublished information could help scientists more effectively tackle key research priorities.
- While not specific to antibiotic discovery, researchers across sectors have a responsibility to find ways to better share negative data.
- It is a challenge to encourage researchers to take advantage of information-sharing software platforms given the time and effort required to upload data, demonstrate to researchers the value of using an information-sharing software platform, and sustain such a resource over time.
- Participants discussed the benefit of linking funding awards to data-sharing recommendations or requirements. While funders such as NIH have data-sharing policies in place to make data as widely and freely available as possible while protecting confidential and proprietary data, recommendations on specific mechanisms for researchers to share data could amplify ongoing investments to advance antibiotic discovery.
- Panelists suggested that scientific journals could consider adding a requirement for publication that supplemental data be shared on a publicly available database.
- It is a struggle to obtain historical industry data. The challenge may not be due to unwillingness on the part of researchers, but rather the considerable time and labor required to access, collate, and curate legacy data sets.
- There are variability and limitations in data recording, management, formatting, and storage across companies. Additionally, missing information, such as detailed experimental conditions, may limit the value of available historical data.
- Even in cases where high-quality data have been generated in the laboratory, quality-control issues remain a concern for any database. Statistical procedures can be used to evaluate the quality of the data.

- To integrate data from across multiple sources, there should be an established process to ensure these data are high quality. A good starting point could be for experts from across research groups to come together to develop data standards and best practices for data collection.
- Robust, high-integrity data that include analyses of compounds based on comparable assays should be compiled. Data, methods, and materials such as strains, conditions, and chemical transport modifiers should be integrated in a consistent manner.
- It is also important to provide context on how a given database was assembled so that end-users are aware of its limitations.
- Researchers who may use an information-sharing platform may not be computational experts, so information should be clearly presented and easily searchable so that chemists and biologists know what data are available and how they can be accessed and used. Panelists noted that data standards may vary across disciplines, so it would be important to reconcile these differences.

Recommendations & next steps

- Participants generally agreed that establishing a web-based software platform that organizes preclinical data, allows for public and private data exchange, builds on published studies, and incorporates prospective research findings could help scientists share information across sectors and disciplines to advance the discovery of antibiotics that target Gram-negative bacteria.
- Importantly, data included on the platform should be curated and targeted—focused on information relevant to addressing key questions that have stalled new drug discovery:
 - What evidence do we have that physicochemical guidelines for more rational antibiotic drug design and optimization could be established?
 - How can we determine structure permeation relationships to better find and design molecules that get into and stay inside of Gram-negative bacteria?
 - What information and tools are needed to fill gaps in understanding?
- Success would likely require:
 - Mechanisms to address the technical, social, and behavioral barriers to sharing data.
 - An advisory committee to set data standards and establish best practices for sharing information.
 - Leading experts to identify promising data sets that should be incorporated into the platform.

- If established, a collaborative information-sharing platform could provide an impetus and nexus for inquiry and mentorship in a field that has experienced substantial downsizing over the past 20 years.

Session 6 references

1. Moses Hohman et al., "Novel Web-Based Tools Combining Chemistry Informatics, Biology and Social Networks for Drug Discovery," *Drug Discovery Today* 14, no. 5/6 (2009): 261-270, <https://pdfs.semanticscholar.org/0526/0d5042c6e67a16475c5dde4cc15e813987ee.pdf>.

Other comments

While outside the scope of this workshop, participants brought up several points that merit further discussion:

- Participants discussed the benefits of diversifying screening approaches (e.g., phenotypic screening, target-based, and fragment-based approaches), focusing on chemical matter that is more likely to yield successful starting points for antibacterial discovery (e.g., lower molecular weight, higher polarity, less aromaticity, more three-dimensionality, more functional groups).
- The field should continue to explore new techniques for generating promising chemical matter, such as methods to produce natural products from previously unculturable microbes, synthetic biology approaches for genome mining, and hypersensitive screening.
- Clinicians and scientists in other biomedical fields, such as HIV/AIDS, tuberculosis, and oncology, have long recognized the value of combination therapy. Combination therapy has not been as well-studied for the treatment of systemic bacterial infection and may offer a promising avenue for new antibacterial discovery and development.
- Single-target inhibitors, or molecules that bind specifically to a particular protein within a cell, often lead to a high frequency of resistance and substantial decreases in the minimum inhibitory concentration (i.e., reduced susceptibility to a given drug), which limits their potential for development. Further studies are needed to determine whether combinations of single-target antibacterials can, at least in principle, reduce the frequency of resistance.
- It is difficult to confirm a single mechanism of action for a given molecule. Off-target activity is common for novel synthetic compounds. Methods for rapid determination of mechanism of action for antibacterials are needed. Additionally, participants discussed the benefits of exploiting drug targets located in the periplasm rather than the cytoplasm.