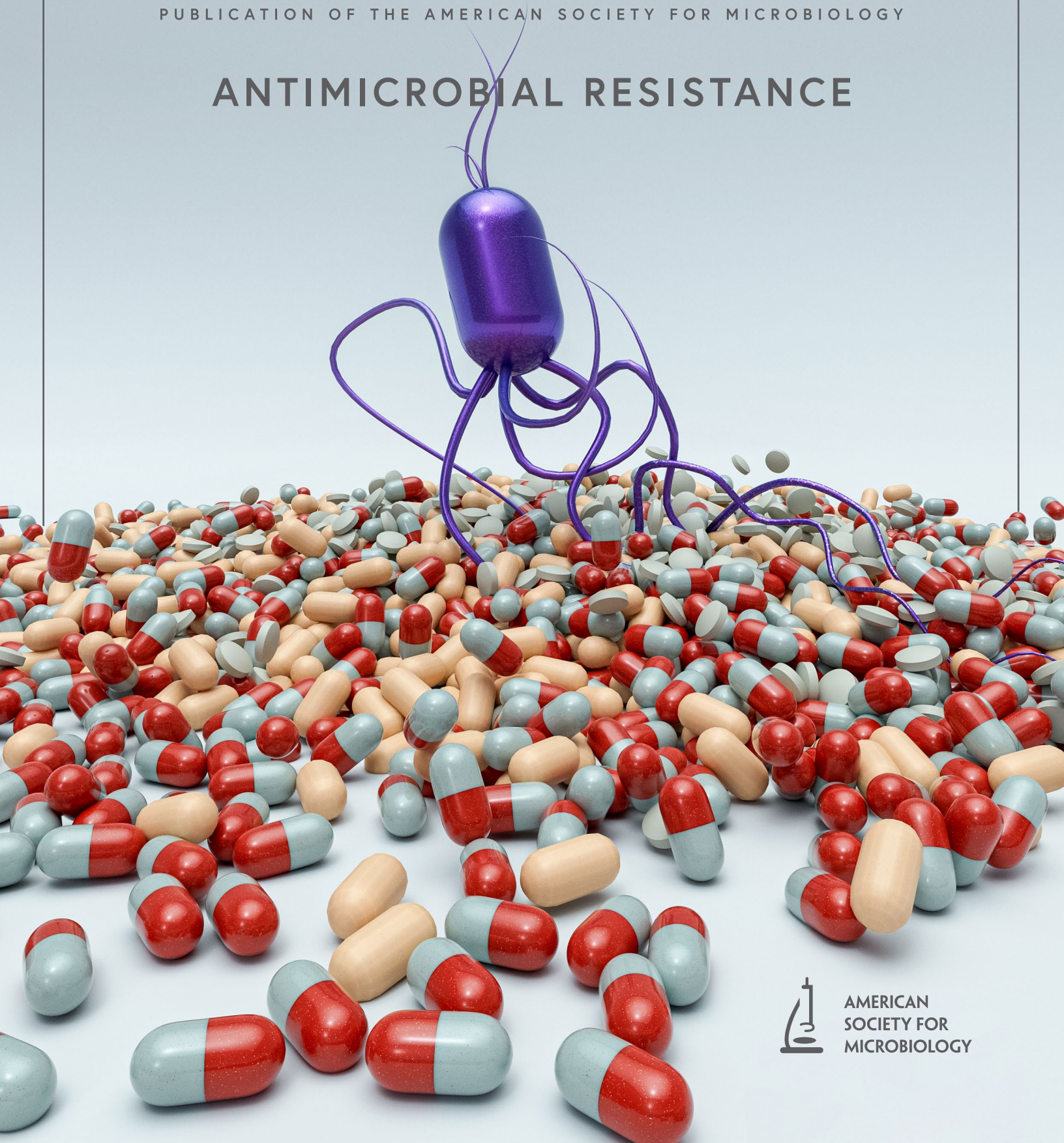


FALL 2023

# microcosm

PUBLICATION OF THE AMERICAN SOCIETY FOR MICROBIOLOGY

## ANTIMICROBIAL RESISTANCE



AMERICAN  
SOCIETY FOR  
MICROBIOLOGY

# microcosm FALL 2023

From discovering new antibiotics, to implementing policies that steward existing drugs in the antimicrobial toolbox, this issue of *Microcosm* explores antimicrobial resistance (AMR) and how to combat one of the largest threats to global public health and the environment.

## CONTENTS

- 3 LESSONS FROM A DECADE OF ANTIMICROBIAL RESISTANCE POLICY
- 6 GROWING RESISTANCE: HOW PLANT AGRICULTURE CONTRIBUTES TO AMR
- 10 UNDERSTANDING THE RISKS OF ANTIFUNGAL RESISTANCE
- 14 EXPERTS IN EAST AFRICA PROPOSE EQUITABLE AMR INTERVENTIONS
- 19 WASTEWATER AS A KEY DRIVER OF AMR
- 23 EXPLORING A ONE HEALTH APPROACH TO AMR IN PAKISTAN
- 27 FEDERALLY QUALIFIED HEALTH CENTERS LEAD AMR STEWARDSHIP
- 31 UPDATING BREAKPOINTS IN ANTIMICROBIAL SUSCEPTIBILITY TESTING
- 35 HUNTING FOR ANTIBIOTICS IN UNUSUAL AND UNCULTURABLE MICROBES
- 38 WHAT'S HOT IN THE MICROBIAL SCIENCES

## CONTRIBUTORS



**CYNTHIA ADINORTEY, PH.D., PGDE** is a senior lecturer in the Department of Molecular Biology and Biotechnology at the University of Cape Coast, where she received her doctorate in molecular biology and biotechnology.



**MADELINE BARRON, PH.D.** is the Science Communications Specialist at ASM. She obtained her Ph.D. from the University of Michigan in the Department of Microbiology and Immunology.



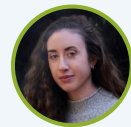
**JOHN BELL** is the Senior Communications Specialist at ASM. He moved to the ASM Science Communications team from ASM's Journals Department. He brings with him many years of experience in copyediting, proofreading and production.



**ASHLEY HAGEN, M.S.** joined the American Society for Microbiology as the Science Communications Specialist in 2020. Today, she is the Scientific and Digital Editor and host of ASM's Meet the Microbiologist and Microbial Minutes. She earned her Master's Degree in Microbiology from the University of Georgia in Athens, Ga.



**STEPHEN ORNES** is a science and medical writer who lives in Nashville, Tenn. He's also the creator and host of "Calculated," a podcast collection of stories about people at the intersection of math, art and culture. Visit him online at [stephenornes.com](http://stephenornes.com).



**LEAH POTTER, M.S.** joined the American Society for Microbiology as the Communications Specialist in 2022. Potter earned a Bachelor of Arts degree in journalism and mass communication from The George Washington University and a Master of Science degree in health systems administration from Georgetown University.



**ANDREA PRINZI, PH.D., MPH, SM(ASCP)** is an infectious disease medical science liaison and works to bridge the gap between clinical diagnostics and clinical practice.



**MUHAMMAD USMAN QAMAR, PH.D.** is an assistant professor at the Institute of Microbiology, Government College University Faisalabad.



**EMILY READY** is the Communications Program Officer at ASM. She earned her Bachelor of Arts degree in Public Relations from the University of South Carolina.

microcosm FALL 2023 ASM LEADERSHIP

MANAGING EDITOR  
ALEEA KHAN

PRESIDENT  
VIRGINIA L. MILLER, PH.D.

PRESIDENT-ELECT  
THERESA M. KOEHLER, PH.D.

SECRETARY  
ROBIN PATEL, M.D.

TREASURER  
VICTOR DIRITA, PH.D.

PRODUCTION EDITOR  
LEAH POTTER, M.S.

CEO  
STEFANO BERTUZZI, PH.D.



# Lessons From a Decade of Antimicrobial Resistance Policy

BY EMILY READY



When antimicrobial agents become less effective, it becomes more difficult—and more costly—to treat infections and control the spread of disease. Over the last decade, as the battle to respond to and combat antimicrobial resistance (AMR) has continued to shift, both U.S. and global policy have evolved with it. How has the view of the public health threat of AMR changed through a policy lens in the last 10 years, and what's next?

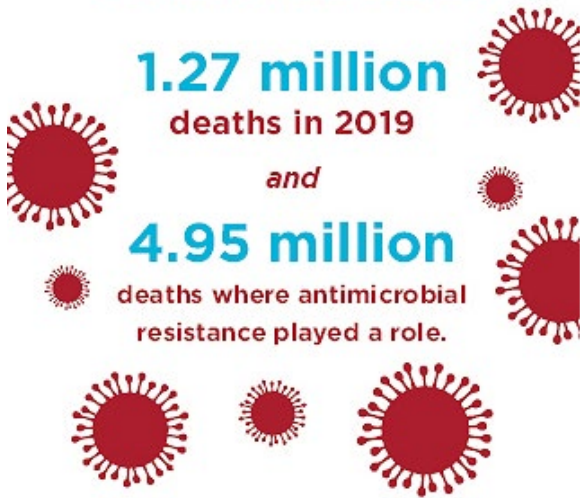
According to a 2022 Lancet study, antimicrobial resistance itself caused

1.27 million deaths in 2019

and

4.95 million

deaths where antimicrobial resistance played a role.



Source: ASM

## A DECADE OF AMR POLICY

In the U.S., Congress and federal agencies have been aware of AMR for years, but even though action has been taken, the goalposts are constantly shifting. In 2013, the U.S. Centers for Disease Control and Prevention (CDC) [released a report on antibiotic resistant threats in the U.S.](#) This report identified 3 bacteria with a threat level of "urgent" at that time: *Clostridium difficile*, Carbapenem-resistant Enterobacteriaceae and drug-resistant *Neisseria gonorrhoeae*. The CDC released [an updated report in 2019](#) in which those bacteria remained on the list of urgent-level threats, and 2 more had been added: *Candida auris* and Carbapenem-resistant *Acinetobacter*.

Note: [In 2016](#) the genus name for *C. difficile* was updated to *Clostridioides*, and [in 2020](#) Enterobacteriaceae was updated to a family within the order of Enterobacterales. The above are listed as they were written in the reports at the time of publication.

Within the 6 years between publication of the first and second reports, the White House released the [National Action Plan for Combating Antibiotic-Resistant Bacteria](#) (March 2015) with the following goals:

- Slow the emergence of resistant bacteria and prevent the spread of resistant infections.
- Strengthen [One Health](#) surveillance efforts to combat resistance.
- Advance the use and development of rapid and innovative diagnostic tests for identifying and characterizing resistant bacteria.

## AMR HAS NO BORDERS

The World Health Organization (WHO) identified AMR as 1 of the top 10 global public health threats, [estimating 10 million deaths related to AMR globally, every year, by 2050](#). It is unlikely that a new, broad-spectrum antibiotic like penicillin, which has been a reliable cornerstone of infection treatment for decades, will be discovered—so a key strategy for battling AMR is following best practices to prevent AMR transmission from the outset and reduce the need for more powerful antimicrobials. Issues that contribute to the challenge of addressing AMR include:

- Over-prescription of antimicrobials.
- Patients not completing prescribed antimicrobial courses.
- Antimicrobial overuse in livestock, fish and [crop farming](#).
- Limited discovery of new antimicrobials.

Though domestic policy relating to AMR has been part of the legislative discussion for a long time, it is increasingly apparent that international collaboration and development of global policy is needed to adequately combat AMR.

- Accelerate basic and applied research and development for new antibiotics, vaccines and other therapeutics.
- Improve international collaboration and capacity for antibiotic resistance prevention, surveillance, control and antibiotic research and development.

The U.S. government also contributed to the [global action plan on AMR](#) endorsed by the WHO World Health Assembly in May 2015, with focus on the following 5 objectives:

- Improving awareness and understanding of AMR.
- Strengthening the knowledge and evidence base.
- Optimizing the use of antimicrobials in human and animal health.
- Using effective sanitation and hygiene measures to prevent infection.
- Developing an economic case for investment that accounts for global needs—increasing investment in new medicines, diagnostic tools and vaccines.

Domestic and global policy, as well as government agency efforts, to address AMR quickly ramped up as it became evident that the threat of AMR was rapidly evolving. In 2019, Congress passed the Pandemic and All Hazards Preparedness and Advancing Innovation Act. The bill [contained language championed by ASM](#), which codified the Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria ([PACCARB](#)), an advisory committee of outside experts that had originally been established in 2014 by [Executive Order 13676](#).

The National Action Plan for Combating Antibiotic-Resistant Bacteria [was updated in October 2020](#), building on the framework from 2015 to prioritize infection prevention and control and reduce the need for antibiotic use. The updated plan supported expanding activities that were shown to slow the spread of AMR, such as improving antibiotic stewardship in hospital and outpatient settings and engaging the animal health and crop protection communities to advance strategies for fostering responsible use of medically important antibiotics. The plan emphasized the interconnected threat of AMR to humans, animals and the environment. It also underscored that AMR is not an issue that can be addressed simply through domestic action in the U.S. but requires international and multisectoral collaboration between public and private entities.

### PROGRESS INTERRUPTED: COVID-19

That same year would bring unprecedented challenges and impacts on the fight against AMR due to the COVID-19 pandemic. The 2019 antibiotic resistance threat report from the CDC highlighted prevention as the most crucial tool to protect against and prevent the spread of antimicrobial resistant infections. However, as health care facilities, health departments and communities grappled with the challenges of COVID-19, there was an increased use of antimicrobials and lower adherence to best practices for infection prevention and control. Moreover, public health resources had to shift from tracking antimicrobial resistance to tracking COVID-19. [The CDC later released a report in 2022](#) analyzing the impacts and setbacks that the COVID-19 pandemic made to the fight against AMR, including increased prescription of antimicrobials that weren't necessary for treatment and lapses in antimicrobial resistance tracking. The report underlined the imperative need to recover from those setbacks to continue to protect human and animal health.

### WHERE ARE WE NOW?

The work to fight AMR continues, with the convening of the 118th Congress on January 3, 2023. Key pieces of legislation on AMR include the [Pioneering Antimicrobial Subscriptions to End Upsurging Resistance \(PASTEUR\) Act](#), reintroduced to Congress in May 2023 by Sen. Sherrod Brown, which would promote new antibiotic development and appropriate use of existing antibiotics to limit the increase and spread of AMR. The Strategies to Address Antibiotic Resistance (STAAR) Act, which has yet to be reintroduced, would strengthen the country's capability to address AMR outbreaks quickly, while bolstering antibiotic stewardship models to preserve their effectiveness against existing pathogen threats.

ASM released a report of policy recommendations in July 2023, [Policy Pathways to Combat the Global Crisis of AMR](#), that underlined the need for a multidimensional approach to facing a problem like AMR and the crucial position of microbiologists as part of the solution. The recommendations assert the need for proper antimicrobial stewardship, harmonization of new domestic antimicrobials and antimicrobial alternatives, such as [phage therapy](#), [microbiome therapeutics](#) and vaccines.

Read the AMR Policy Paper



Rep. Buddy Carter of Georgia joins ASM members and staff at the breakfast reception during ASM's AMR-focused Hill Day on September 28, 2023. Source: ASM.

ASM is a leader in advocating for science and evidence-based policy and recognizes the essential role of microbiology in addressing public health issues, including AMR. ASM members and staff spent an entire day on Capitol Hill in September 2023, meeting with 50 Congressional offices to discuss the global public health threat of AMR.

"The expertise of microbiologists is crucial to the development of policy surrounding the mounting threat of antimicrobial resistance," said [ASM past president Robin Patel, M.D.](#), who participated in ASM's Hill Day in September "Meeting our members of Congress face to face, and bringing expertise directly to them to show the impact that policy has on the fight against AMR, is important to ensure the health of all people living in the United States."

When microbiologists meet with legislators, they can explain how legislation directly impacts their research and work and contribute their first-hand scientific experience to the development of sound policy. ASM is [actively tracking and contributing to the policy development surrounding AMR](#) and continues to work with members of Congress and their staff members, government agencies and ASM members to develop key public policies that address the challenges of AMR.

[Learn About ASM Advocacy](#)

# Growing Resistance: How Plant Agriculture Contributes to AMR

BY STEPHEN ORNES

If you tell Anuradha Chowdhary, Ph.D., to eat an apple a day, she's likely to tell you about pathogenic, drug-resistant strains of the fungus *Candida auris* that might be lingering on the surface. "An apple is great," she might say, "but be sure you wash it first."

Apples might not seem like the most obvious reservoir for pathogenic microbes that don't respond to most of today's potent antimicrobials. But at the same time, according to Chowdhary, a mycologist at the [V.P. Chest Institute in Delhi, India](#), it's not surprising. "We're looking at sources in the environment because everything is connected," she explained. "Fruit is 1 example."

Research points to practices in plant agriculture and crop production that may help pathogenic, resistant microbes reach people. According to Chowdhary, who studies the molecular ecology of pathogenic fungi, it's a global problem for which developing countries may have fewer resources, and it's an avenue of transmission that's often overlooked. But we neglect it at our peril: research focusing exclusively on human health and transmission in places like hospitals and other health care settings only captures a cross-section of the larger, interconnected system by which antimicrobial resistance (AMR) moves through the world.



Practices in plant agriculture and crop production may assist pathogenic, resistant microbes in reaching humans and animals. Source: iStock.

## FRUITS, FUNGUS AND FARMS

Between March 2020 and September 2021, [Chowdhary worked with microbiologist Jianping Xu](#), Ph.D., at the McMaster University in Hamilton, Ontario, to look for *C. auris* in ordinary places. The research group visited markets and orchards in Delhi and across northern India. In total, they collected 84 fruits, all grown on trees. Those included 62 apples, 20 of which had been picked from orchards, and 42 of which had been purchased from a market. Some of the orchards used organic farming methods; others didn't. The researchers tested samples from the surfaces of the fruits and found fungal species on all of them. These findings were reported in *mBio* in March 2022.

On 8 of the 62 apples—representing 13% of the sample—they found pathogenic, drug-resistant strains of *C. auris*. This fungus can cause severe infections and, because it colonizes the skin, spreads easily, especially in health care settings. If those strains manage to infect people, they can be dangerous. In an analysis of 192 hospitalizations in the U.S. related to *C. auris*, [researchers from the U.S. Centers for Disease Control and Prevention \(CDC\) found a mortality rate of 34%](#).

"When we think about AMR, most of us think about the consequences in terms of health, in particular for humans and animals," said [Jorge Pinto Ferreira, D.V.M., Ph.D.](#), food safety officer at the Food and Agriculture Organization of the United Nations in Rome. "And for the good and the bad, the global dimension of a health issue is often evaluated by the number of human deaths it causes." But that is not the whole picture.



Researchers found that 13% of their apple samples contained pathogenic, drug-resistant strains of *C. auris*. Source: iStock.



Resistant bacteria have many ways to get to the soil if they aren't already there: manure used as fertilizer, organic soil additives, the water used in agriculture, the seeds themselves and the workers who plant and harvest the crops. Source: iStock.

The intersection of AMR and plant production affects the food chain and human health around the planet, and experts argue that the best solutions to addressing the spread of AMR will require a wide view. "The focus must be on the global nature of the problem," Ferreira said. "It is more than well-known and recognized today how interconnected everything is."

### FROM SOIL TO SALAD: OPPORTUNITIES FOR RESISTANCE

Plants begin with a seed in the soil, and to really understand how agricultural processes for growing plants can contribute to the spread of AMR, researchers are studying resistant genes and microbes that aren't pathogenic to humans. "Antibiotic resistant bacteria associated with plants in the greatest numbers are not human pathogens, but rather commensal bacteria often associated with food spoilage," said [Karl Matthews, Ph.D., a food microbiologist at Rutgers University](#) in New Brunswick, N.J.

By studying how these microbes spread, researchers can better see how AMR moves through the food cycle. "Recognizing that non-pathogenic bacteria associated with food crops can be antibiotic-resistant is essential," he said. His research has 2 main arms: developing plant-derived antimicrobials and understanding how the use of antibiotics in crop production facilitates the spread of AMR.

Resistant bacteria, pathogenic or not, have plenty of places to live, including in and around the soil. They also have many ways to get to the soil if they aren't there in the first place—e.g., the seeds themselves and the workers who plant them, as well as organic soil additives, water that is used in agriculture and manures that are used as fertilizer.

For example, antibiotics are regularly added to livestock feed to prevent or treat bacterial infections, and waste from those animals can be used as fertilizer. [A review by an international collaboration of researchers](#) connected the use of fertilizers, including cattle manure, chicken manure, swine manure and sewage sludge, to the presence of antibiotics—tetracyclines and fluoroquinolones—and corresponding antibiotic resistance genes in the soil.

Microbes (and those corresponding resistance genes) that are introduced to the soil via such agricultural practices can then hitch a ride on the plants in the surrounding environment. [A study published in July 2021](#) connected the dots: researchers from the University of Nebraska-Lincoln found that the use of animal manure directly contributed resistant microbes and resistance genes to the soil. In fact, altered soil composition accounted for more than 60% of the AMR genes found on the outside of leaves of lettuce that grew in the sampled soil.

In addition, plants might be treated with antibiotics themselves. Similar to the way antimicrobials have led to major gains in health care by turning previously fatal infections into treatable, survivable ones, they've also led to healthier, more productive crops. For example, tetracyclines, a family of antibiotics used to treat bacterial infections in humans, are also injected into tree trunks to prevent diseases like huanglongbing (HLB) disease. They are also applied to pears and apples to prevent fire blight.

After harvest, the food might be treated with antibacterials or antifungals for packaging and shipping, which both reduces the bacterial population and offers an opportunity for resistance to persist. Azoles are potent drugs used to treat human infections by *Aspergillus* fungi; they're also the most-used antifungals in agriculture, applied to fruit to prevent the growth of mold during shipping and storage. Some researchers worry that the use of azoles in agriculture may be making matters worse by killing off

susceptible fungi and leaving resistant strains to flourish. Importantly, the CDC has reported that [those infected with azole-resistant strains of \*Aspergillus\* are up to 33% more likely to die from the infection](#) than those infected with non-resistant strains.

Furthermore, according to Ferreira, plasmids, which are tiny loops of DNA that can carry antibiotic-resistant genes, have been identified in both plant and human pathogens. As a result, harmless microbes that live on the plants may develop resistance, and then pass that resistance on—likely through horizontal gene transfer—to neighboring, pathogenic microorganisms that could transfer resistance to humans and animals. Fruits and vegetables eaten raw and unwashed offer a potential opportunity for resistant microbes and genes to enter the human gut, and [some studies](#) hint at the possibility of gene transfer to the existing gut bacteria.

Researchers hope the growing understanding of how resistance travels through the plant food cycle will inform tomorrow's strategies for lowering risk.

The increasing menace of antimicrobial resistance has led researchers around the world to track how AMR factors into the food cycle, crop production and farming practices. "It is an issue that goes clearly beyond health," Ferreira said. "Food safety, food security, food production, one way or another, all rely in higher or lower degrees on the use of antimicrobials."

At the local, national and even international levels, food safety experts have begun to think deeply about stewardship, sketching out a set of recommendations about best practices and methods for reducing the spread of AMR, and the risks associated with it. "Ultimately, it has to be a global effort," Ferreira said. "Microbes do not recognize boundaries."

### LOOKING TO ONE HEALTH FOR BEST PRACTICES

Experts point to the management of antibiotics in agriculture as an opportunity to use transdisciplinary approaches—including [those in the One Health concept](#)—to stem the spread of resistance.

By trying other methods before turning to antibiotics, farmers could promote the growth of healthy crops without disrupting the ecosystem. For example, integrated pest management uses knowledge about threats to crops and involves strategies like rotating crops or identifying those weeds and pests that require control. It also includes recommendations for how to keep antimicrobials to a minimum, and how to minimize contamination and risk to people and animals. Recent studies also suggest bacteriophages may be useful in eliminating harmful, resistant bacteria.

Of course, antimicrobial stewardship won't be easy. The use and misuse of antibiotics in medicine and livestock is well-documented, but in horticulture and plant agriculture the impact and spread of resistance are less well-studied. Plus, regulations vary by region and country. [Brazil does not allow antibiotics to be used in pesticides; neither does the European Union](#). Other countries limit their use to certain crops, while still others have no legislation at all. "These countries [without legislation] could be disproportionately affected by AMR because the use of antimicrobials is not monitored, and regulations are either minimal or not enforced," Ferreira said.

Matthews, at Rutgers, concurred that closer monitoring of the agriculture production environment could help stem the problem. "Measures at the field or processing levels will contribute to reducing antimicrobial-resistant bacteria to crops, but some of these measures may not be implementable in countries with limited resources," he said. Matthews also noted that education could help. Greater awareness that unwashed, uncooked fruits and vegetables could harbor pathogenic, resistant microbes "must be achieved," he emphasized.



Experts emphasize the importance of a One Health approach, which brings together considerations of humans, animals and the environment. Source: iStock.



Experts agree that what will likely be most beneficial is a global, transdisciplinary, One Health approach, which brings together considerations of humans, animals and the environment at the local, national and even international levels.

"We need to be realistic, and to be aware that there is no 1 simple solution that will magically make the AMR issue disappear in a fast way," said Ferreira. Still, recent findings that elucidate the movement of microbes through plant agriculture suggest a way forward—supporting science-based practices that can be translated at the local level (e.g., informing farmers of the dangers of the overuse of antimicrobials). But everyone involved in farming—from farmers to governments—will need to support international efforts to curb the risk to human and animal health.

"Global strategies for stewardship focus on the development of integrated, sustainable agrifood systems," Ferreira said. "And on the implementation of the idea that antimicrobials are global common goods that we are all responsible for."

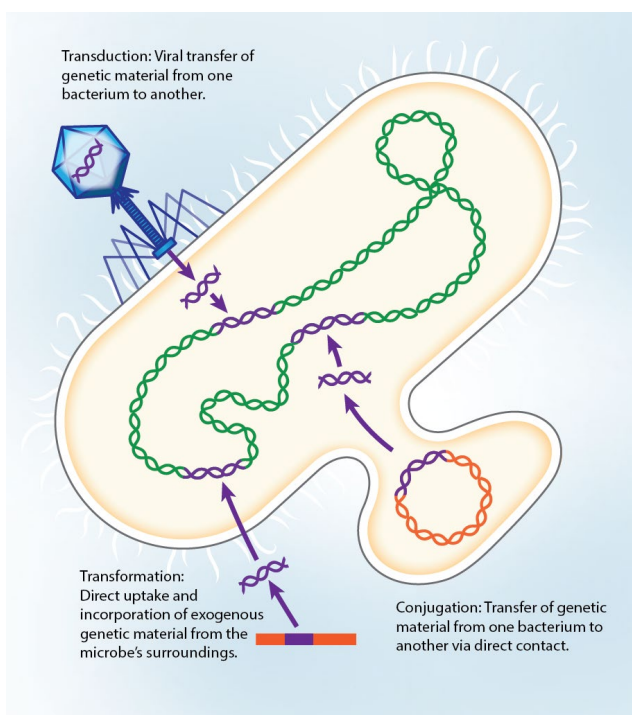
[Read the USDA's Reflection on a Decade of AMR Response](#)

# Understanding the Risk of Antifungal Resistance

BY ASHLEY HAGEN, M.S.

Invasive fungal infections, though less frequently encountered than bacterial and viral infections, can be devastating, particularly in immunocompromised patients. The rise of antifungal resistant pathogens, like *Candida auris* and *Aspergillus fumigatus*, has only heightened this concern. But how common is antifungal resistance? Who is at risk? What pathogens are of particular concern, and how significant is the overall threat?

ASM's "Meet the Microbiologist" podcast breaks down the topic of antifungal resistance with [Gary Procop, Ph.D.](#), CEO of the American Board of Pathology and professor of pathology at the Cleveland Clinic, and [Shawn Lockhart, Ph.D.](#), Senior Clinical Laboratory Advisor in the Mycotic Diseases Branch at the U.S. Centers for Disease Control and Prevention (CDC).



## HOW COMMON IS ANTIFUNGAL RESISTANCE?

According to Procop, antifungal resistance is very different from antibacterial resistance. "I mean, these really are apples and oranges," Procop explained. The reason? Fungi are eukaryotic organisms that possess relatively stable genomes compared to their bacterial counterparts. When it comes to the acquisition of antimicrobial resistance (AMR), that stability makes a difference.

Prokaryotic organisms are considered to be highly promiscuous in the exchange of genetic material—taking advantage of methods of [horizontal gene transfer](#). Although some fungi can, and do, exchange genetic material, the relative stability that eukaryotes possess, compared to prokaryotes, makes the acquisition and development of antifungal resistance rarer than that of antibiotic resistance.

"We did a study years ago, looking at *Cryptococcus*, and CDC did a similar study. We both had the same finding," said Procop.

In this study, [scientists looked at 3 different cryptococcal isolates from 3 different areas](#) of the globe—the U.S., Thailand and Malawi. The sites differed in antifungal use in the following manner:

1. One site required a prescription for patients to acquire antifungal drugs.
2. One site distributed antifungals over the counter.
3. One site fell somewhere in the middle—antifungals were not as readily available as over the counter, but also not overly difficult to obtain.

Importantly, despite these differences in antifungal stewardship practices, there was no significant difference in *Cryptococcus* resistance to a slew of antifungals (amphotericin B, fluconazole, itraconazole, 5-flucytosine and ketoconazole) among isolates collected from the 3 locations.

"We did that same study when ESBL, extended spectrum beta lactamases, were just coming out in gram-negatives," Procop said. This time, researchers evaluated antimicrobial susceptibility of gram-positive cocci and gram-negative bacillus in bloodstream isolates collected from 5 hospitals in Cairo, Egypt where, at the time (1999-2000), antibiotics were available over the counter. The team found significant differences in susceptibility profiles compared to samples collected in locations where a prescription was required for antibiotic use. "Of course, where there were more antibiotics, there was more resistance," Procop summarized.

He explained that this knowledge logically shifts his focus in the clinical laboratory from antifungal susceptibility testing to fungal identification. "We will often say, 'If you can get to the identification, you've got some good information about the likelihood of responses to certain drugs.' In my mind, anyway, identification becomes priority to antifungal susceptibility testing."

Still, he acknowledged that this doesn't hold true for all organisms. The profiles of some fungi (i.e., *Fusarium*), are much harder to predict and that's where the [CLSI Antifungal Susceptibility Subcommittee](#) comes into play. CLSI repeatedly tests rare fungi to determine accurate breakpoint information if/when sufficient clinical trial data does not exist. Essentially, the group identifies the normal distribution of rare fungi, with respect to minimal inhibitory concentrations (MICs), against different antifungals and publishes the resulting epidemiologic cut off values (ECVs).

"From that, you can kind of see how these fungi are probably going to act," Procop explained, adding that the CLSI has also developed lists of fungal groups that possess intrinsic resistance. "For example, you know that *Canada krusei* is intrinsically resistant to fluconazole. So, I think knowing the intrinsic resistance profile is very important. Then, if you have an identification, that can be used to help guide therapy."



## WHO IS AT RISK OF CONTRACTING INVASIVE FUNGAL INFECTIONS?

Both Procop and Lockhart emphasized that, although invasive fungal infections are rarer than bacterial and/or viral infections, and the development of antifungal resistance is less frequent, fungal infections can be devastating, especially in vulnerable populations (e.g., people with weakened immune systems or who are immunocompromised, those who have experienced stem cell or organ transplant and those who have cancer or are undergoing chemotherapy). It is a fact that provides a new metric for evaluating the severity of the threat of antifungal resistance.

"Normally, healthy patients don't get fungal infections—at least fungal infections that are beyond the superficial. But that makes them especially disturbing, because the people who are susceptible to fungal infections are the people who already have serious underlying conditions, and that makes them less likely to survive a fungal infection," explained Lockhart.

This vulnerability may be compounded by delays in diagnoses, due to the simple fact that fungi tend to be considered after viral and bacterial candidates have been ruled out in the differential diagnosis. If any type of drug resistance is added to the equation, the unrestrained pathogen wreaks havoc.

"At that point, a lot of times it can be too late," said Lockhart, whose clinical microbiology fellowship included a distinct emphasis on antifungal resistance.

## WHAT FUNGI ARE OF PARTICULAR CONCERN FOR ANTIFUNGAL RESISTANCE?

Lockhart pointed out that, relatively speaking, acquired antifungal resistance is rare, even in *Candida* (~ 7%). Yet, *C. auris*, *Candida parapsilosis* and *A. fumigatus* are 3 fungal pathogens that have "broken the mold" on antifungal resistance.



### CANDIDA AURIS

"What we're seeing now is this rise in a brand-new bug called *C. auris* that very easily acquires resistance. We've even seen pan-resistance in *C. auris*, which is something we've never seen or experienced before. And yet, here it is, all of a sudden. It's literally spreading across the world," Lockhart said, adding that new states where *C. auris* is detected in the U.S. are being reported each year.

*C. auris* carries a high mortality rate, [killing more than 1 in 3 people with infections](#), and has demonstrated [pan-drug resistance to at least 4 classes of antifungal agents](#). According to the CDC, about 90% of U.S. isolates are resistant to fluconazole, 30% are resistant to amphotericin B and >5% are resistant to echinocandins.

*C. auris* is typically found in hospital or health care settings and is capable of causing hospital acquired infections in immunocompromised people. When asked about the course of infection, Lockhart explained that *C. auris* follows an outside-in phenomenon. "Most of these patients have multiple central lines—they often have feeding tubes and they're often on ventilators. You have all these devices that go from the outside of the patient to the inside of the patient that are just providing portals for *C. auris* to get to the inside." According to Lockhart, 5-10% of patients who become colonized with *C. auris* go on to develop bloodstream infections, which can be life-threatening.

### CANDIDA PARAPSILOSIS

Resistant *C. parapsilosis* is another *Candida* species that is raising alarm bells. "It tends to be single clones that rise in a geographic area and spread hospital-to-hospital. We don't know how or why yet, but that's something that we have an interest in," Lockhart explained.

*C. parapsilosis* typically functions as a skin and gut commensal organism, but once again, in hospital settings and immunocompromised patients it may follow an outside-in course of infection that leads to bloodstream and/or internal organ infection. Still, according to the Cleveland Clinic, [less than 0.01% of people get invasive candidiasis](#) of any type each year.

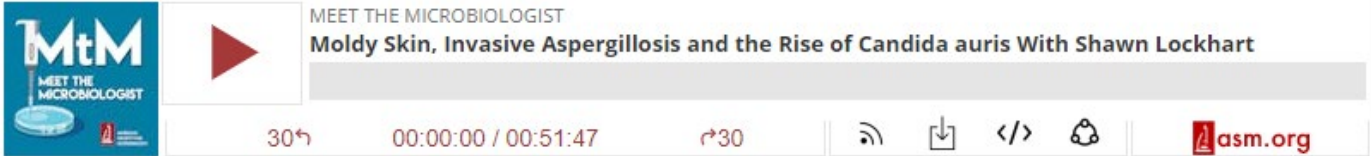
### ASPERGILLUS FUMIGATUS

Importantly, not all antifungal resistance is experienced (or spread) in the hospital. "The other bit of acquired resistance that we've never seen before is azole-resistant *A. fumigatus*, and that's caused by the use of azole antifungals or fungicides that are sprayed on agricultural fields," Lockhart stated.

Today, the U.S. alone is using 4 times as much fungicide as it was using just 5 years ago, and as these fungicides continue to be sprayed, the repeated exposure ultimately selects for resistant *A. fumigatus* clones over susceptible ones. Furthermore, according to Lockhart, *A. fumigatus* is everywhere, and there doesn't appear to be a fitness cost to the organism carrying azole resistance.

"We catch *Aspergillus* from the environment," he explained. "The spores are literally all over us. You can stomp on your carpet in front of you right now, and I guarantee you will be spreading *A. fumigatus* spores—they're just ubiquitous. And so, we're going to see more and more patients that show up at the hospital with an already resistant case of pulmonary aspergillosis."

Should *A. fumigatus* spores be inhaled, the innate immune system of healthy individuals is usually effective at warding off infection. However, aspergillosis may become localized or invasive in more vulnerable populations. Similar to the examples of candidiasis mentioned above, symptoms (coughing up blood, fever and chills, headaches, chest pain, shortness of breath, among others) largely depend on the organs that are infected, and the mortality rate of proven invasive pulmonary aspergillosis in immunocompromised patients [is very high \(between 40-90%\)](#).



### THE TAKEAWAY: ANTIFUNGAL RESISTANCE IS A GROWING THREAT THAT MUST BE MANAGED

While antifungal resistant infections remain significantly overshadowed by antibacterial infections in terms of frequency, the severity of disease experienced by immunocompromised patients with invasive fungal infections presents a confounding factor when evaluating the threat of antifungal resistance. Early diagnosis can help, but use of antifungals in the environment and in health care settings must be properly managed in order to protect vulnerable populations and retain the strong arsenal of antifungal classes that exist.

[Read More About Combating Antifungal Resistance](#)

# Experts in East Africa Propose Equitable AMR Interventions

BY LEAH POTTER, M.S.

The [effects of antimicrobial resistance \(AMR\), such as fewer treatment options for bacterial and fungal infections, disproportionately impact under-resourced countries.](#) This is often [compounded with a higher burden of disease and systemic health inequities in some regions.](#) Considering this convergence, a One Health approach that incorporates policy support, workforce development and effective laboratory capacity-building is critical to mitigating the impact and spread of this global public health threat. A collaborative and informed strategy means meeting communities where they are, advancing sustainable interventions and listening to local microbiologists and health care professionals.

"We need to improve coordination across the globe, including providing scientists the opportunity to share their data to advance microbiology and bolster health equity," said [Wes Kim, Ph.D., Director of ASM's Global Public Health Programs \(GPHP\).](#) "It's not just about creating equity for the sake of it; it's also about learning from local experts."

Here, experts from Ethiopia, Mozambique and Tanzania—countries in East Africa that face similar health inequities and observe many of the same drug-resistant microbes in their communities—offer advice and expertise on combating AMR. They emphasize the need for equitable and accessible health care resources and sustained laboratory and workforce development.



A lab technician in Maputo, Mozambique at INS National TB Reference Lab applies new skills acquired from ASM's tuberculosis training. Source: American Society for Microbiology.

## HOW HEALTH INEQUITIES IMPACT AMR

The health care landscape in East Africa is shaped by a complex interplay of socio-economic, geographical and systemic factors. For any location, [health inequities are often created because of the unequal distribution of health resources.](#) For countries in East Africa—like Ethiopia, Mozambique and Tanzania—rural communities have limited access to health centers and diagnostic facilities, let alone affordable care options, which can lead to worsened health outcomes.

"Access to care is uneven, with variations in what is available at different levels of health care services and a need to pay for services," [Mtebe Majigo, M.D., MMed, a senior lecturer of microbiology and immunology at Muhimbili University of Health and Allied Sciences in Tanzania](#), said. "These variations drive health choices for providers and patients and may affect the effort to combat AMR."

With the steep cost of visiting a health care provider to treat an infection, many patients self-diagnose their suspected infection and opt to go directly to a pharmacist. In Ethiopia, for example, [it is common to obtain an antimicrobial without a prescription from a health care provider](#).

"There is currently no standard restriction," explained Surafel Fentaw, former ASM local consultant and lab lead for the Ethiopian Public Health Institute's (EPHI) national bacteriology and mycology reference laboratory (NRL) in Addis Ababa and regional sentinel laboratories in Adama and Bahir-Dar. "If I needed [or asked for] something, the pharmacy would just dispense it." The assumption is that self-diagnosing will ultimately lead to reduced health care costs (i.e., skipping the physician's office means the only cost is the pharmaceutical, which can be quite expensive on its own). However, this practice can have long-term negative effects. If the patient has incorrectly identified their infection, their condition will not only stay the same or worsen, but taking an antimicrobial they don't need could further contribute to the spread of AMR. Even if the patient's self-assessment is correct, the drug they are given might not be the most effective choice, and they might not be provided guidelines on the appropriate length of treatment.

Similar findings have emerged throughout communities in [Tanzania](#) and [Mozambique](#), where, even with guidelines that stipulate antimicrobials should not be dispensed without a provider's prescription, drugs are often provided at the patient's request. One [study conducted in Maputo City, Mozambique](#) described how this practice puts pharmacists "between a rock and a hard place"—

helping patients that cannot access care means bypassing regulations, and ignoring guidelines could mean poor health outcomes in the future.

For patients who can visit health facilities, gender and age bias may affect the likelihood of antimicrobial misuse and/or overuse. "[Complications related to AMR] seem to favor certain groups based on their health-seeking behavior," said [Agricola Joachim, M.D., MMed, Ph.D., a professor in the School of Diagnostic Medicine at Muhimbili University of Health and Allied Sciences](#), noting that children and people assigned female at birth visit health facilities more frequently than people assigned male at birth.



A patient in Mozambique stands outside Nampula Central Hospital. Source: American Society for Microbiology.

## MICROBES ON THE WATCH LIST

While dozens of microbes are identified on "watch lists" for being resistant to antimicrobials, global health and microbiology experts in Tanzania, Ethiopia and Mozambique cited 11 pathogens as priority candidates for AMR surveillance.

Several culprits of health care associated-infections (HAIs) were noted, including [Acinetobacter baumannii](#), [Klebsiella pneumoniae](#), [Pseudomonas aeruginosa](#) (especially for its role in ventilator-associated pneumonia) and [Staphylococcus aureus](#) (a common cause of skin and blood infections).

Pathogens impacting the intestinal tract (and leading to acute diarrheal disease) include [Escherichia coli](#), [Salmonella spp.](#) and [Shigella spp.](#) [Enterococcus spp.](#), which is commonly associated with urinary tract infections (UTIs), and [Neisseria gonorrhoeae](#), which causes the sexually transmitted infection (STI) gonorrhea, have also been flagged.

Lastly, [Neisseria meningitidis](#), the causative agent of meningitidis, and [Streptococcus pneumoniae](#), which causes community-acquired pneumonia, can be resistant to 1 or more antimicrobials.

With high-profile microbes identified, what's next? Local experts advocate for sustainable efforts to monitor these microbial foes, equitably diagnose and treat patients and enhance best practice guidelines for AMR stewardship.

## OPPORTUNITIES AND STRATEGIES TO ADDRESS THE GLOBAL SPREAD OF AMR

Strategies to combat AMR require collaborative and complimentary efforts that span multiple sectors. Microbiology experts based in East Africa provided the following recommendations:

- Improve awareness and education about AMR and its impacts, both in and outside of clinical settings (e.g., via community groups, journalists, health care providers, university students, social media, awareness campaigns, clinical training sessions). "At the health care provider level, there is awareness about AMR mainly in tertiary hospitals," said Geremew Tasew, Ph.D. the Director of Bacterial, Parasitic and Zoonotic Diseases Research Directorate at the Ethiopian Public Health Institute. "However, the practice still lags and needs more effort, mainly on the overuse and misuse of antibiotics. At the community level, there still needs to be more awareness interventions."
- Disseminate clear guidelines for both health care professionals and patients regarding appropriate antimicrobial use.
- Bolster current infection prevention and control (IPC) and stewardship programs in health care facilities.
- Increase access to quality lab supplies and infrastructure and introduce guidelines and policies for AMR reporting programs. "There needs to be more confidence among clinicians, as the laboratory often needs more quality supplies, which can contribute to inconclusive patient test results," said [Isabel Pinto, Ph.D., Director of the National Directorate of Medical Assistance \(DNAM\) at the Mozambique Ministry of Health.](#)
- Improve access to health care resources, especially for under-resourced communities.
- Develop human-animal surveillance systems that support a [One Health approach](#) for addressing AMR.
- Improve access to clean water, which will in turn lead to better "sanitation and hygiene for both humans and animals," said [Sofia Omar Viegas, M.Sc. Ph.D., Deputy Director-General of Public Health Laboratories for the National Institute of Health of Mozambique.](#)
- Strengthen guidance on antimicrobial use for livestock. "The irrational use of antibiotics in animals for treatment, growth promotion and disease prevention [contributes to AMR]," said Erick Komba, the Director General of Tanzania Livestock Research Institute (TALIRI). "Several studies have shown that there are high amounts of residues (e.g., tetracycline) in milk, cattle meat, broiler chickens and eggs."

## ASM'S GLOBAL PUBLIC HEALTH PROGRAMS (GPHP)

With opportunities to improve stewardship, health care access and laboratory capacity in mind, ASM is committed to cross-disciplinary collaboration with local experts and community leaders, sustainable funding efforts and implementing [One Health-focused programs](#) from concept to practice. "Part of advancing equity is getting everyone on board in terms of stewardship," Kim, ASM's GPHP Director, emphasized.



ASM GPHP Director Wes Kim receives a certificate of appreciation for support in combating AMR in Tanzania. Source: American Society for Microbiology.





Surafel Fentaw supporting ASM's AST training for EPHI's NRL staff. Source: American Society for Microbiology.



EPHI-ASM-CDC kickoff collaborative for AMR surveillance support in Ethiopia. Source: American Society for Microbiology.



A lab technician in Mozambique tracks test results at Nampula Central Hospital. Source: American Society for Microbiology.

## ETHIOPIA

Since 2017, ASM has been instrumental in establishing the AMR surveillance network in Ethiopia. ASM local experts and consultants aided the Ethiopian Public Health Institute (EPHI) in developing the 2016-2020 AMR Surveillance Plan and supported basic microbiology diagnostics and AMR training at the national and regional levels. This training equipped staff with the knowledge and skills to sustain the surveillance system and routine microbiology lab services.

Through structured mentoring and training, ASM worked with EPHI's national bacteriology and mycology reference laboratory (NRL) in Addis Ababa and regional sentinel laboratories in Adama and Bahir-Dar to improve quality management. All 3 laboratories achieved 5-star status (the highest level) in the Stepwise Laboratory Quality Improvement Process Towards Accreditation (SLIPTA), contributing to the national accreditation of EPHI's NRL in 2017. The staff members who were trained expressed that the ASM mentoring support made them feel more confident in their work and allowed them to continue teaching and instructing others in best practices in the lab. ASM's training materials also helped them focus on improving quality assurance and competency building.

"The support of ASM is not only time and technical support provided, but they are always there for us," Amete Mihret Teshale, a microbiologist at EPHI's NRL and former ASM Young Ambassador of Science, said. "Time will not be enough to thank ASM for all their contributions and support they have given to our profession, and I shall stop here by saying that ASM is the backbone of microbiology in Ethiopia."

To support the establishment of continuing education and professional development, ASM initiated the [Extension Community Healthcare Outcome \(ECHO\) program in the region](#), which promoted regular interaction and real-time exchange of information between the NRL and the sentinel sites and enabled the program's continuity under EPHI's responsibility. ASM also empowered trained microbiologists to take concrete steps toward improving scientific knowledge by facilitating international scientific exchange programs, allowing Ethiopian staff to gain international experiences, including encouragement to submit abstracts and present their work at the [ASM Microbe](#), ASM's flagship annual conference.

## MOZAMBIQUE

Between 2010-2015, ASM worked alongside local experts to improve tuberculosis (TB) surveillance laboratories in Mozambique. After 3 years of ASM-sustained training and mentoring efforts to the TB National Reference Lab (NTRL) in Maputo, the lab became the first to hold [ISO 15189 international accreditation](#). For patients, laboratory accreditation ensures that the test results are delivered quickly and accurately, which ensures proper diagnosis and treatment of disease. For the broader community and health system, accreditation provides a guarantee of quality. This accreditation engenders confidence that the data collected is reliable. In the following years, from 2015-2020, ASM helped TB regional reference labs in Beira and Nampula meet important laboratory milestones, with Nampula obtaining ISO 15189 accreditation in 2020.

"[ASM's] support [helped create] a foundation [and a] culture of quality in all lab activities performed that will be implemented in all public health emergencies," said Viegas at the National Institute of Health of Mozambique.

Through its local experts, ASM also focused on delivering customized microbiology training to enhance antibiotic susceptibility testing in Mozambique. The training further developed clinical laboratory quality control and external quality assurance systems by equipping over 100 lab technicians across all 9 country provinces. As a result, antibiotic susceptibility testing was decentralized to reach Mozambique's geographic regions, contributing to the country's response to AMR and COVID-19 testing.

"With the support received, it was possible to train technicians and elaborate on guidelines for microbiological techniques, as well as [standardize testing procedures] and the acquisition of means and procedures," Pinto of DNAM at the Mozambique Ministry of Health said. "We were also able to design a strategy of revitalization of the area of microbiology."

## TANZANIA

Since 2009, ASM has supported microbiology laboratory diagnostic services in Tanzania. This commitment extends to 11 regional laboratories, enhancing their capabilities and fostering local expertise in laboratory mentorship. ASM also supported laboratories in Tanzania in collecting and reporting animal surveillance data to global databases, which had not previously been done in the region.

More recently, through [the Fleming Fund](#), ASM is enabling the execution of Tanzania's National AMR Surveillance Framework. This involves building laboratory capacity to generate top-tier microbiological data for patient care and to facilitate surveillance efforts across human and animal domains.

"These initiatives have greatly impacted combating AMR by improving the quality of laboratory service and the ability to generate AMR data to inform policy," Majigo of [Muhimbili University of Health and Allied Sciences](#) said.



ASM staff members attended Tanzania's 3rd National AMR Symposium, "Preventing AMR Together." Source: American Society for Microbiology.

## MAINTAINING THE MOMENTUM

The weight of addressing AMR should not be on a single profession, country or sector. Effectively combating AMR will require a collaborative, sustained global effort that draws from local expertise and community needs. Local experts asserted that beyond writing and enforcing antimicrobial stewardship guidelines, access to laboratory diagnostics and health centers must improve so that a patient does not have to choose between a diagnosis or treatment.

Donate to ASM's Global Public Health Programs

# Wastewater As a Key Driver of AMR

BY CYNTHIA A. ADINORTEY, PH.D.

**W**astewater—[used water](#) resulting from rainwater runoff and human activities—serves as a dynamic vehicle for the spread of antimicrobial resistant microbes, antimicrobial drug residues and antimicrobial resistance (AMR) genes in the environment. [High concentrations of AMR genes and AMR organisms](#) have been detected in environmental samples recovered from hospital and urban-treated wastewaters.

The spread of AMR in the environment represents an immense threat to the survival of living organisms, including humans. This is, in part, because antimicrobial drug residues have ecotoxicological effects on the environment, thereby contributing to loss of microbial diversity, pollution and waste generation. Consequently, AMR has been recognized by the [United Nations General Assembly](#) and the World Health Organization (WHO) as [a global threat requiring urgent attention](#). This calls for pragmatic measures to be taken against this menace through the implementation of sustainable interventions.



Environmental discharge of wastewater. Source: U.S. Department of Agriculture.

## HOW DOES WASTEWATER CONTRIBUTE TO AMR?

Wastewater is typically categorized based on the way it is produced (e.g., domestic, industrial, hospital or rainwater runoff). It is a common feature among [5 main pollutant sources](#) that play a major role in the emergence, transmission and dispersal of AMR in the environment.

1. **Sewage:** Antimicrobial drug residues excreted in feces are released in the environment through improper waste disposal, inadequate wastewater management and poor sanitation. These minimal doses of antimicrobials exert selective pressures on microbial communities in domestic and industrial drainage systems, rivers, seawater and the soil. Consequently, sewage and [effluent](#) (liquid waste) from septic tanks, as well as sewage treatment plants, have been found to contribute significantly to AMR pollution in the environment.
2. **Pharmaceutical ingredients:** Pharmaceutical companies contribute to effluent and produce waste that can contain active pharmaceutical ingredients. [These active ingredients have been detected in the environment](#) at concentrations that could potentially increase the abundance of resistant microbes and AMR genes.

3. **Health care facilities:** Effluent and waste from health care facilities can [contain concentrations of AMR genes and microbes](#) up to 10 times higher than wastewater from surrounding communities.
4. **Crop treatment:** The use of antibiotics and fungicides—as well as manure resulting from inadequately treated or untreated wastewater—in crop production could introduce antimicrobial drug residues and resistant microbes into the environment. Additionally, the use of soil fertilizers resulting from [dried, digested, activated sludge](#) may cause antibiotic contamination in surface runoff, groundwater and drainage networks.
5. **Livestock farming:** In a quest to ensure livestock health and productivity, [intensive terrestrial and aquatic animal production systems often use antimicrobial drugs](#). Yet, the wrongful use of antimicrobial drugs in animal husbandry for growth promotion and disease prevention, instead of the recommended sole use in the treatment of infected animals, greatly contributes to the rapid emergence of AMR. A high percentage of these antimicrobial drugs are excreted through urine and feces, which are subsequently released into the environment either directly or indirectly through wastewater.

### THE SPREAD OF AMR IN WASTEWATER: WHY IS IT CONCERNING?

As a result of human activities, coupled with poor sanitation, sub-therapeutic doses of antimicrobial drugs and disinfectants accumulate in the environment over time. Even though the concentrations of antimicrobial drugs in effluent is too low to be lethal to the exposed microbes, it may be enough to promote the emergence and spread (via vertical or [horizontal gene transfer](#)) of microbes with mutations that confer resistance to the corresponding drugs.

While vertical gene transfer occurs when genes are passed from 1 generation to another, horizontal gene transfer facilitates the acquisition of AMR genes by non-resistant microbes from other microbes that are not necessarily genetically related. The resulting resistant microbes may then be transmitted to (and between) humans and animals. For example, human exposure to AMR from the environment can take place following [consumption of food and/or water](#) that have become contaminated by resistant microorganisms and can [lead to human infections](#).

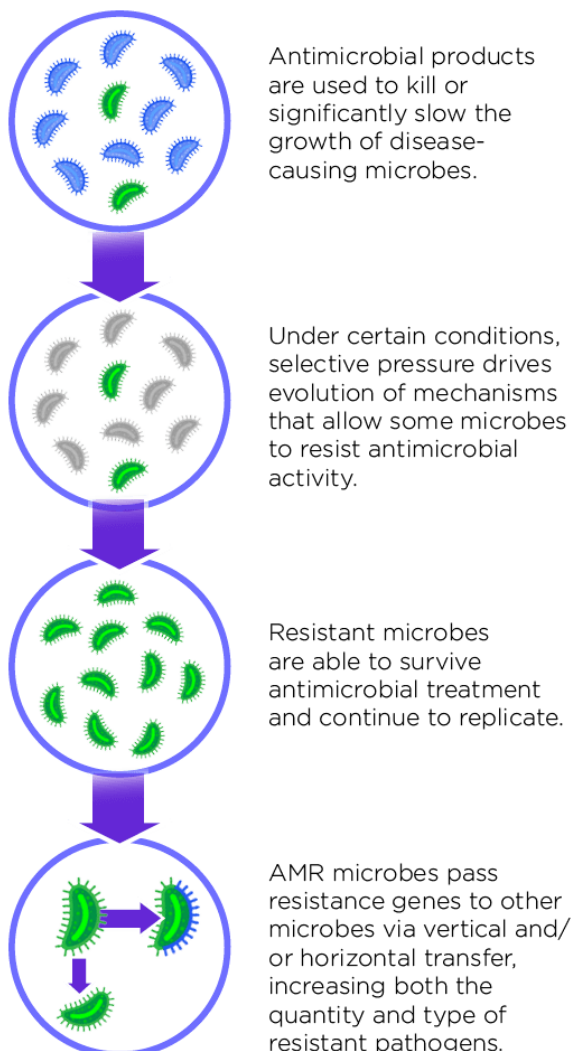
The introduction of antimicrobial drugs to the environment via disposal practices that contaminate waterbodies and soil varies from country to country. In countries with developed sewer networks, antibiotics used in clinical settings and large-scale farms end up in collected wastewater, which is treated before being discharged into the environment.

However, even though conventional methods of wastewater treatment kill live microbes, they usually do not eliminate antimicrobial drug residues or pieces of environmental DNA, including AMR genes. Rather, following treatment, most antimicrobial drugs are reduced to their individual molecular units via hydrolysis. This is usually observed for residues of commonly used antibiotics, like beta-lactams, sulphonamides, tetracyclines and quinolones. These individual molecular units accumulate over time in the environment, thereby increasing the exposure of environmental microbes to these traces and contributing to the emergence of AMR.

Inadequate treatment of wastewater exacerbates the spread



### Development of Antimicrobial Resistance (AMR)



The development of AMR. Source: ASM.

of AMR. In many [under-resourced countries](#), less than 10% of wastewater is adequately treated. In fact, on a global scale, [over 56% of domestic and industrial wastewater](#) is released into the environment with little or no treatment. Furthermore, untreated or inadequately treated wastewater is a potential source of toxic compounds and can contain high levels of nutrients, like phosphorus and nitrogen, which can cause [eutrophication](#). The health of freshwater and marine organisms is also at risk if direct or indirect discharge of untreated wastewater makes its way into streams and oceans. Yet, [untreated or inadequately treated wastewater](#) is often used for irrigation purposes and greatly contributes to water and food security in developing countries.

Considering the complex composition of wastewater, and the need to safeguard aquatic ecosystems, it becomes important that wastewater is treated before it is released into the environment. When wastewater management is enhanced, the health of agricultural workers will be improved, as the risk of pathogen exposure and chemical contaminants will also be reduced.

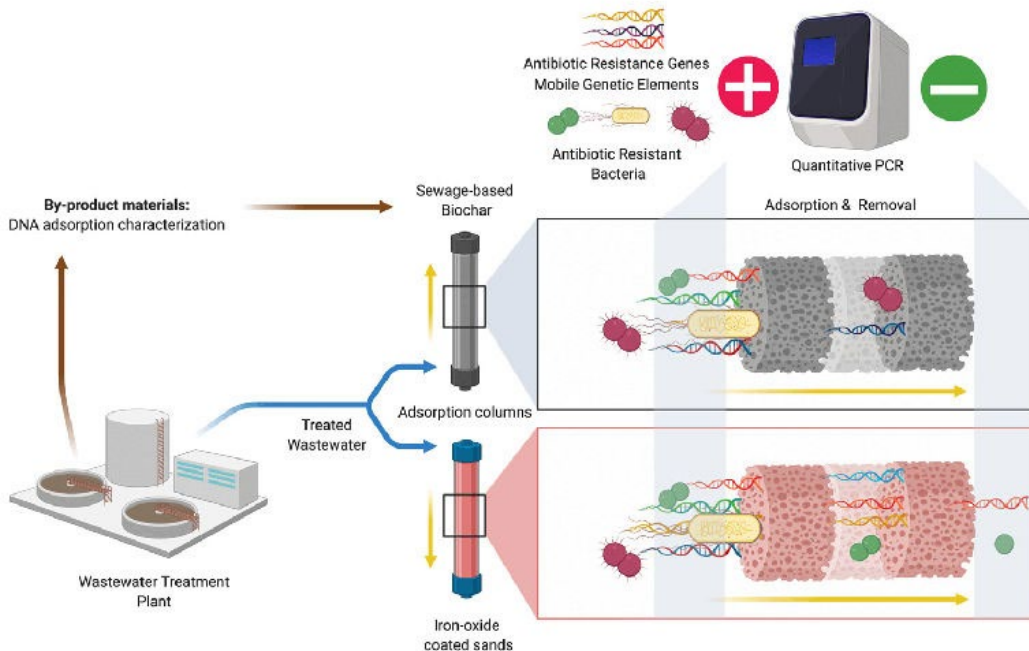
### HOW CAN THE ENVIRONMENTAL SPREAD OF AMR BE CURBED SUSTAINABLY?

[Millions of lives across the globe will be at risk from AMR by 2050](#). The [One Health](#) approach, which is a collaborative strategy encompassing environmental, animal and human health, is crucial to curtail the rise in the emergence of AMR. Everyone in society has an important role to play in ensuring that AMR is combated in a sustainable manner.

Deliberate efforts to ensure that antimicrobial drugs are used responsibly, can reduce the concentration of antimicrobial drugs that end up in wastewater in the first place. Still, given the global status of AMR, further action is needed. The [removal of pathogenic microbes—as well as drug residues and AMR genes](#)—must be a focus in the [treatment of wastewater](#).



The spread of AMR in the environment. Source: UNEP, 2020.



The removal of AMR genes from wastewater can be achieved via nucleic acid adsorption using various materials. Source: Calderón-Franco, et al., *Science of the Total Environment*, 2021 under a CC BY-NC-ND 4.0 DEED license.

## WHAT'S NEXT? APPLICATIONS IN AMR GENE REMOVAL

Given that conventional wastewater treatment methods are essential but ineffective against the complete removal of AMR genes, innovative, cost-effective and environmentally friendly techniques are being developed. One such [intervention is based on the adsorption for nucleic acids](#) during wastewater treatment. Adsorption is a separation method whereby total environmental DNA, including AMR genes and mobile genetic elements, fuse iron oxide-coated sand and are removed from treated wastewater.

There are various nucleic acid-adsorbing materials that have been studied and applied to target AMR reduction from wastewater, including [bio-based adsorbents](#) like [biochar](#) (the lightweight black product resulting from the burning of any organic matter, including sewage sludge), iron oxide-coated reclaimed sand and modified eggshells. For example, iron oxide-coated sand and sewage sludge biochar were respectively reported to [retain 54%-85% of antibiotic resistant genes](#), mobile genetic elements and free-floating extracellular DNA from wastewater. In other studies, high adsorption capacity and inhibition of AMR genes has also been reported for non-biobased adsorbents, such as graphene oxides and ceric oxide (CeO<sub>2</sub>).

Despite the promising nature of these methods in the removal of antimicrobial resistant genes from wastewater, a number of critical factors including material toxicity, cost-effectiveness, affordability, leaching property and scalability have been identified that are worthy of consideration. Consequently, the search for biological and cost-effective materials that would efficiently remove AMR genes from wastewater continues.

[Learn More About How Microbes Help Us Reclaim Our Wastewater](#)

# Exploring a One Health Approach to AMR in Pakistan

BY MUHAMMAD USMAN QAMAR, PH.D.

**A**ntimicrobial resistance (AMR) is a major public health concern worldwide. Bacterial resistance to antibiotics can lead to ineffective medical treatment, often requiring the use of more toxic medications and resulting in increased treatment failure and mortality. These complications are already having a severe impact. [A 2019 report](#) indicated that nearly 5 million deaths worldwide were associated with drug-resistant bacteria, with almost 1/4 of these deaths (1.27 million) being directly attributed to AMR. Importantly, AMR-related deaths are not distributed equally around the globe: nearly 90% occurred in under-resourced countries.

This means [global efforts to counter AMR will only be successful if they can be applied across all countries](#), regardless of economic status. This article will use the situation in Pakistan (an under-resourced country) as a case study, examining the current impact of AMR in Pakistan, discussing previous plans to combat AMR and highlighting examples of how the challenge of AMR is being addressed in the country. The hope is that these examples will provide key insights into strategies that can be spread worldwide, including a One Health approach.



In Pakistan, where AMR has led to tens of thousands of deaths annually, efforts to fight antimicrobial resistance could offer insights into strategies that could be used worldwide. Source: iStock.

## THE STATUS OF AMR IN PAKISTAN

[Pakistan ranks 176th out of 204 nations](#) in AMR-related mortality per 100,000 people. In 2019, the Global Research on Antimicrobial Resistance Project [reported 59,200 deaths in Pakistan directly attributable to AMR](#), with an additional 221,300 deaths listed as AMR-associated. AMR was therefore the [third-leading cause of death for Pakistanis in 2019](#), behind only cardiovascular disease and maternal/neonatal disorders.

The majority of AMR-related deaths in Pakistan are caused by a select group of bacteria, including *Salmonella enterica*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and carbapenem-resistant Enterobacterales and non-Enterobacterales, that appear on the [World Health Organization's \(WHO\) list of "priority pathogens."](#) The growing threat represented by these deadly microbes is tangible. In 2016, a report out of Hyderabad in Pakistan highlighted the [first case of extensively drug-resistant \*Salmonella enterica\*](#) serotype Typhi (XDR typhoid). This XDR strain was resistant to ampicillin, cotrimoxazole, quinolone, chloramphenicol and third generation cephalosporins.

Yet, while these are the most prominent, they certainly are not the only drug-resistant microbes in Pakistan. New Delhi metallo- $\beta$ -lactamase-1 (NDM-1) is a  $\beta$ -lactam enzyme that provides resistance to several antibiotics, such as carbapenems, which are broad-class antibiotics that are often used against drug-resistant bacteria. [First identified in 2008](#), the NDM-1 gene has subsequently [been found in pediatric and neonatal septicemia patients in Pakistan](#).

*mcr-1* is a plasmid-encoded gene that imparts resistance to polymyxin E (colistin), which is considered a last resort antibiotic for carbapenem-resistant bacteria infections. After first being identified in animals and humans in China in 2016, the *mcr-1* gene was soon found in Pakistan, [first in a clinical isolate of \*Escherichia coli\*](#), and followed shortly thereafter in [human clinical samples](#).

## THE CHALLENGES OF COMBATING AMR IN PAKISTAN

Several factors raise challenges to addressing AMR in the country. [With a population of 241 million people](#), Pakistan is the world's fifth most-populated country and has a [doctor-to-patient ratio of approximately 1:1300](#); by comparison, [the doctor-to-patient ratio in China is approximately 1:400](#).



The low doctor-to-patient ratio contributes to low health literacy and a lack of patient education about AMR. Source: iStock.

Such a low doctor-to-patient ratio contributes to low health literacy and a lack of patient education about AMR. Overall, [around 63% of the population in Pakistan is literate](#). In addition, there is a [low awareness among medical experts in Pakistan of effective treatments for resistant organisms](#).

Economics presents another challenge. Pakistan is one of the world's poorest countries. As of 2022, [per capita income was measured at \\$6,836](#), ranking 135th out of 204 countries. This means that the average person living in Pakistan cannot afford the cost of a doctor's appointment.

## OVERUSE AND MISUSE OF ANTIBIOTICS

The overall result of these combined factors is a high prevalence of misuse and overuse of antibiotics, 2 main drivers for the development of AMR in bacteria. [Physicians administer antibiotics to 70% of patients](#). This prescribing pattern is particularly prevalent among general physicians in public sector hospitals, [who tend to prescribe more than 3 drugs per patient](#).

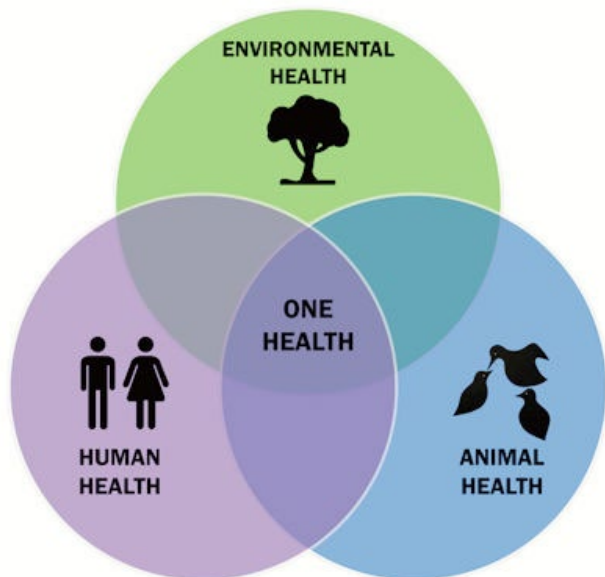
In addition, [more than 60% of the population in Pakistan self-medicates](#), given the prevalence of unlicensed pharmacies. Companies in Pakistan currently possess 647 operational licenses for drug production from the Drug Regulatory Authority of Pakistan (DRAP). However, none of these licensed production units have obtained approval from the United States Food and Drug Administration (FDA). By contrast, neighboring Bangladesh has [5 pharmaceutical plants approved by the FDA, while India has around 200](#).

Pharmacists in Pakistan are also able to provide antibiotics, often without a physician's prescription. Patients therefore have easy over-the-counter access to "Watch" (azithromycin, ceftriaxone, ciprofloxacin, meropenem and vancomycin) and "Reserve" (ceftazidime/avibactam, colistin, Fosfomycin, linezolid and polymyxin B) antibiotics.

The use of antimicrobials in livestock production, known as antimicrobial utilization (AMU), also contributes to the dissemination of AMR. Nearly [70% of antibiotics worldwide are used in livestock](#) to prevent sickness, encourage growth and increase feed production. One study found that AMU by growers in Pakistan for broiler chickens alone was [greater than every country in the world other than China](#), reaching nearly 568 tons annually.



## SUCCESS STORIES OF ONE HEALTH APPROACHES IN PAKISTAN



One Health is an interdisciplinary concept that recognizes the relationship between human health, animal health and environmental health. Source: Wikimedia Commons.

Despite these challenges, Pakistan is making significant progress toward addressing and combating AMR. Like other nations, Pakistan is emphasizing a [One Health approach to AMR](#). One Health is an interdisciplinary concept that recognizes the relationship between human health, animal health and environmental health.

The [Pak One Health Alliance \(POHA\)](#), a non-governmental organization, promotes One Health strategies, policies and interventions across the country. POHA regularly conducts meetings and workshops to [raise public awareness about One Health-related issues](#) and the emergence and re-emergence of infectious diseases in Pakistan, working with stakeholders like ASM.

Additionally, the U.S. National Academy of Sciences, Engineering and Medicine (NASEM), in partnership with the Pakistan Academy of Sciences, has established a [fellowship program to build the capacity of early- and mid-career scientists in Pakistan](#) in One Health research. The program also encourages collaboration and cooperation in preparedness and response to zoonotic diseases and other shared environmental health risks.

In 2017, Pakistan released its [National Action Plan on AMR \(NAP\)](#), testifying to the nation's firm commitment to battling this global crisis. The NAP included initiatives for health care, agriculture and the environment to combat AMR. It also aimed to promote sensible antibiotic usage, strengthen surveillance and improve infection prevention and control across health care settings in the country.

Antibiotic production has also improved. In 2018, the moxifloxacin tablets manufactured by Getz Pharma Pvt., Ltd. attained the distinction of being the first-ever pharmaceutical product from Pakistan to [receive prequalification by the WHO](#).

The [National Institutes of Health, Pakistan](#) has also played a crucial role. The implementation of the [Global Antimicrobial Surveillance System](#) in 2016 led to the establishment of the [Pakistan AMR Surveillance System](#) in 2018, which has provided a strong platform for monitoring and controlling AMR. Moreover, Pakistan's efforts extend to initiatives such as the [AMR Tricycle program](#) and [point prevalence surveys on antimicrobial consumption](#).

Pakistan is engaged in various global initiatives, including developing a microbiology laboratories database for microbiologists and a data sharing portal for the Pakistan AMR Surveillance System. Whole-genome sequencing is utilized to monitor antimicrobial resistance, and resources like the ["Microbiology and Antimicrobial Resistance" Virtual Journal Club](#) hosted by the NIH, Pakistan are available for health care workers, early to mid-career microbiologists and infection disease control experts.

## FUTURE PROSPECTS

Pakistan's ongoing efforts and initiatives demonstrate the country's collaborative approach toward addressing AMR, both at the national and international levels. The NAP-AMR is a comprehensive plan that addresses future strategies and regulatory challenges related to AMR, using a One Health method through its core objectives of the Global Action Plan.

To effectively implement this plan, it is necessary to have ongoing stakeholder participation from politicians, policymakers, pharmaceutical industries, agriculture sectors, civil society, media and international organizations, like the WHO, the [World Organization for Animal Health](#) and the [United Nations Food and Agriculture Organization](#). Dedicated funding from the government of Pakistan and international collaborators is also required for specified AMR reduction efforts.

Coordination between central and provincial governments within Pakistan is crucial for achieving the aims and outcomes of the NAP on AMR. Furthermore, pharmaceutical companies, agriculture sectors and non-governmental organizations should collaborate to achieve the desired outcomes. Health care personnel should also support antimicrobial stewardship and infection prevention and control programs. Measures to limit the self-purchasing of antibiotics and the excessive use of broad-spectrum antibiotics at the community and hospital levels are expected to lower overall consumption.

My research has focused on AMR for the past 15 years, leading to the publication of more than 75 scholarly papers on the subject. My current research is focused on the surveillance and investigation of the molecular mechanisms that lead to the development of antimicrobial resistance in pathogens, within the context of the One Health approach. I am also studying the patterns of antibiotic usage in both human and veterinary sectors, determining antimicrobial residue in food-producing animals, examining how AMR impacts hospital-acquired infections (HAIs) and investigating bloodstream infections in children. Furthermore, my research group is actively involved in exploring new therapeutic options, such as phages, nanoparticles, synthetic compounds and natural products.

By continuing to undertake these efforts, Pakistan can provide an example for the rest of the world to follow in the fight against AMR.

[Read More About One Health](#)

# Federally Qualified Health Centers Lead AMR Stewardship

BY LEAH POTTER, M.S.

With [approximately 60% of total antibiotic use in the U.S. associated with outpatient settings](#), and millions of antibiotic prescriptions written annually, it has become crucial to address the issue of overuse and misuse of antimicrobials both within and outside the walls of health systems. In fact, of the over 200 million antimicrobial prescriptions written each year in the U.S., [almost 30% are considered unnecessary, and 50% are considered inappropriate](#) (i.e., the dosage, duration or selection of the pharmaceutical is considered unfitting).

As outpatient settings are responsible for a substantial portion of antimicrobial usage, health centers are turning their sights to stewardship programs to promote responsible prescribing practices, enhance medication safety and, ultimately, mitigate the impending concern of antimicrobial resistance (AMR). While the adoption of such programming has been slow across various health systems, [Federally Qualified Health Centers \(FQHCs\)](#), which serve historically marginalized populations, are well-positioned to contribute to equitable care while leading stewardship efforts.



Implementing outpatient and inpatient antimicrobial stewardship programs requires multidisciplinary teams, support from leadership and sustained educational efforts. Source: iStock.

## GUIDING FRAMEWORK FOR ANTIMICROBIAL STEWARDSHIP PROGRAMS

To tackle health-care related AMR challenges, the Centers for Disease Control and Prevention (CDC) released the report, [Core Elements of Outpatient Antibiotic Stewardship](#), in 2016. These core elements provide a framework for outpatient practices to focus on 4 key pillars:

1. Commitment.
2. Action for policy and practice.
3. Tracking and reporting.
4. Education and expertise.

The target audiences for these core elements include primary care clinics, retail health clinics, urgent care facilities, emergency departments and dental offices.



Of the over 200 million antimicrobial prescriptions written each year in the U.S., 30% are considered unnecessary and 50% are considered inappropriate. Source: Flickr.

Even with the CDC's framework, the adoption of outpatient stewardship programs has been slow. To address this, [The Joint Commission](#) introduced [new standards for ambulatory health care in 2019](#) to encourage accredited organizations to establish outpatient stewardship programs. These standards emphasize the importance of identifying stewardship leaders, implementing evidence-based practice guidelines, providing necessary resources and collecting data related to stewardship goals.

Acknowledging the longstanding inequities that impact patient populations affected by AMR (with [overlap between patients served by FQHCs and those who are disproportionately impacted by AMR](#)), health care professionals and advocates of stewardship programming are turning their focus to FQHCs, which aim to provide affordable care to historically underserved patient populations.

## HOW FQHCs CAN SUPPORT MULTIDISCIPLINARY STEWARDSHIP TEAMS

FQHCs [serve over 30 million patients annually, with nearly 1,400 centers](#) across the U.S. These centers play a pivotal role in reducing health disparities. "FQHCs are uniquely positioned to provide equitable care to underserved patients, with the aim of promoting health equity," said Kierra Wilson, Pharm.D., BCPS, AAHIVP, pharmacy clinical coordinator at Chase Brexton Health Care, a 501(c)(3) nonprofit medical center based in Maryland.

However, implementing stewardship programs in FQHCs comes with several challenges, including funding constraints, staffing shortages, limited access to diagnostic tools and high patient volume. Despite these limitations, Wilson noted several emerging strengths for these health centers. The overall medical landscape of FQHCs has changed over the past decade, with expanded onsite services (e.g., pharmacy, dental, behavioral health, case management) and an increasing emphasis on comprehensive care. Wilson emphasized that, with these robust clinical offerings, it is essential for stewardship teams to be multidisciplinary.

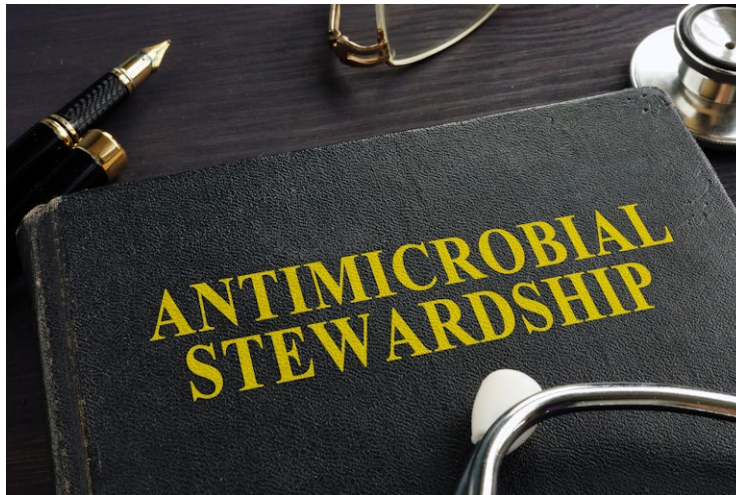
"Stewardship programs warrant multidisciplinary collaboration in order to be successful, and we need that in terms of allowing us to be able to optimize the resources that we provide, allowing for more people at the table and more diverse perspectives, and to allow for interprofessional communication," Wilson explained. "[Multidisciplinary teams have been shown to \[improve\] management and outcomes](#) for patients with many chronic diseases. And that involves medical providers—not just physicians, but also your advanced practice providers, pharmacy and nursing staff. Each of us has a unique perspective."

When it comes to antimicrobial stewardship involvement, Wilson said there are 2 groups, in particular, that should be included: pharmacy staff and nursing staff. Between 2016 and 2020, [FQHC pharmacy staff increased from 21.8% to 25.2%](#). The number of advanced practice providers, like nurse practitioners and physician assistants, has also grown in recent years, with [health experts noting their heightened importance as physician and nursing shortages loom](#). Both pharmacy staff and advanced practice providers are often responsible for a significant portion of antimicrobial prescribing, so why aren't they always included in antimicrobial stewardship efforts? Wilson offers 1 possible answer: a lack of AMR-related education and training.

## IMPLEMENTING STEWARDSHIP EDUCATION

Educational initiatives for FQHC staff members that are not one-off occurrences, but rather sustained year-round, are critical for stewardship programs, Wilson said. This training should include staff from a variety of clinical and nonclinical teams, emphasizing the desired multidisciplinary approach. Wilson offered several recommendations for educational programming during a [scientific session](#) at [ASM Microbe 2023](#):

1. Focus on the health system's commitment to antimicrobial stewardship, emphasizing why this work is important to public health. "[You can] disseminate that information throughout your organization to show that you are a champion of stewardship," Wilson said.
2. Collaborate with marketing teams to develop educational materials and posters for providers and patients.
3. Explain antimicrobial selection and appropriateness, with an emphasis on infection control and patient monitoring.
4. Outline roles and responsibilities in antimicrobial management for a variety of positions within the health system.
5. Discuss health inequities and social determinants of health, and how this relates to antimicrobial stewardship and public health outcomes.
6. When applicable, collaborate with information technology (IT) teams to provide guidance on electronic health record (EHR) use when making prescription decisions.

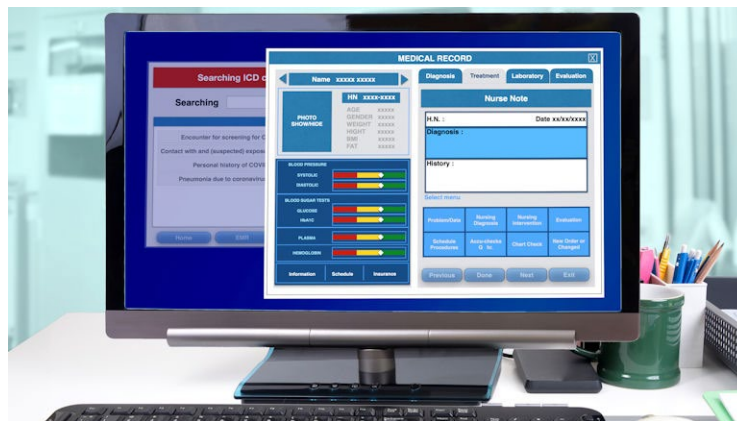


Antimicrobial stewardship education should include staff from across a variety of clinical and nonclinical teams. Source: iStock.

### ASSISTANCE FROM ELECTRONIC HEALTH RECORDS

IT emerges as a "hidden hero" in antimicrobial stewardship programs, according to [Anna Zhou, Pharm.D., BCIDP, co-director of the adult antimicrobial stewardship program at Loma Linda University Medical Center and an assistant professor of pharmacy practice at Loma Linda University](#). Zhou highlighted IT's role in supporting activities, like [prospective audit and feedback](#), [antibiotic timeout](#) (i.e., reviewing a patient's response to an antimicrobial a few days after starting treatment) and clinical decision support. In particular, she noted how [clinical decision support systems](#) (i.e., software that assists health care workers in making decisions by matching patient data with clinical recommendations) can enhance guideline adherence and reduce antimicrobial consumption.

"As human beings, we simply cannot remember every guideline and every pivotal study that's out there," Zhou explained. "Research shows that 30-60% of decisions would actually be different if we had new information at the time of decision-making. And that's where clinical decision support comes into play."



Clinical decision support systems provide evidence-based recommendations categorized by the infectious disease indication in the form of an empiric antibiotic order set. Source: iStock.

Clinical decision support systems can include a vast set of evidence-based recommendations categorized by the infectious disease indication in the form of an empiric antibiotic order set. This takes into account the local antibiogram data (e.g., empiric antimicrobial susceptibility) and patient-specific factors, like an allergy to penicillin or the patient's renal function.

What does this look like in practice? Let's say a patient comes in with community-acquired pneumonia. For medication considerations, all the clinician has to do is click under "pneumonia" in the EHR and establish several factors that may impact the patient's treatment, like if the patient has a penicillin allergy or not. Then, the software will populate antimicrobial orders for review and placement.

## HOW GENDER BIAS INFLUENCES ANTIMICROBIAL STEWARDSHIP

Even with steadfast programming in place, unconscious biases can shape a patient's experience and health outcomes, and possibly counteract stewardship efforts at FQHCs (or any health center, for that matter). Sara Alosaimy, [a postdoctoral scholar at Wayne State University](#), examined gender bias in her research, in particular, noting that gender bias against both patients and providers can impact antimicrobial stewardship.

Gender bias is a socially constructed set of norms and rules, varying across societies and cultures. This type of bias is driven by prejudice and stereotypes, affecting the health and well-being of individuals, particularly historically underrepresented gender identities. Alosaimy explained that this can lead to "[bikini medicine](#)," where marginalized gender identities are treated based on their sex assigned at birth, with much of the focus being on reproductive and sexual health, while "everything else is forgotten."

"Typically, when we talk about antimicrobial resistance, we acknowledge terms that are very surrogate, like data and aggregate surveillance and health outcomes, but we forget to explore the correlation between sex, gender and antimicrobial resistance," Alosaimy said. "Antimicrobial stewardship programs can be biased. Think about all the reports selected, all the instruments designed—the [diversity of] people [that are chosen] to lead those programs and [who serve] on multidisciplinary teams is sometimes forgotten."

Several studies highlight [gender disparities in antimicrobial prescribing](#). For example, patients who are assigned female at birth are often prescribed antimicrobials at a higher rate. Recommendations for treatment are also [less likely to be accepted from health care professionals who are not cis-men](#). With these data in mind, the World Health Organization (WHO) released a report in 2018 [explicitly outlining the need to address gender bias and inequities when combating AMR](#).

Alosaimy stressed the need to investigate and address gender bias in antimicrobial stewardship programs systematically. This includes calling for gender-conscious decision-making in health care and the inclusion of gender bias training in education and reporting. Furthermore, Alosaimy advocates for achieving gender equality in health care teams to create a more inclusive and effective antimicrobial stewardship environment.

"[Many] health care systems with antimicrobial stewardship programs reflect and reinforce gender biases, which compromises the safety of [patients and providers]. Gender bias primarily affects women and marginalized genders, causing a high burden of illness among these groups," she said. "Investigating gender bias in [antimicrobial stewardship programs] is an unmet clinical need. We need more descriptive variables and categories for gender, and we need to be able to assess this [with the context of] gender norms and behavior in order to protect the patient's well-being, and therefore impact the ability to prescribe in a very effective way that prevents the overuse of medication."

## THE FUTURE OF HEALTH CENTER STEWARDSHIP

Looking ahead, ensuring the sustainability of equitable antimicrobial stewardship programs will be key. FQHCs could start embedding stewardship into job descriptions, ensuring protected time for program leaders, as well as provide ongoing education, training and collaboration with experts from various disciplines. The creation of an outpatient and inpatient stewardship program also necessitates strong leadership support. Collaborative efforts between stewards and leaders should align with several common goals, including improving clinical outcomes, addressing health inequities, reducing hospitalizations and lowering health care costs.

As efforts expand, future projects may aim to strengthen partnerships with laboratories for rapid diagnostics, local health departments for surveillance, health care payers for reimbursement opportunities and other health centers for idea-sharing and research.



Jill Biden, Ph.D., visits Whitman-Walker Health, a federally qualified health center in Washington, D.C. Source: Wikimedia.

*Research in this article was presented at [ASM Microbe](#), the annual meeting of the American Society for Microbiology, held June 15-19, 2023, in Houston.*

Read About Clinical Education in the Stewardship Movement

# Updating Breakpoints in Antimicrobial Susceptibility Testing

BY ANDREA PRINZI, PH.D., MPH, SM(ASCP)

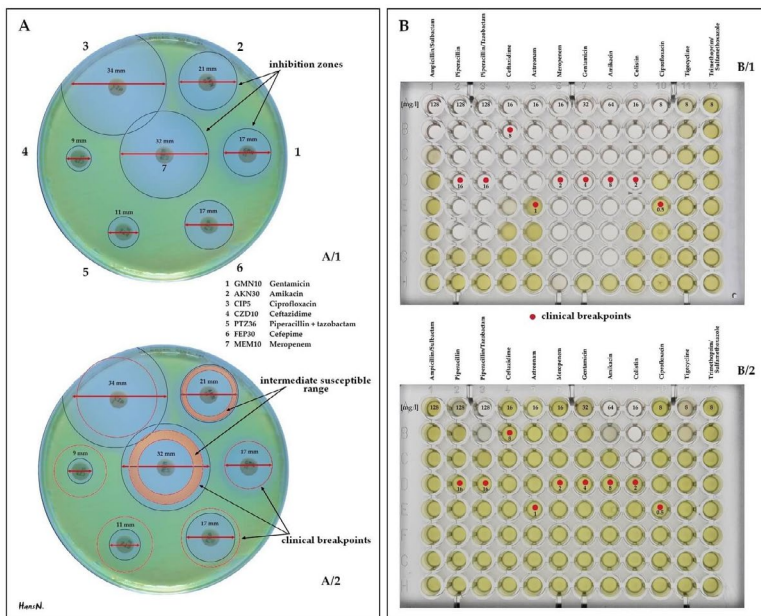
*This article was originally published on February 22, 2022 and has been updated by the author.*

In January 2022, a publication was released that [summarized the worldwide impact of antimicrobial resistance \(AMR\) in 2019](#). This study concluded that there were an estimated 4.95 million deaths associated with bacterial AMR across the globe in 2019, considerably more than previously estimated. This highlights the importance of antimicrobial susceptibility testing, reporting and surveillance in preventing and managing infections caused by resistant organisms. Data transparency and susceptibility testing in the clinical laboratory are more important than ever before.

## ANTIMICROBIAL SUSCEPTIBILITY TESTING IN THE CLINICAL LAB IS ESSENTIAL TO PATIENT CARE

Imagine that a patient comes to a hospital seeking care for a bloodstream infection. Blood cultures are collected and sent to the microbiology laboratory, where microbiologists identify the organism causing the infection and set up susceptibility testing, the process by which the organism will be tested against antibiotics in varying dilutions to determine which dilutions prevent growth. The lowest dilution with no growth is known as the minimum inhibitory concentration (MIC) or, [in the case of disk diffusion](#), a zone of inhibition. Alone, these numbers may not mean much to a clinician, but when paired with clinical breakpoints (a pre-determined range that classifies an organism as susceptible or not), they provide information that helps determine which antibiotic is best for their patient. In this patient's case, the MIC of the clinician's drug of choice is interpreted as "susceptible," and the drug is used to treat the patient.

Now imagine the patient's health worsens, and they are moved to another hospital to receive the care they need. New blood cultures are collected and are positive with the same organism. This time, the interpretation of the MIC is resistant. Based on this, the provider switches the antibiotic therapy to a drug to which the isolate is susceptible. This intervention is life-saving for the patient, since the organism was not susceptible to the original antibiotic used. The same bacterial isolate was tested at 2 different locations; how could the results be different? The answer is the use of different breakpoints. In this scenario, the first hospital used outdated breakpoints that classified the organism as susceptible to a particular antibiotic when it was not. Evidence about optimal treatment approaches changes over time, and so too should clinical breakpoints. While this story serves as an example, it is not fiction. It is estimated that each year, [thousands of patients become colonized by drug resistant organisms](#), and [using outdated clinical breakpoints](#) comes with the risk of mismanaging those patients.



Left to right: Disk diffusion susceptibility testing and microbroth dilution panels for automated susceptibility testing. Source: Wikimedia.

### CLINICAL BREAKPOINTS AND HOW THEY RELATE TO MINIMUM INHIBITORY CONCENTRATIONS

Clinical breakpoints are used to categorize MICs for different "bug-drug" combinations into 3 primary interpretive categories based on clinical data and research. These categories are: susceptible, intermediate (or in the case of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), "susceptible, increased exposure") and resistant. An additional category, "susceptible-dose dependent" suggests that the organism can be treated with higher or more frequent dosing of antibiotic. Three primary agencies determine these interpretive categories: the U.S. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), the Clinical and Laboratory Standards Institute (CLSI) and EUCAST. Breakpoints may change when new data or resistance mechanisms emerge.

### CHALLENGES OF UPDATING BREAKPOINTS IN THE MICROBIOLOGY LABORATORY

Four primary agencies determine these interpretive categories: the U.S. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), the Clinical and Laboratory Standards Institute (CLSI) and EUCAST. In the U.S., the FDA regulates drugs and devices used in medical practice. The regulations around automated susceptibility testing (AST) devices used in the microbiology laboratory can be challenging because each time a drug is added to the system or changed, FDA clearance is required. In addition, processes may seem disjointed between the FDA, CLSI and AST systems manufacturers. For example, the process for setting breakpoints for new drugs is different from the process for revising breakpoints for an antimicrobial that is already in use clinically. The time it takes a manufacturer to perform an internal study and receive approval from the FDA can be years and varies between manufacturers. Sometimes, manufacturers may even have to reformulate their test because what is currently available does not have a MIC range that accommodates new breakpoints. Testing in the clinical microbiology laboratory never stops, and while FDA clearance for new breakpoints is underway, laboratories have to decide whether to use outdated breakpoints on their device, perform a labor-intensive validation of the current breakpoints or switch to another method altogether.

### NEW COLLEGE OF AMERICAN PATHOLOGISTS (CAP) CHECKLIST REQUIREMENT FOR AST BREAKPOINTS

Due to the safety concerns and impact on patient care associated with using obsolete breakpoints, New College of American Pathologists (CAP) has developed a new checklist item that requires all clinical laboratories to update their systems and AST processes to use current breakpoints by January 1, 2024. Laboratories will have 3 years from the time a new breakpoint is published by a standards development organization (e.g., FDA) to make updates and will be required to be aware of which breakpoints (e.g., FDA, CLSI or EUCAST breakpoints) they are applying. Although the process can seem overwhelming, it is imperative for quality patient care.

The following resources provide additional context and background on AST Breakpoints:

- AST Breakpoints: A Case of Not Aging Gracefully highlights the important of AST breakpoint updates and the associated challenges.
- The AST and safety at core of microbiology checklist changes describes the reasoning behind the CAP checklist requirement update and why it is important for patient care.



- These presentation slides, [Laboratory Detection and Reporting of Carbapenem-Resistant Enterobacteriaceae \(CRE\)](#), provide a comprehensive summary of CREs.
- [AST Rationale Documents](#) further explain breakpoint changes.
- [Antimicrobial Susceptibility Test Breakpoint Updates: Challenges and Considerations for Laboratory Validation](#) describes how breakpoints are determined and describes the process of an AST breakpoint validation.

## VALIDATION AND VERIFICATION STEPS FOR UPDATING BREAKPOINTS

### Step 1: Identify Which Breakpoints Are [Obsolete](#)

The first step in this process is identifying the method of MIC interpretation in your laboratory. For example, it is possible that [interpretations could be driven](#) by the AST instrument, the laboratory information system (LIS), the electronic medical record (EMR) or manual entry. Understanding which system is responsible for MIC interpretations will help laboratorians identify where updates are needed. Once identified:

1. Compare breakpoints to those listed in the [CLSI M100](#) document or provided on the [EUCAST website](#).
2. Check the breakpoints posted on the FDA [STIC website](#) if they do not match with CLSI M100 or EUCAST.
3. If the breakpoints routinely reported by the laboratory do not match CLSI, EUCAST or FDA, they are obsolete and must be updated to at least 2021 breakpoints before January 1, 2024. [Breakpoints Matter: Understanding CLSI Efforts and New CAP Requirements to Ensure Appropriate Antimicrobial Treatment for all Patients](#) further discusses how to identify obsolete breakpoints.

### Step 2: Engage Industry Partners

If the laboratory is using a commercial AST system, reaching out to the manufacturer for guidance can be helpful. For example, manufacturers can inform laboratories of which breakpoints are FDA cleared and expound upon the testing capabilities of their current system. In addition, it is essential to discuss the following with a manufacturer:

Which breakpoints (identified in step 1) are [FDA cleared, and which are not](#). Updating breakpoints to those cleared by the FDA on an automated system is considered on-label use. Labs should perform verification to demonstrate that assay performance is comparable to what was shown by the manufacturer during the FDA clearance process.

Updating breakpoints to those not FDA cleared on a device is considered [off-label use and a modification of the test](#). Laboratories must perform a [validation, which is a more extensive evaluation than a verification](#). Laboratories may choose to validate breakpoints that are not FDA-cleared due to clinical relevance or local needs.

Whether the current formulation of the susceptibility panel or card has a testing range that can accommodate the new breakpoints. If not, the manufacturer may provide information about when new panels or cards may be available or provide alternative testing options until the appropriate testing range is available.

### Step 3: Make a Plan With the Clinical Team

1. Prioritize which breakpoints should be updated first. For example, updating carbapenem breakpoints for the Enterobacterales is considered a top priority, as the adverse clinical and public health outcomes associated with not doing so are significant.
2. Identifying whether an antibiotic is used at an institution, dosing requirements and understanding use in different patient populations can help determine whether breakpoint updates need to be performed. Not reporting the drug MIC might be a preferable option to completing a verification or validation. All decisions to not report a drug should be documented in the laboratory standard operating procedure.

The [Journal of Clinical Microbiology](#) articles below describe tactics to help develop a plan for updating breakpoints in your laboratory:

- [Understanding and Addressing CLSI Breakpoint Revisions: a Primer for Clinical Laboratories](#) explains the entire breakpoint revisions process and provides background and action items.
- [Summary of Strategies for Implementing Current Breakpoints](#) breaks down strategies for each antimicrobial/organism combination.
- [Proposed Decision Tree for Revised Breakpoint Adoption on Commercial ASTs](#) includes a flowchart to help laboratorians think through the breakpoint update process.

#### Step 4: Perform Validation or Verification Testing and Update Breakpoints

Once a plan of action has been decided, the laboratory can perform validation or verification testing. All data from steps 1-3, and data collected during validation or verification, must be documented and will likely be required during future CAP inspections.

The following resources contain additional information about how to plan for and perform validation and verification testing:

- [Planning a Method Verification Study in Clinical Microbiology Labs](#) breaks down how to perform a clinical laboratory verification study with attached template.
- [Verifications and Validations: How to bring a new test to the lab aiming at clinical stewardship and compliance](#) shares a real-life example of validation and verification.
- [Verification of Antimicrobial Susceptibility Testing Methods "A practical approach"](#) is a comprehensive walkthrough of validation and verification.
- [AST Breakpoint Update Template](#) is a blank template provided by APHL, which can be used for updating carbapenem breakpoints for Enterobacterales.

#### Step 5: Monitor for Quality and Repeat

The CAP checklist update (found specifically under MIC.11385) [requires that laboratories do breakpoint updates within 3 years of the most recent update by the FDA, CLSI or EUCAST](#). This means that by January 1, 2024, [all breakpoints must be up-to-date](#), with breakpoints considered current as of 2021 (at a minimum). By January 1, 2025, all laboratories must be using current breakpoints as of 2022. This review process should occur yearly, and laboratories should update breakpoints regularly.

### PATIENT CARE AT THE CORE OF ALL CLINICAL MICROBIOLOGY PRACTICES

In light of the COVID-19 pandemic, clinical microbiology laboratories are grappling with burnout, staffing shortages and high demand. Updating AST breakpoints may feel like an overwhelming task that is difficult to accomplish under these conditions. While it is true that AST validation is a challenging task for clinical microbiology laboratories, there are a wealth of resources available to provide guidance and support throughout the process. Providing accurate and up-to-date susceptibility data is one of the many key roles the microbiology laboratory plays in public health and direct patient care. In the face of a looming AMR crisis, it is imperative that laboratories take the lead in preventing and mitigating the spread of drug resistant organisms, as well as helping ensure that patients receive optimal antimicrobial therapy and high-quality care.

*The statements and opinions expressed in this article are those of the author and do not necessarily reflect those of bioMerieux, Inc., nor of the American Society for Microbiology.*

# Hunting for Antibiotics in Unusual and Unculturable Microbes

BY MADELINE BARRON, PH.D.

The emergence of antibiotic-resistant pathogens has far outpaced the discovery of new antibiotics to combat them. This is partly because antibiotic discovery efforts generally focus on screening culturable environmental microbes (e.g., bacteria from soil) for antimicrobial compounds. However, most environmental microbes cannot be grown in the lab, and thus are useless from a drug discovery standpoint—or are they? Aided by clever culturing techniques, scientists are gaining access to once inaccessible bacteria and, from these microbes, uncovering a spate of new antibiotics.

As outpatient settings are responsible for a substantial portion of antimicrobial usage, health centers are turning their sights to stewardship programs to promote responsible prescribing practices, enhance medication safety and, ultimately, mitigate the impending concern of antimicrobial resistance (AMR). While the adoption of such programming has been slow across various health systems, Federally Qualified Health Centers (FQHCs), which serve historically marginalized populations, are well-positioned to contribute to equitable care while leading stewardship efforts.



Actinomycetes bacteria. Source: Oregon Caves/Wikimedia Commons.

## THE GOLDEN AGE OF ANTIBIOTIC DISCOVERY

There was a time when it seemed like antibiotics were being uncovered left and right. This ["golden age" of antibiotic discovery](#) took off in the 1940s when [Selman Waksman, Ph.D.](#), a Nobel Prize-winning microbiologist, [discovered the broad-spectrum antibiotic, streptomycin](#), from a species of soil-borne actinomycetes. Waksman's discovery pointed to soil actinomycetes as potential sources of new antibiotics and motivated efforts throughout the pharmaceutical industry to mine the bacteria for promising leads.

These efforts [led to the discovery of many of the main classes of antibiotics](#) used today (e.g., aminoglycosides, tetracyclines,  $\beta$ -lactams, etc.). However, in the 1960s, progress tapered off. Soil actinomycetes were tapped out of novel antibiotics that could be uncovered with standard screening methods. Subsequent screens for synthetic antimicrobials were also largely unsuccessful; most synthetic molecules are unable to bypass the bacterial cell membrane (especially the repellent charges and pumps of the outer membrane in gram-negative bacteria), and thus are ineffective.

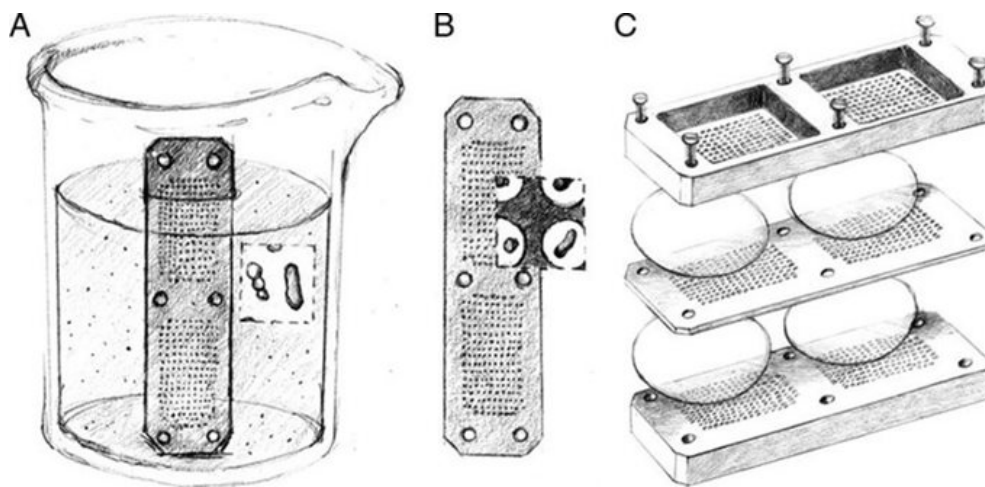
Since then, progress in antibiotic discovery has been marginal—or, as stated during [ASM Microbe 2023](#) by [Kim Lewis, Ph.D.](#), a University Distinguished Professor and Director of the Antimicrobial Discovery Center at Northeastern University, "We are not in a great place to be."

All is not lost, though. For Lewis and his colleagues, the key to jump-starting natural product discovery is looking where scientists have never looked before. "One simple proposition is to start screening outside the actinomycetes and see what we can find," Lewis said. "And if you're going outside of actinomycetes, why not target uncultured bacteria?"

## CULTURING THE UNCULTURABLE

Only 1% of environmental bacteria can grow on a petri dish, leaving a whopping 99% uncultured. Most of these bacteria cannot be grown in the lab using traditional cultivation techniques; if scientists can't grow them, they can't access their potentially useful products. Over the past 20+ years, however, Lewis and his collaborators have developed methods for cultivating uncultivable soil microbes. The ticket, Lewis explained, is to make the microbes feel at home—that is, "trick" the cells into thinking they are growing in their natural environment, where they have access to nutrients and other growth factors. With his colleague, [Slava Epstein, Ph.D.](#), Professor of Biology at Northeastern University, Lewis invented what he jokingly referred to as a "very sophisticated device."

The [device consists](#) of a semi-permeable membrane, dabbed with a mix of agar and environmental cells (i.e., a diluted soil sample), sandwiched between 2 metal washers. The sandwich can be placed in an outdoor sampling site or in a simulated natural environment in the lab. The membrane allows molecules from the environment to diffuse in and out. After incubation for several weeks, bacterial microcolonies populate the membrane and can be isolated. Notably, once a cell population has been established, the bacteria are more apt to grow on a petri dish in the lab (up to 40% growth recovery). Another iteration of the technology, known as the [Isolation Chip \(ichip\)](#), is comprised of hundreds of tiny diffusion chambers that hold approximately 1 bacterial cell each, thus streamlining the process by allowing scientists to both grow and isolate individual bacteria.



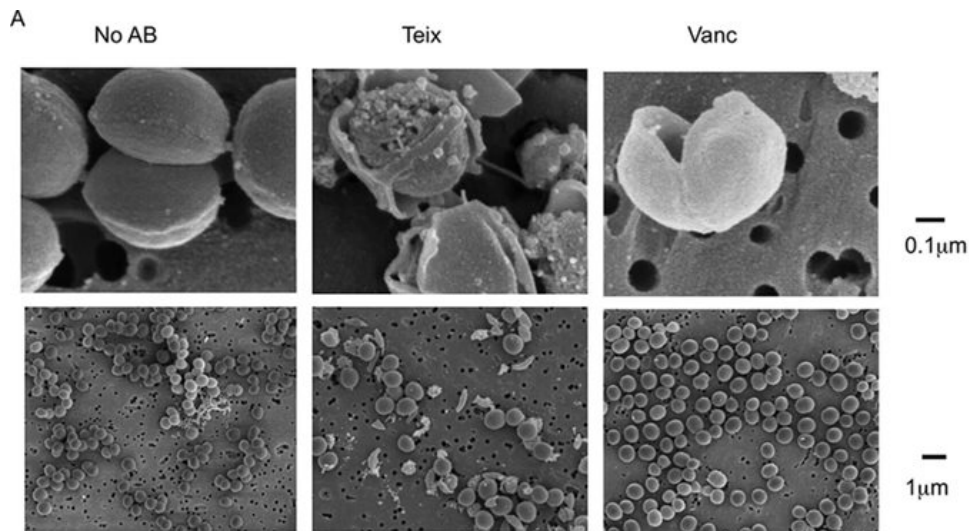
The ichip. To assemble the device, a plate covered in tiny holes is dipped into a suspension of environmental cells, covered in membranes and sealed between 2 additional plates. Source: Nichols D., et al/*Applied and Environmental Microbiology*, 2010.

[NovoBiotic Pharmaceuticals](#)—a biotechnology company, co-founded by Lewis and Epstein, which focuses on the discovery and development of new drugs from natural sources—has used the diffusion chamber technology to screen soil samples at the industrial scale. The company now has a collection of over 64,000 unculturable bacterial isolates and, from those unusual isolates, has identified several promising antibiotics.

## DRUGS FROM UNCULTIVABLE BUGS

The company's leading antibiotic, [teixobactin](#), [was isolated from a previously uncultivated soil bacterium called \*Eleftheria terrae\*](#). Lewis highlighted that the compound shows excellent activity against a slew of gram-positive pathogens, regardless of their antibiotic resistance profile, is nontoxic to eukaryotic cells and, based on current evidence, appears to kill pathogens without detectable resistance. This is likely because teixobactin's targets on the cell membrane (lipid II and lipid III—precursors of peptidoglycan and teichoic acid, respectively) are immutable. That is, they are not proteins (i.e., are not directly coded by genes), and therefore do not acquire genetic mutations that can confer antibiotic resistance. This discovery suggests that "the paradigm that bacteria will always develop resistance to everything is incorrect," Lewis said.

Teixobactin's efficacy is also linked to its unique [2-pronged mechanism](#). Molecules of teixobactin don't just bind their targets, which inhibits cell wall synthesis, but also associate to form sheet-like supramolecular structures. "The membrane thins beneath the supramolecular structure," Lewis explained. "We figured that [this] may disrupt the membrane—and it does." He highlighted that "teixobactin is giving us a recipe for how to develop safe, membrane-active compounds," which have remained somewhat elusive, despite scientists' best efforts to find them. Scientists have since uncovered another antibiotic, [clovibactin](#), that similarly targets lipid II and "zips up into a supramolecular structure," albeit one that is a bit different from teixobactin.



Teixobactin damages the cell membrane. Here, *Staphylococcus aureus* cells in the absence of antibiotic (No AB) or treated with teixobactin (Teix) or vancomycin (Vanc), another antibiotic that targets lipid II and disrupts peptidoglycan synthesis. Source: Homma T, et al./Antimicrobial Agents and Chemotherapy, 2016.

"The conclusion from these compounds is...[that] nature clearly developed compounds that evolved to avoid resistance," Lewis said. "And our notion of what is a suitable or druggable target is irrelevant because nature's oblivious to that [notion]."

Teixobactin is currently under late-stage preclinical development. Compounds from the NovoBiotic collection that [target \*M. tuberculosis\*](#) have also been discovered, and the company recently received funding to [mine their collection for antifungal drugs](#) to combat the fungal pathogen, *Candida auris*.

### TAKING ON THE GRAM-NEGATIVES

Discovering antibiotics against gram-positive bacteria is notable. However, Lewis acknowledged that there is a paramount need for compounds that target gram-negative pathogens, [which are especially concerning from an antimicrobial resistance standpoint](#) (3 out of the 5 pathogens [listed as "urgent" antimicrobial resistance threats](#) by the U.S. Centers for Disease Control and Prevention are gram-negative). Yet, when screening soil, the "hit rate" for compounds targeting gram-negative bacteria is 2x lower than for gram-positive. Lewis estimates it would take 100 years to find leads against gram-negative bacteria with the standard soil sampling pipeline.

To address this, Lewis and his collaborators are narrowing their scope, honing in on bacteria they know have similar requirements for antibiotics as humans (e.g., active against gram-negative bacteria, low toxicity, in vivo efficacy). It turns out, bacteria living in the guts of [entomopathogenic nematodes](#) are good candidates. Antimicrobial compounds produced by these gut microbes must have low toxicity against their nematode host, be able to travel through tissues and must work against gram-negative pathogens, which are key competitors in the nematode gut environment.

So far, this approach has been successful. For example, [a screen of nematode gut isolates belonging to the \*Photorhabdus\* genus uncovered an antibiotic](#), darobactin, that is active against prolific gram-negative pathogens (e.g., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and others) in vitro and in mice, but shows limited activity against gram-positive organisms and other symbionts. Importantly, darobactin targets a complex on the gram-negative bacterial surface ([the BAM complex](#)), which overcomes the need to bypass the outer membrane—a formidable barrier to many compounds. Lewis noted that additional *Photorhabdus*-derived compounds are under development.

Overall, the work of Lewis and his colleagues—from growing uncultivable soil microbes to capitalizing on nematode gut bugs—points to 1 key message: new, effective antibiotics are out there. It's simply a matter of where (and how) one looks.

*Research in this article was presented at ASM Microbe, the annual meeting of the American Society for Microbiology, held June 15-19, 2023, in Houston. Ready to share your science at ASM Microbe 2024?*

Submit an Abstract Now

# What's Hot in the Microbial Sciences

BY MADELINE BARRON, PH.D. & JOHN BELL

## INVESTIGATING CLOVE OIL (EUGENOL) AS AN ADJUVANT TO OVERCOME COLISTIN RESISTANCE



Clove oil has traditionally been used to bolster health. A compound called eugenol is one of its primary components. Source: Wikimedia Commons.

A recent study published in [Microbiology Spectrum](#) described an investigation of the combined activity of colistin, the "drug of last resort" used to treat infections by carbapenem-resistant members of the Enterobacteriaceae family, and eugenol, a naturally derived aromatic phenolic compound, against clinical isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

The misuse of antibiotics in human medicine and in agriculture has contributed to antimicrobial resistance (AMR), according to the [World Health Organization](#) (WHO). An urgent concern, in this regard, is that pathogenic bacteria are developing resistance to colistin, an antibiotic of great importance for patients lacking viable treatment alternatives, such as carbapenems and cephalosporins. The increasing frequency of AMR infections clearly demonstrates the [need for new antimicrobials](#), yet few are in development in the pharmaceutical industry.

As a result, researchers are casting the net far and wide for existing substances that can serve the purpose, including [natural antimicrobial agents](#) produced by plants, fungi and bacteria. Products from the natural world have served as useful therapeutic agents against pathogenic bacteria since the [golden age of antibiotics](#) in the mid-20th century.

[Eugenol](#), also known as clove oil, is the major constituent (83%-95%) of the aromatic oil extracted from cloves (*Syzygium aromaticum*). It derives also from other plant products, such as nutmeg, cinnamon, ginger, turmeric, basil, bay laurel leaves and peppers (Solanaceae). Eugenol possesses known pharmacological properties and is used in antimicrobial, anticancer, antioxidant, anti-inflammatory and analgesic applications. Eugenol derivatives already have applications as local anesthetics and antiseptics.

In the study described in *Microbiology Spectrum*, 9 commonly used antibiotics were each tested in combination with eugenol against 14 colistin-resistant *P. aeruginosa* and *K. pneumoniae* clinical isolates. Cell viability assays indicated that eugenol at concentrations of up to 500 µg/mL was not toxic toward murine macrophage cells, and the colistin-eugenol combination displayed effectiveness against colistin-resistant bacteria in vivo in an infection model using greater wax moth larvae (*Galleria mellonella*). Treatments delayed larval death and increased their survival rates by 20%-30%.

Leakage of protein and DNA from bacteria significantly increased in the presence of eugenol, as measured by alkaline phosphatase (ALP) leakage assays, leading researchers to conclude that eugenol and colistin, in combination, increased bacterial membrane permeability. Furthermore, crystal violet staining and scanning electron microscopy revealed that, in concert, colistin and eugenol were found to disrupt biofilms, which act as bacterial shields from antibiotic pressure and are known to produce recalcitrant infections. Further studies will evaluate the safety of eugenol for clinical use.

The researchers concluded that colistin and eugenol in combination represent a formidable treatment option for colistin-resistant clinical infections involving *P. aeruginosa* and *K. pneumoniae*. While natural compounds alone may not demonstrate significant antibacterial activity, they can be used as adjuvants with conventional drugs to boost the drugs' antimicrobial properties. Such use of [adjuvants](#) can circumvent antibiotic resistance, prevent its spread and reduce the adverse effects of drugs.

## COULD ENGINEERED LIVING MATERIAL BEING USED TO CREATE SELF-HEALING BUILDINGS?

Researchers at the University of California and the Scripps Institute of Oceanography in San Diego used synthetic biology to design an engineered living material consisting of a strain of cyanobacteria suspended in a gel matrix that can sense and decolorize a common textile dye pollutant, [indigo carmine](#) (IC). Such a system could be used for environmental remediation in the near term and, in the future, may even be expanded to produce [biomimetic building materials](#) that can sense and respond to their environment—such as walls that can sense structural damage or wear and automatically repair themselves ("self-healing"). The researchers reported their findings in [Nature Communications](#).



Engineered living materials may one day be used to construct human habitations. Source: Freepik.

IC, a coloring agent popular in the pharmaceutical, textile, leather and food industries, is widely used to [dye denim fabric](#). Yet, it functions as an environmental contaminant, exerting [toxic effects](#) in aquatic ecosystems, and the discharge of textile wastewater into the environment can lead to [adverse health conditions](#) in humans, such as skin and eye irritation, corneal and conjunctiva lesions, dermatitis and even cancer with constant and prolonged contact. Effective methods to remove the dye from wastewater would prove useful.

To address this concern, researchers introduced a synthetic riboswitch to regulate expression of a yellow fluorescent protein reporter in the cyanobacterium, *Synechococcus elongatus*. Fluorescence of the reporter protein indicated successful introduction of the gene, as well as a gene encoding a laccase enzyme. [Laccases](#) are broad-spectrum enzymes that can degrade compounds in wastewater effluents from industries and hospitals. Then the researchers used 3D printing to fabricate a transparent and porous biocomposite material, an alginate hydrogel matrix, into which was "printed" a gel-based "ink" containing a strain of *S. elongatus* that can sense IC and respond by producing laccase.

The transparent and porous alginate hydrogel matrix surrounding the laccase-producing cyanobacteria allowed for photosynthesis and the transport of nutrients and gases, respectively. The gel-based "ink" protected the cells from mechanical shear stress during extrusion ("printing") of the cyanobacterium-containing hydrogel matrix, minimizing cell membrane damage and enhancing cell viability in the printed pattern.

Upon completion of its bioengineering, subsections of the hydrogel (2 interconnected hollow squares) were suspended in medium containing IC. The responsive biomaterial produced laccase and decolorized the IC in the hydrogels. A "kill switch," or [inducible cell death](#), was engineered into the responsible cells so that they conveniently perished once the task was completed. Inducible cell death constitutes an important aspect of the process to contain the organism and reduce potential environmental impact.

This experiment constituted proof of concept that [engineered living material](#) can produce useful products and/or perform desired tasks in response to environmental cues. Such material has the potential to serve in environmental bioremediation of other pollutants and might one day be harnessed to produce self-healing components incorporated into built environments. Imagine a housing unit or laboratory facility constructed of engineered living material that can sense damage to itself and subsequently repair its own structure.

Engineered living materials that combine the structural properties of traditional building materials (such as concrete and cement) with living systems, which possess the ability to rapidly grow, sense their environment and even self-heal in response to environmental cues, have potential to solve numerous challenges associated with construction and maintenance of built environments. For example, development of advanced engineered living materials could improve methods for manufacture and maintenance of large physical systems, such as military bases and transport vehicles and, perhaps, even self-repairing scientific research and housing units far from Earth.

## DO VITAL ANTIBIOTICS LURK AMONG EXTINCT MOLECULES?

[Molecular de-extinction](#), a new field of science, involves the retrieval of organic molecules, such as nucleic acids, proteins and other compounds, from extinct organisms. In a recent study published in [Cell Host & Microbe](#), University of Pennsylvania researchers investigated potentially beneficial applications of molecular de-extinction for drug discovery.



Molecular de-extinction may allow us to reproduce useful antibiotic compounds from organisms that lived in the distant past. Source: iStock.

Unlike larger scale [de-extinction of whole organisms](#), which has been discussed in the literature, these researchers concentrated on reintroducing bioactive molecules from nonextant organisms, hypothesizing that such molecules conferred benefits to extinct organisms and could, once again, be of use but in the modern world.

Recreating extinct molecules has the potential to reinforce human defenses against future challenges that resemble challenges from ancient environments, such as climate change and outbreaks of infectious diseases.

Previous researchers have defined methods for sequencing [ancient DNA](#). However, with the advent of artificial intelligence (AI), the past 5 years have yielded an explosion of possibilities for drug discovery, including the rediscovery of antibiotics from remains that are hundreds of thousands of years old.

Utilizing an approach referred to as "[paleoproteome mining](#)," the researchers employed the [PanCleave Python pipeline](#), a protein informatics, open-source machine learning tool, to prospect for antimicrobial encrypted peptides in the secreted proteins of modern humans, as well as in the archaic proteomes of our closest extinct relatives, Neanderthals and Denisovans.

The team discovered small protein subsequences that displayed antibiotic qualities encrypted within the proteins of our long-gone relatives, and subsequently synthesized the corresponding molecules. They then tested the efficacy of these resurrected antibiotics. In both murine skin abscess infection models and thigh infection models, some of the protein fragments demonstrated efficacy against [Acinetobacter baumannii](#), a known antimicrobial resistant pathogen that often functions as a hospital acquired infection (HAI) and causes blood, urinary tract and lung infections.

Molecular de-extinction shows promise in drug discovery by reintroducing unique antimicrobials from the distant past and opening the door to an entirely new pathway for future antibiotic discovery.



## CAN PHAGES TREAT ACNE?

Ah, adolescence. A time characterized by immense growth, a sprinkling of awkwardness and, [for up to 80% of teens](#), acne. Though the development of acne is tied to multiple factors, from genetics to hormones, the skin-dwelling bacterium *Cutibacterium acnes* plays a key role in exacerbating inflammation. Antibiotics aimed at controlling *C. acnes* growth have been [widely used to treat moderate-to-severe acne](#), yet the emergence of resistant strains has limited their efficacy. A study published in [Nature Communications](#) suggests that phages (viruses that infect bacteria) targeting *C. acnes* may offer a solution.



Acne is a common scourge of adolescents. Scientists are exploring whether phages could be used to treat it. Source: iStock

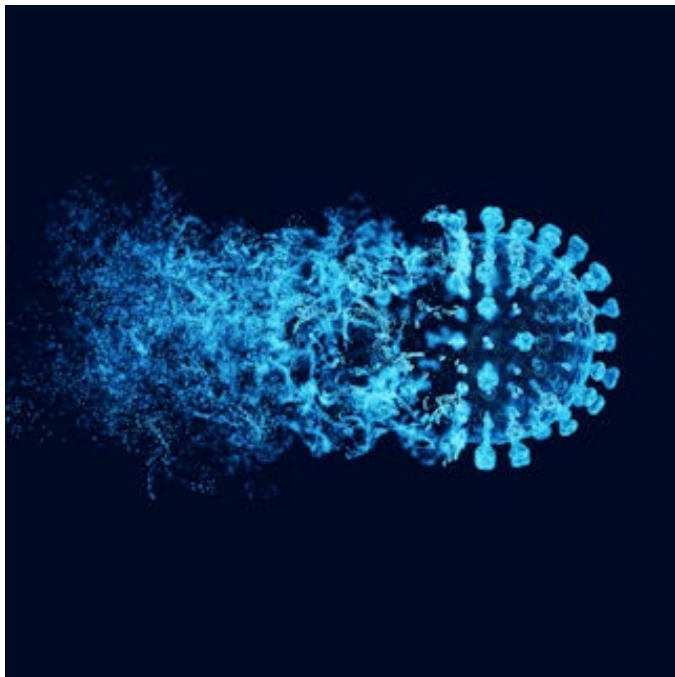
In the study, scientists screened skin swabs from patients with acne to uncover 8 phages with lytic activity against *C. acnes*. In vitro experiments with clinical *C. acnes* strains revealed that a majority (32/36, or 88%) were sensitive to the phages, including strains that were resistant to at least 1, and some that were resistant to all, antibiotics commonly used to treat acne (e.g., clindamycin, tetracycline and others). These findings highlighted the phages' potential for combating *C. acnes*, including when antibiotics may be ineffective.

To test in vivo efficacy, researchers turned to a mouse model. Phages were suspended in a gel, which was then smeared onto *C. acnes*-induced, acne-like lesions on mice once a day for 5 days. Compared to animals treated with gel alone (control), phage treatment led to significant improvement in lesions, including a decrease in lesion bacterial load, diameter, elevation and presence of necrotic tissue. It also dampened *C. acnes*-triggered inflammation, as indicated via reduced neutrophil migration and expression of inflammatory molecules within the lesions. From these results, the scientists concluded that topical administration of phages could be a powerful method for treating acne—something that had never been shown before.

So, will doctors soon be prescribing phage-infused zit cream? Not quite. The study acknowledged several limitations, including differences in the characteristics and progression of acne-like lesions in the mouse model compared to human acne. Thus, clinical investigation of topical phage acne treatment in a patient population is warranted.

## HOW CAN ANTIMICROBIAL PEPTIDES LEAD TO NEW ANTIVIRALS?

The COVID-19 pandemic underscored the need for effective antivirals to combat existing and emerging viral threats. In a recent [study published in ACS Infectious Disease](#), scientists describe antimicrobial peptide (AMP)-inspired molecules, called peptoids, that exhibit broad-spectrum antiviral activity by targeting conserved, host-derived lipids in the viral membrane. This mechanism may lower the potential for generation of resistant variants. The findings provide a basis for the development of new antiviral agents.



New antiviral compounds are needed to combat current and emerging viral threats.  
Source: iStock.

AMPs are released by diverse host cells to kill pathogens, including viruses. Though AMPs have been explored for clinical use, they have various drawbacks in their natural state, including poor bioavailability and the potential to trigger unwanted immune responses against the peptides themselves, which can reduce their efficacy. Yet, peptoids can be synthesized to mimic desirable aspects of AMPs and maintain structural differences that may make them more clinically applicable (e.g., greater stability and membrane permeability).

In this study, scientists examined the activity of 7 AMP-like peptoids against a diverse range of viruses, including Zika virus (ZIKV), Rift Valley Fever virus (RVFV), chikungunya virus (CHIKV) and coxsackie B3 virus (CVB3). Peptoids that had [documented activity against SARS-CoV-2 and herpes simplex virus-1](#) were selected for the study.

While the efficacy of individual peptoids varied for each virus, 1 thing was consistent: the peptoids only inactivated enveloped viruses (ZIKV, RVFV, CHIKV). CVB3, a non-enveloped virus, was unaffected. Additional experimentation revealed that the peptoids showed high specificity for a host-derived lipid in the viral membrane, called phosphatidylserine (PS). In host cells, PS

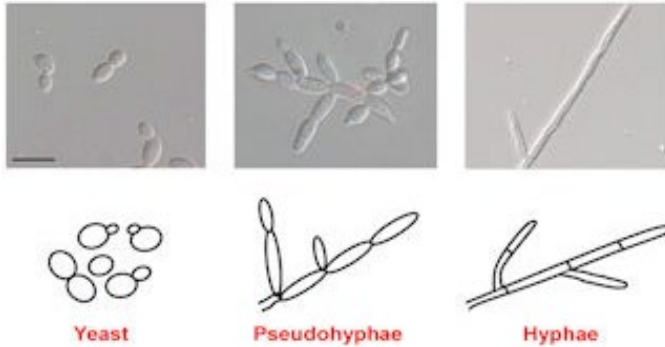
is normally maintained on the inner leaflet of the plasma membrane. During apoptosis, however, PS is flipped to the surface, where it serves as a signal for other cells to engulf their dying comrade.

Viruses can [take advantage of this process](#)—when PS is expressed on their membrane, they "look" like apoptotic cells, and are thus taken up by host cells. Once inside, the viruses have all the cellular machinery they need to reproduce. Notably, viruses do not have the enzymes needed to control where PS localizes. This means that, in contrast to host cells, PS is often present in high concentrations on the virus surface. With that in mind, the researchers proposed that the peptoids were not attracted solely to PS on the viruses, but how much of it was present. Indeed, CHIKV viruses expressing a higher proportion of PS on their surface were more sensitive to peptoid treatment than those expressing normal levels.

Besides highlighting the antiviral potential of antimicrobial peptoids, these findings are significant for several reasons. For 1, given viruses obtain membrane lipids from the host (i.e., they do not encode their own membrane proteins), designing antivirals that target conserved, host-derived membrane components may limit the development of resistant variants, as there is no selective pressure on the virus itself. Capitalizing on differences in localization and concentration of lipids, like PS, between host and virus may also be advantageous for promoting selectivity and limiting host cytotoxicity.

## HOW DOES COMMENSAL CANDIDA STAY COMMENSAL?

*Candida albicans* is a common fungal member of the gut microbiome, present in about 70% of people. While it can be pathogenic, *C. albicans* normally exists in a state of commensalism with its host. What underlies the maintenance of this commensal relationship? [New research published in Science](#) provides some clues, suggesting that a form of a hormone that controls appetite, called Peptide YY (PYY), plays a role.



*C. albicans* exhibits several morphologies, including a commensal yeast form and a pathogenic hyphal form. Source: ASM Journals.

PYY is secreted by enteroendocrine cells in the gut; upon its release, it is cleaved by a protease, and the resulting fragment helps regulate appetite satiety. In this study, scientists found that the full-length version of PYY is also secreted by a subset of gut cells, called Paneth cells (PC), which release a slew of antimicrobial peptides to control microbial growth in the gut. This led the researchers to wonder: does PC-secreted PYY (PC-PYY) have antimicrobial powers too?

While PC-PYY showed limited activity against various bacterial species, it was effective against *C. albicans*, indicating it could play a role in modulating growth of the fungus in the gut. Indeed, gut colonization by *C. albicans* in mice lacking PYY was 2-3 times higher than in mice expressing PYY.

However, there was a catch: in vitro experiments revealed that the peptide only worked against *C. albicans* hyphae (a branched, virulent form of the fungus), not the commensal yeast form of the microbe. Why? It appears the peptide is attracted to the negatively charged surface of the hyphal membrane—a characteristic not shared by the yeast. Once bound, the peptide wreaks havoc on the hyphal cell membrane. RNA-seq analyses showed that PC-PYY also interferes with hyphal expression of biofilm and virulence genes; in yeast, PC-PYY downregulates genes involved in the transition to the pathogenic hyphal form.

These data point to a mechanism whereby the host maintains *C. albicans* commensalism by specifically targeting the pathogenic version of the microbe, while leaving the non-pathogenic version unharmed. The authors found that PC-PYY exhibited similar activity against other fungal species, suggesting it could play a broader role in shaping the gut mycobiome.