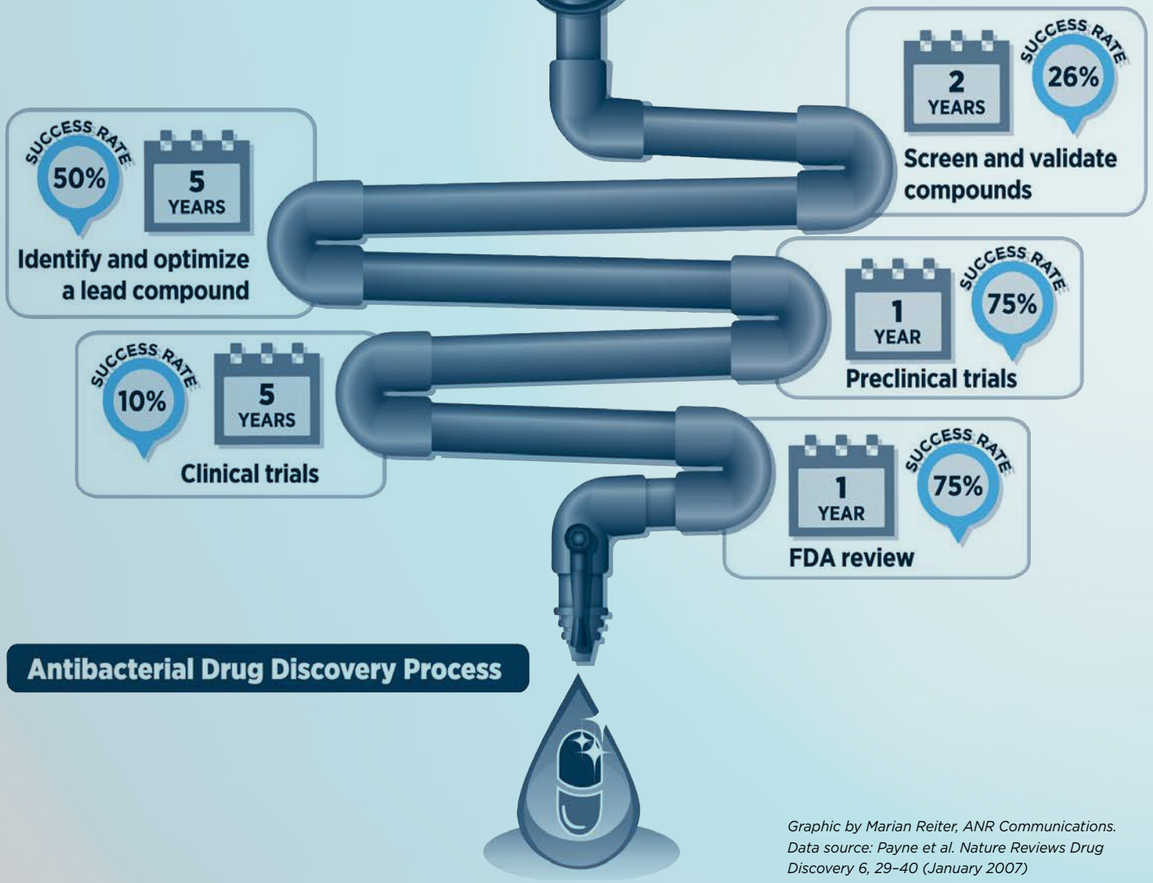
PRIMING THE PIPELINE . . .



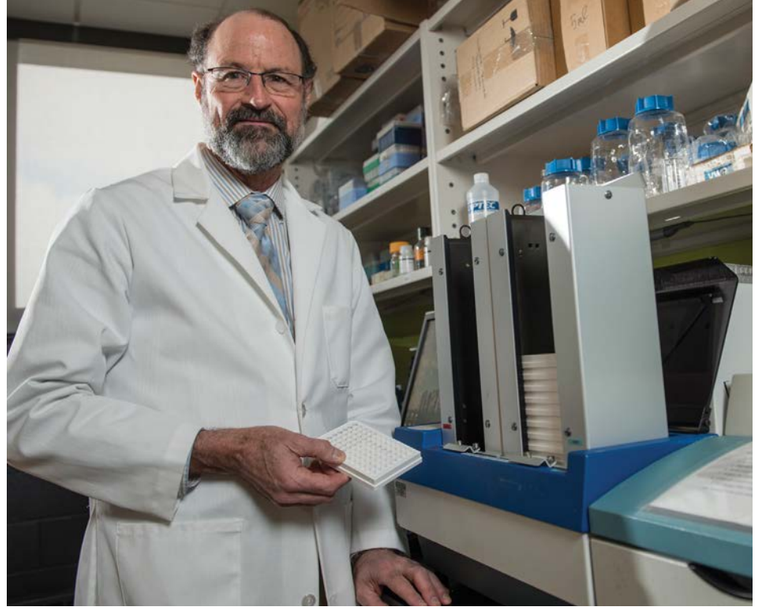
Graphic by Marian Reiter, ANR Communications. Data source: Payne et al. Nature Reviews Drug Discovery 6, 29–40 (January 2007)

The promising role of the university in drug development

The introduction of penicillin in the 1940s is considered one of the greatest advances in therapeutic medicine, marking the inception of an era of antibiotic production that revolutionized healthcare. Over the following decades, other types of antibiotics followed, flooding the medical market with what many called "miracle drugs." Together, these transformative medicines all but removed the fear of life-threatening complications caused by bacterial infections.

Today, that rosy outlook has begun to fade. A marked decline in development of new antibiotics in the past 30 years, coupled with the heavy use and misuse of antibiotics, has experts concerned that the current drug supply will no longer suffice. Reports of diseases that are resistant to one or more antibiotics are on the rise, and the severity of the situation has even garnered the attention of several prominent world leaders:

BY NATASHA BERRYMAN
Writer



LEFT: Richard R. Neubig, MSU professor and chairperson of the Department of Pharmacology and Toxicology, is establishing a new drug-discovery lab MSU researchers can use to explore viable drug candidates — a process he believes MSU scientists are well positioned to achieve because of a strong foundation in basic science.

- President Barack Obama acknowledged the need to address drug-resistant bacteria in his 2014 State of the Union address.
- The Centers for Disease Control and Prevention and Prevention (CDC) issued a detailed report describing the extent of bacterial drug resistance in the United States in 2013.
- Margaret Chan, World Health Organization (WHO) director, warned, "In the absence of urgent corrective and protective actions, the world is heading towards [an] era in which many common infections will … once again, kill unabated" on World Health Day in 2011.

The CDC explained that, during the past 70 years, bacteria have shown the ability to develop resistance to every known antibiotic. Not only are drug-resistant bacteria capable of rendering many routine therapies ineffective, but they also increase the risk of complications for patients undergoing life-saving procedures.

Several Michigan State University (MSU) researchers are responding to this urgent call for new antibiotics and other novel drug therapies.

Constructing a niche

The process of developing and introducing a new antibiotic from concept to market can take as many as 14 years and costs upwards of a billion dollars — not including the investments in the fundamental science required to understand disease mechanisms. Couple the high costs with low success rates (see page 22) and most pharmaceutical companies avoid such endeavors altogether. University researchers, however, aren't as timid.

Richard R. Neubig, MSU professor and chairperson of the Department of Pharmacology and Toxicology, is well aware of the risk but recognizes the important role that research universities such as MSU can play in this under-occupied niche. Neubig left his position with the University of Michigan in the spring of 2013 and came to work at MSU, bringing more than 15 years of drug discovery expertise.

"We're trying to open the door for MSU academics to use powerful drug discovery tools to attack long-term problems that don't necessarily fall into the immediate plans of the pharmaceutical industry," he said.

Neubig, president of the American Society for Pharmacology and Experimental Therapeutics, explained that pharmaceutical companies often focus on developing drugs for persisting diseases (such as heart disease) or working to improve already-existing drugs. In contrast, university researchers often nudge away at unconventional approaches that pharmaceutical companies might typically ignore.

> *"Faculty may be able to find new targets that work and push them forward in a way that pharmaceutical groups hesitate to."*
>
> *— Richard Neubig*

"By having academics explore off-beat drug targets, they reduce the risk of commercial ventures that explore a completely new approach," he said. "Faculty may be able to find new targets that work and push them forward in a way that pharmaceutical groups hesitate to."

Neubig is establishing a high-throughput screening lab at MSU that will enable researchers to quickly screen thousands of compounds and identify those that could be developed into new drug candidates.

"The faculty already has expertise in the biology — either the proteins they're working on or the biological mechanisms — so we can help them convert that understanding into an assay [an analysis used to measure the activity of a protein molecule or biological response in a cell] they can use in high-throughput screens," he explained.

Researchers will use the new lab to screen compounds for applications in many types of drug therapies, not just antibiotics. Neubig noted, however, that antibiotic drug discovery presents an interesting challenge.

"There are a number of known cell mechanisms: penicillin affects the cell wall of the bacteria; others affect the way DNA is handled inside the cell," he said. "However, many other processes are important for pathogenesis of bacteria that allow them to cause damage or survive in the host — mechanisms that are risky for commercial groups to explore but that fit in well with the academic's creative pursuit of answers to basic biological questions."

Embracing risk

Tuberculosis (TB) — caused by *Mycobacterium tuberculosis* (MTB) — is among the most common infectious diseases and frequently causes death worldwide, according to the CDC. It is estimated that one-third of the global population (2.3 billion people) is infected with latent TB, a form of the disease that has no symptoms but allows the bacteria to live in the body for decades.

In the past 40 years, only one new drug has been successfully developed to treat TB. **Robert Abramovitch**, MSU AgBioResearch microbiologist, is in the second stage of screening compounds that he hopes will lead to a new, fast-acting, affordable antibiotic treatment for TB.

"When MTB infects humans, our immune system walls off the infection by building a granuloma — a tumor — around the bacteria, which is why you seem healthy if you have latent TB," explained the MSU assistant professor of microbiology and molecular genetics.

The granuloma does not kill the bacteria; instead, the bacteria sense environmental cues and substantially slow their growth, changing the way they use and make energy to survive inside the tumor. Abramovitch believes that oxygen may play a pivotal role in this process.

"MTB needs oxygen to grow," he said. "We believe the bacteria have the ability to sense when oxygen levels around them decrease — a state known as 'hypoxia.' When they sense that the environment has become hypoxic, that's their cue to hunker down."

TB bacteria in this dormant, slow-growing state are difficult to kill with antibiotics. Additionally, current therapies require patients to take antibiotics daily for six months. Missing several doses causes patients to remain ill longer and inadvertently breeds drug resistance.

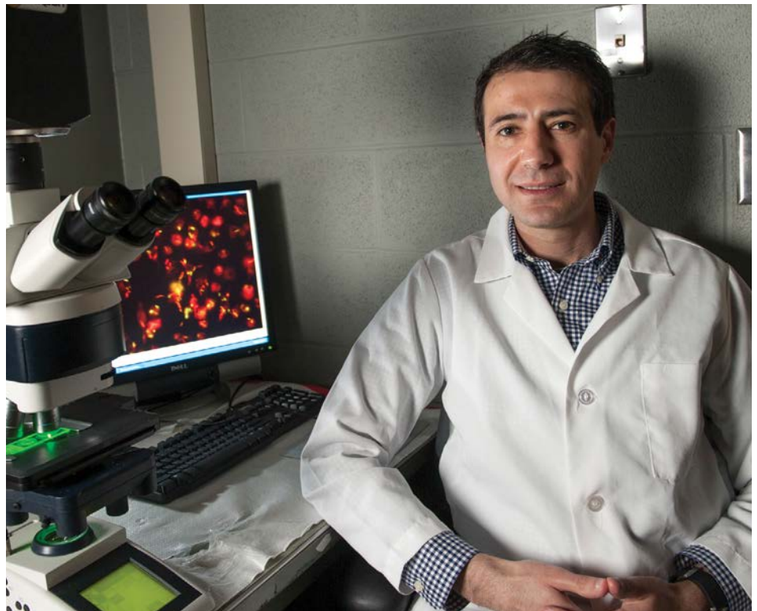
To target MTB's hypoxia-sensing ability, Abramovitch genetically engineered an MTB strain that glows green when it transitions to a dormant state. Abramovitch was recently awarded the 2014 MSU Innovation of the Year award for creating this inventive assay, which he uses to screen for compounds that turn off the glow. Obstructing this signal indicates that a compound has successfully prevented the bacteria from sensing its hypoxic environment and entering a dormant state.

"Inhibiting the ability of the bacteria to establish dormancy may shorten the course of antibiotic treatment, thus eliminating the disease more quickly and reducing the emergence of drug-resistant TB," he explained.

Abramovitch used the assay to screen 273,000 compounds and, after running many validation tests, has identified several compounds that turn off the green signal, are non-toxic to humans and work inside the cells in which TB grows. He and his lab then investigated how each compound targeted a specific pathway in the bacteria.

"That part is really exciting from the scientist's perspective because it leads you back to basic science," he explained. "By figuring out how the compounds work, we learn something about the biology of the bacterium we never knew before. It's a virtuous circle, and the hope is that basic biology will lead to some new ideas for other treatments."

Abramovitch is now focusing on identifying a lead compound to navigate through the rest of the development process. Because drug discovery is prone to failure, Abramovitch said it is not uncommon to choose one lead compound and prepare one or two other alternatives.



> *Because drug discovery is prone to failure, Abramovitch said it is not uncommon to choose one lead compound and prepare one or two other alternatives.*

LEFT: Robert Abramovitch, MSU assistant professor of microbiology and molecular genetics, is working to develop new antibiotics to treat tuberculosis, which has only seen one drug treatment option in the last 40 years. Abramovitch stresses the important role basic biological discoveries play in the process of developing new antibiotics.

"Any sort of drug discovery operation is high-risk, high-reward," he concluded. "There has been one new drug approved for TB in the past 40 years — it's a field littered with failed projects, but you still have to try. Our goal is to have something come out of the lab, but the joy of the lab is in making basic biological discoveries — that's the importance of it. You have to be able to find purpose in that process."

Exploiting weakness

On an 1831 expedition to South America and the South Seas, Charles Darwin formed the basis of his theory of evolution. He hypothesized that natural selection is the mechanism by which evolution occurs: in the competition to survive, individuals within a species adjust to their environment and perpetuate genetic qualities that enable them (and future generations) to survive. Bacteria also experience this pressure to select genetic qualities for survival.

Christopher Waters, MSU assistant professor of microbiology and molecular genetics, has been studying a genetic quality that nearly all bacteria select for: biofilm formation. It is a process in which communities of bacteria attach to a surface and secrete extracellular matrix material. The substance acts as a shield, empowering the bacteria to tolerate a range of environmental stresses including human immune defenses and antibiotic drugs.

> *"Bacteria will eventually develop resistance to any new drug. It's inevitable — but that just means we need to develop lots of compounds and use them judiciously."*
>
> *— Christopher Waters*

"Biofilms are a big problem during infection," he explained. "To bacteria, there's no distinction between humans and any other environment they're adapting to. In this particular case, our environment is trying to get rid of them, so if causing disease allows them to stay and spread, then it will be selected for."

These bacterial communities appear in a variety of chronic inflammatory and infectious diseases, including diabetic foot ulcers, cystitis, endocarditis and infections caused by medical implants. They are also responsible for the pulmonary infections that cystic fibrosis patients endure that ultimately end in lung transplants.

Waters has devoted much of his career to understand the chemical signals bacteria employ as they adapt to changing environments. In the hunt to unravel the role of chemical signaling in infection, he has stumbled upon the Achilles' heel of biofilms — and he plans to take advantage of it.

He explained that chemical signaling is imperative to synchronizing the activities of large groups of cells. Bacteria rely on a process called "quorum sensing," which enables them to monitor their environment for other bacteria through self-generated messenger molecules. When enough bacteria are present and concentrations of these messenger molecules reach a certain level, the bacteria begin to behave collectively rather than as individual cells.

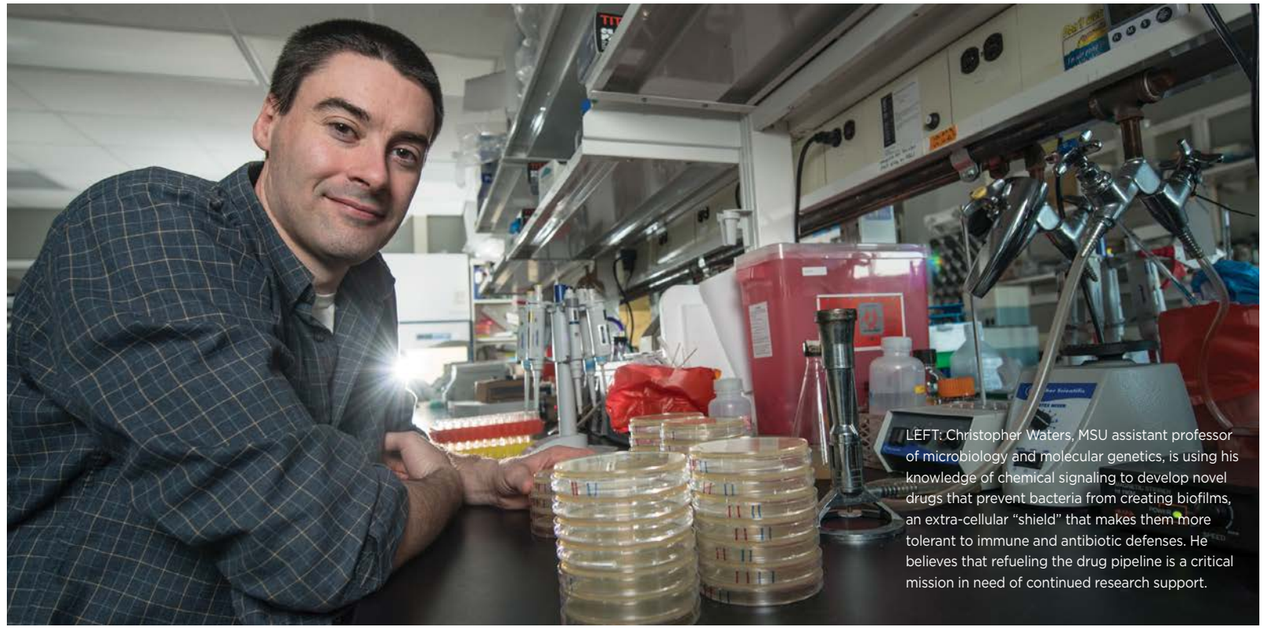
"In many species of bacteria, biofilms are controlled by quorum-sensing," Waters said. "As a postdoctoral scholar, I started studying how quorum sensing impacts biofilm formation and found that one way it works is through a messenger molecule called 'cyclic di-GMP.' This appears to be a key in bacteria switching between the biofilm state and a planktonic (or motile) state."

When bacteria are in an unattached, roving state, they are more likely to be susceptible to natural immune defenses and antibiotics.

"If we can tip the balance away from the biofilm to the planktonic state, we have a way to treat infection," he explained.

In 2009, Waters screened 66,000 compounds and identified seven that inhibited cyclic di-GMP synthesis.

"This was the first time that cyclic di-GMP inhibitors had been described," he said. "Now, we're working to optimize the compounds so they more effectively inhibit cyclic di-GMP production. We're also trying to determine if they have any efficacy in animal disease models — that's the big hurdle to getting [pharmaceutical] companies excited about your compounds."



LEFT: Christopher Waters, MSU assistant professor of microbiology and molecular genetics, is using his knowledge of chemical signaling to develop novel drugs that prevent bacteria from creating biofilms, an extra-cellular "shield" that makes them more tolerant to immune and antibiotic defenses. He believes that refueling the drug pipeline is a critical mission in need of continued research support.

Pharmaceutical compounds ready for inclusion in a second-round, high-throughput screen. Researchers routinely employ high-throughput screens to sift through thousands of drug-like compounds in large batches, enabling them to quickly identify those with promising biological or biochemical activity. These screens play a foundational role in the drug-discovery process.

Waters is also searching for compounds that increase the effectiveness of antibiotics against biofilms.

"If you treat a biofilm infection with antibiotics, you might kill 95 percent of the cells — so it works, but it doesn't kill everything because some of the cells in the biofilm are in a dormant state," he explained. "These cells are not targeted well by antibiotics, so we're also trying to find compounds that work with antibiotics to kill any remaining cells."

Waters has developed an assay and screened 6,000 compounds in an initial screen to identify this second class of compounds. He plans to follow up on the promising hits from the screen.

When asked about the possibility of reverting to a pre-antibiotic era, Waters said he thinks of a time when amputation was often the only way to survive an infection.

"That isn't too far off [from where we are today] if we lose the ability to treat infections with antibiotics," he concluded. "Bacteria will eventually develop resistance to any new drug. It's inevitable — but that just means we need to develop lots of compounds and use them judiciously. There are many academic institutions developing antibiotics, and if we can keep up the federal funding for research, we can continue to develop them and get them in the pipeline."

Leveraging favorable traits

Staving off the loss of antibiotic effectiveness will require a systems-based effort from medicinal, agricultural, governmental and public health leaders — the pressures of evolution are far too great for a solution to come from a single source. The pharmaceutical industry and research universities will play equally important roles in this endeavor. The antibiotic drug pipeline is drying up at an alarming rate because of the high costs and large investment of time involved in developing new drugs, and extremely high failure rates.

Despite these obstacles, some large pharmaceutical companies have remained committed to the process. Others are slowly making their way back to it, and small private and non-profit groups are actively pursuing prospective treatments. Still, many wonder if more can't be done.

With a strong tradition of embracing risk and the ability to garner extramural funding, researchers such as those at MSU are ideal candidates to jumpstart the flow of this drying drug pipeline.

"Academics don't necessarily start with the idea that they're going to cure disease," Neubig concluded. "They come from a place of innate curiosity: they want to understand a process or biological function. They may not be able to predict the therapeutic indications of their results, but they are well positioned to take their findings and move forward. The practical application of basic discoveries often only becomes obvious in time."

For more information:

On Neubig's efforts, visit phmtox.msu.edu.

On Abramovitch's progress, visit mmg.msu.edu/abramovitch.

On Waters' research, visit msu.edu/~watersc3.

futures
Michigan State University • AgBioResearch
SPRING/SUMMER 2014 • Volume 32, Nos. 1 & 2

ANTIBIOTIC RESISTANCE

SEEKING SOLUTIONS
in a time of
GROWING CONCERN