

*Four methicillin-resistant Staphylococcus aureus bacteria being enveloped by a human white blood cell
Credit: US National Institute of Allergy and Infectious Diseases*

Antimicrobial Resistance: Investigating the Environmental Dimension

What is antimicrobial resistance?

According to the World Health Organization, we may be entering a post-antibiotic era when simple, and previously treatable, bacterial infections can kill and when routine medical procedures, such as joint replacements and chemotherapy that rely on antibiotic preventative treatment, will no longer be possible.¹ The 2014 O'Neill report commissioned by the UK government estimated that antimicrobial resistant infections may become the leading cause of death globally by 2050.²

Antibiotic pharmaceuticals are used around the world to treat and prevent bacterial infections in humans, animals and even plants. They have also been used widely as growth promoters to increase meat production, although this practice

was banned in the European Union in 2006.^{3,4} While the misuse of antibiotics in medical and agricultural practices has been linked to increasing resistance, the role of the natural environment in the emergence and spread of resistance has received relatively little attention.

Resistance to antimicrobials can be intrinsic or acquired. Acquired resistance can occur through a mutation in bacterial DNA or by gaining the resistance genes through horizontal gene transfer when DNA moves from one bacterium to another. Acquired resistance that leads to failure of infection treatments in clinical and veterinary settings is currently a subject of concern.

Many antibiotics are natural, such as the original penicillin found in bread mould, while many are synthesized or



chemically modified from natural antibiotics for enhanced activity and stability.⁵ Antibiotics are a subset of antimicrobials—substances that kill or suppress the growth of microorganisms. The terms are commonly used interchangeably.

Competition always occurs between microorganisms by way of producing antibiotic molecules to inhibit others from thriving. For bacteria to survive, they have successfully developed mechanisms to resist the antibiotic assault. Research indicates that resistance to antibiotics, including some utilized in modern medicine, has existed for millions of years, suggesting that antibiotic resistance is natural, ancient, and hard wired in the shared genome of microbes.⁶

Without human interference, selection for resistance already occurs naturally in microbial populations in soil, water and other habitats. However, current antibiotic use in the hundreds of thousands of tons per annum and subsequent release of antibiotic residues into the environment produce a step change in the magnitude of selection pressures that lead to the increase in antibiotic resistant bacteria.⁷ Once consumed, most antibiotic drugs are excreted unmetabolized, along with resistant bacteria. They can then pass either through sewage systems or more directly into water and soils, and mix with environmental bacteria in the presence of other pollutants that may add further pressure to help select for antibiotic resistance, directly or indirectly. The extent to which the environment contributes to this problem is under intense investigation, but the answers will in part depend on the level of environmental contamination, and how long antimicrobial residues persist in an active form.

Bacteria in water and soil naturally possess a huge diversity of resistance genes. Research has found that previously susceptible pathogens are able to acquire resistance genes from environmental bacteria.⁸⁻¹¹ The genetic basis of antibiotic resistance in bacteria and how resistance can spread between environment and clinic are now subjects of enormous interest.¹¹⁻¹³

Human exposure to environmental bacteria and to antibiotic resistance genes can take place through drinking water, food consumption or through direct contact with the environment. A further question is to what extent transmission of resistant



What is an antimicrobial?

Any substance of natural, semisynthetic or synthetic origin that kills or inhibits the growth of microorganisms, such as bacteria, viruses, protozoa and fungi. Antimicrobial substances are used in the form of pharmaceutical drugs such as antibiotics, antivirals and antifungals; or of chemicals such as antiseptics, disinfectants, and sterilants.

What is an antibiotic?

An antimicrobial substance naturally produced by bacteria or fungi that can kill or inhibit the growth of other microorganisms. People make use of many types of antibiotics as medicines to prevent and treat infections caused by pathogenic bacteria, fungi and certain parasites. The majority of antibiotics are primarily used against bacteria.

Because antibiotics are a type of antimicrobials, the two terms are often used interchangeably.

What is antimicrobial resistance?

Antimicrobial resistance occurs when a microorganism evolves to resist the effects of an antimicrobial agent and multiply in its presence. Globally about 700 000 people die of resistant infections every year because available antimicrobial drugs have become less effective at killing the resistant pathogens.

What is selection for resistance?

Natural selection is a mechanism which drives adaptation of organisms to better survive in their environment in order to thrive and multiply. In the context of antimicrobial resistance, antimicrobial substances exert a selective pressure on microbes that drive evolution of resistance. Those able to resist the effects of antimicrobials survive and reproduce, whereas susceptible ones are killed or their growth is inhibited. Overuse and misuse of antibiotics increase selection for antibiotic resistance among bacteria.

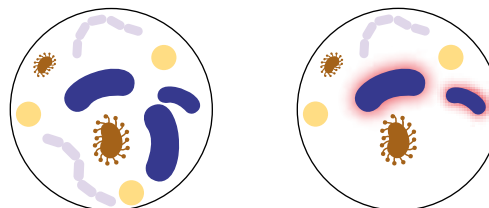
bacteria occurs through the food chain or by direct contact with the environment. For instance, research showed that, even with high levels of investment in wastewater treatment, an estimated 6 million exposure events to one type of antibiotic resistant *E. coli* occur each year in the United Kingdom's coastal recreational waters.¹⁴ There are also well-documented cases of the evolution of antibiotic-resistant bacteria within food animals with subsequent spread to humans.¹⁵

Antibiotics, co-selectors and resistant bacteria in the environment

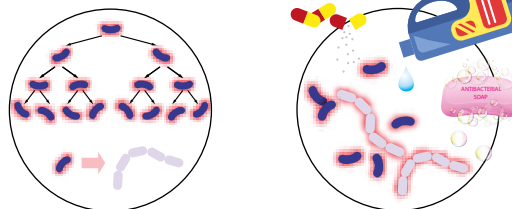
The discharge of antibiotics and other antimicrobial compounds, such as disinfectants and heavy metals, into natural environments has the potential to drive the evolution of resistant bacteria. These compounds are present in waters and soils at a wide range of concentrations, depending on source and behaviour in terms of degradation rate and adsorption to solids.^{16,17} Municipal wastewater contains a vast array of contaminants: pharmaceuticals and personal care products from households; hospital waste with high concentrations of antibiotics and disinfectants; and compounds from industrial activity, including heavy metals. Some pharmaceutical production facilities discharge very large amounts of antibiotics directly into the environment, resulting in concentrations up to or exceeding levels used to treat infection in humans.^{18,19} The elevated level of resistance found in the discharge vicinity is definitive evidence that selection for antibiotic resistance occurs in polluted environments.²⁰ However, antibiotic concentrations in most effluents, surface waters and soil environment could be 1 000 times lower than levels used in the clinic or raw industrial effluent.¹⁶ It is the low-concentration contamination that is of particular importance—the concentration is too low to be lethal to exposed bacteria, but sufficient to select for resistance.²¹ The question is where is the threshold at which antibiotics have no selective effect on microbial communities. At low antibiotic concentration, the acquisition of resistance may be reliant more on gene transfer from another bacterium, known as horizontal gene transfer. Therefore, single species studies of bacteria on agar plates are unlikely to give meaningful insights about the development of resistance in complex microbial communities present in the natural environment.

Concentrations in river water depend on wastewater treatment facilities as well as antibiotic use in the populations they serve. Treatment plants are generally designed to remove conventional pollutants, such as nutrients, organic matter, suspended solids and, to some extent, pathogens, but not antibiotics.²² Agricultural waste such as animal manure may also contain concentrations of antibiotics in the same order of magnitude as are used to treat infection. However, after adsorption to soil particles, some antibiotics get neutralized, while some remain active and exert a selection pressure on

Natural selection and antibiotic resistance



In the microbial world, competition always occurs between organisms by way of producing antibiotic molecules to inhibit others from thriving. Susceptible organisms perish. However, bacteria and fungi are known to have developed defence mechanisms to resist the antibiotic attack and survive, or in other words, become antibiotic resistant.



Resistance genes can pass to the next generation, and even between un-related bacteria via horizontal gene transfer. Overuse and misuse of antibiotic drugs as well as increased exposure to antimicrobial substances in the environment increases selection for antibiotic resistance among bacteria.

Video: Antibiotics and the environment: The quiet crisis



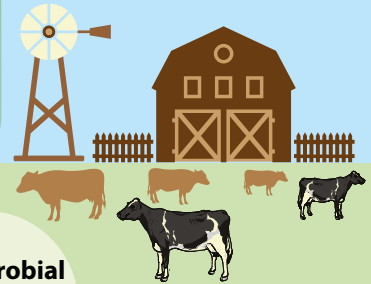
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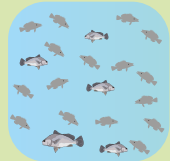
Antimicrobial resistance and the environment

The environment is key to antibiotic resistance. Bacteria in soil, rivers and seawater can develop resistance through contact with resistant bacteria, antibiotics, and disinfectant agents released by human activity. People and livestock can then be exposed to more resistant bacteria through food, water, and air.

Human antibiotic use jumped **36%** in the 2000s



Up to **75%** of **antibiotics** used in aquaculture may be lost into the surrounding environment



70% of **antibiotics** are used by **animals**

Manure fertilizers cause antibiotic contamination in surface runoff, groundwater and drainage networks

Antimicrobial use for livestock will jump **67%** by **2030**

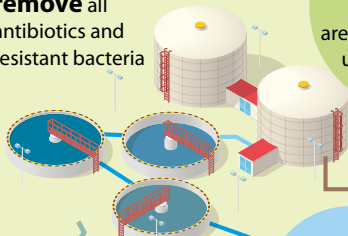
Antibiotics are increasingly used to boost animal growth in intensive farming, especially in developing countries

Antibiotics can be absorbed by plants and crops



Major waste flows including wastewater, manures and agricultural run-off contain antibiotic residues and antibiotic-resistant bacteria

Wastewater treatment plants **cannot remove** all antibiotics and resistant bacteria



Up to **80%** of **consumed antibiotics** are excreted through urine and faeces

30% of antibiotics are used by **humans**



Antibiotic resistant bacteria may be present in **raw source water** and **treated drinking water**

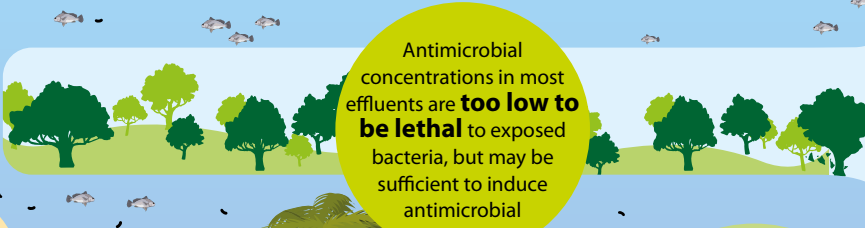


A vast array of **contaminants in municipal and industrial wastewater** increases pressure on bacteria to become resistant



More than 50% of **municipal solid waste** ends up in landfills and open dumps. This can include unused or expired drugs.

Antimicrobial concentrations in most effluents are **too low to be lethal** to exposed bacteria, but may be sufficient to induce antimicrobial resistance



Multi-drug resistant bacteria are prevalent in marine waters and sediments in close proximity to aquaculture, industrial and municipal discharges



▶ Video: Bacterial resistance and its impact on health



<https://www.youtube.com/watch?v=eDhhv31vuV8>

Photo credit: James Gathany

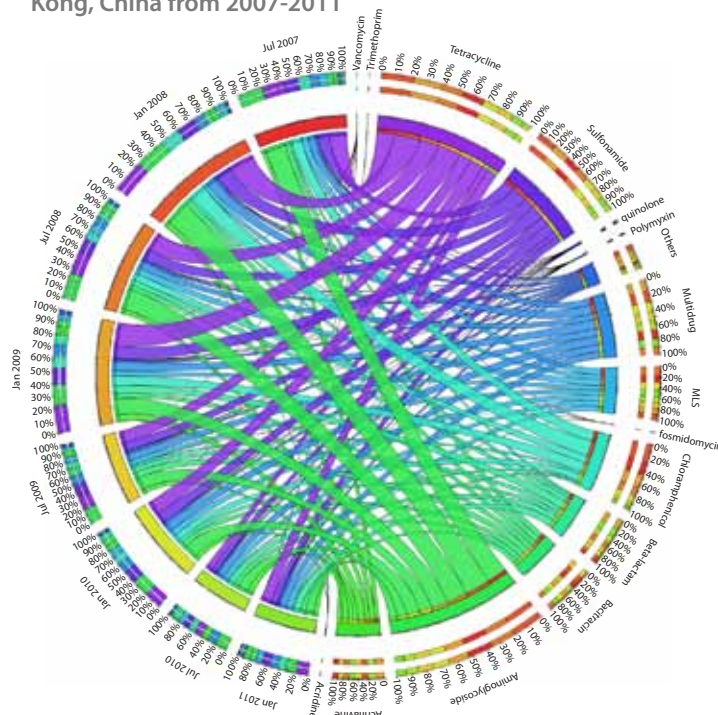
© University of Exeter

bacteria in soil.²³ Generating reliable temporal and spatial data on the exposure of microbial communities to antimicrobial residues in soil and water is vital to better understand the extent of selection that occurs in natural environments.^{24,25} The matter is further complicated by mixtures of antibiotic residues and other pollutants, which may combine to produce increased selection pressures compared to individual substances.²⁶ Evidence for indirect or co-selection for antibiotic resistance is accumulating for heavy metals such as silver, cadmium, copper, mercury and zinc, and for compounds with antimicrobial properties such as disinfectants and biocides.²⁶⁻³⁰ Heavy metals are ubiquitous in agricultural, industrial and urban environments. Therefore, it is likely that antimicrobial resistance may rise in exposed bacteria even when direct selective pressure from antibiotics is absent.

Because antibiotics and antibiotic-resistant bacteria come from the same source, they are often found together. Major waste flows including wastewater, animal manures and agricultural run-off also contain antibiotic-resistant bacteria. The discharge of untreated sewage is likely to be an important driver of increasing antibiotic resistance in the environment, however it is a very challenging problem to solve. Even in countries with high wastewater treatment investment and management strategies to reduce aquatic pollution from farming, large variations in numbers of antibiotic resistant bacteria are still found within river catchments. Conflicting

results have been found regarding the ability of wastewater treatment to reduce the amount of antibiotic resistant bacteria in effluent, with some studies showing efficient removal and others finding increased numbers of resistant bacteria in effluent compared to influent.²² The latter results suggest that wastewater treatment plants may be hot spots for horizontal gene transfer due to high bacterial density and nutrient richness.^{31,32} Therefore, wastewater and sewage sludge are important surveillance tools, allowing assessment of the abundance of antibiotic resistant bacteria and resistance genes in the human population.^{33,34}

Abundance of antimicrobial resistance genes in activated sludge from the Shatin wastewater treatment plant in Hong Kong, China from 2007-2011



Courtesy of Prof. Tong Zhang, University of Hong Kong

See also Yang et al. (2013)³³

The crisscrossing lines illustrate the abundance of resistance genes in eight sludge samples. The thicker the line is, the more abundant the class of resistance genes. For instance, genes resistant to aminoglycoside and tetracycline are the most dominant types detected in all samples.



Mitigating the discharge of antimicrobials into the environment

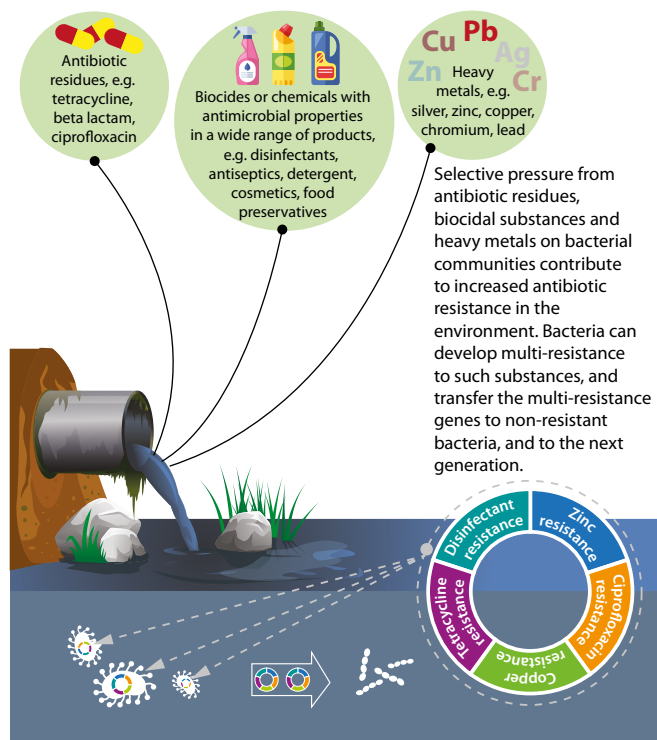
Current regulatory policies have been slow to properly consider discharge of antibiotics and antibiotic-resistant bacteria. Growing awareness of the potential for antibiotic residues to damage aquatic organisms has led to three antibiotic compounds being placed on the European Union Watch List of emerging water pollutants in 2015.³⁵ There are voluntary initiatives to reduce effluent concentrations of antibiotics by some drug manufacturers.²⁵ In September 2016 several leading pharmaceutical companies signed an antimicrobial-resistance roadmap presented to the United Nations with the environmental management of antibiotic-related production as a central theme.³⁶

Some co-selecting compounds such as triclosan, used in a wide range of consumer products, have been banned or restricted in various markets. The Association of Southeast Asian Nations has put a restriction on the maximum concentration of triclosan in cosmetic and personal care items.³⁷ The United States' Food and Drug Administration ruled in 2016 that over-the-counter antibacterial products containing triclosan and 18 other compounds should no longer be marketed due to findings that long-term exposure to these active ingredients could pose health risks such as bacterial resistance or hormonal effects.³⁸

Increased regulation of antibiotics, and of co-selective compounds, could drive the development of affordable mitigation and risk reduction solutions and stimulate discussion of responsibility for antibiotic residues and for the resistant bacteria they engender. Arguably, the antibiotic manufacturers, prescribers, farmers and even patients bear some responsibility for the damaging effects of antibiotic residues that reach the environment. Such significant changes in the way we tackle antibiotic resistance, particularly in the context of a One-Health approach, could transform incentives to reduce antibiotic use and to improve waste management practices.

Many mitigation strategies already exist that reduce or remove antibiotics and resistant bacteria from waste streams entering the environment: secondary and tertiary

Co-selection of resistance to antibiotics, metals and biocides



Video: Why did the FDA ban antibacterial soap?



Link: www.youtube.com/watch?v=9dExiRwh-DQ
Photo credit: Galushko Sergey/Shutterstock.com

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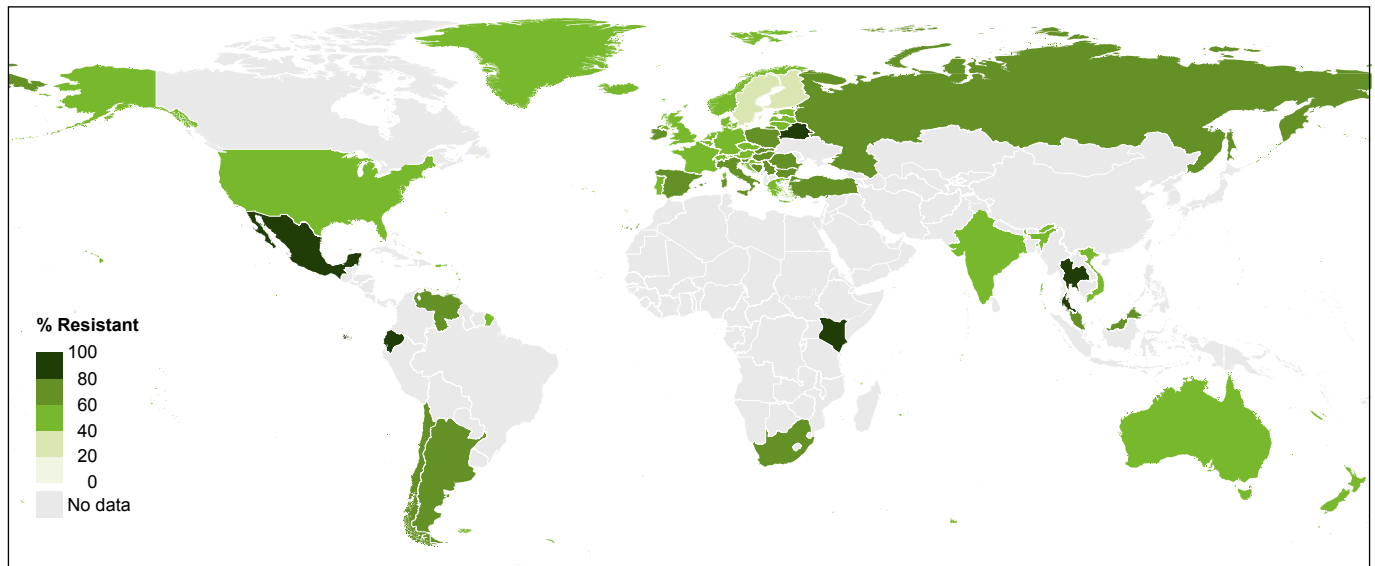
wastewater treatment; membrane filtration and ozonation that remove antibiotics and bacteria; and UV disinfection and heat treatment, which are even more effective at removing viable bacteria. These approaches have variable levels of effectiveness and some can produce unintended consequences, such as toxic by-products. Treating animal waste before application to land and simple methods to reduce aquatic pollution can also be used. Barriers to these approaches are largely financial and relate to the ability or desire of societies to change. There is an urgent need to better understand the risks posed by antimicrobial resistance in the environment and to develop sustainable mitigation technologies.

It has been argued that the complexity of the problem is so great as to be unfathomable, considering the likelihood of interactions among unimaginable numbers of bacteria with a seemingly endless facility for gene transfer, the complex mixtures of selecting compounds, and the diversity of resistance-building mechanisms.³⁹ With sufficient data this is

probably untrue, but the question remains whether we have time to wait for enough data to be generated before making decisions.

We know that wherever human activity occurs increased levels of antibiotics and antibiotic resistance emerge in the environment. We know that under laboratory conditions some antibiotics select for antibiotic resistance even at concentrations found in the natural environment. We also know that clinically important resistance genes that have recently emerged in pathogens originated in bacteria from the natural environment. Data are already available that show transmission is likely to occur via the food chain and through exposure to polluted environments. There is often a call for evidence-based decisions; but with a problem as complicated as antibiotic resistance, how much evidence is enough? Acquiring sufficient evidence of the type generated by clinical trials may be impossible or such a challenging task that we risk huge delays in the regulation of antibiotic use and implementation of mitigation strategies.

Percentage of invasive *E.Coli* isolates resistant to Aminopenicillins



Courtesy of The Center for Disease Dynamics, Economics & Policy (CDDEP). For more resistance maps, visit <http://resistancemap.cddep.org/AntibioticResistance.php>



Future research and activities to inform policy

Antibiotic resistance is increasingly found in clinically important pathogens, livestock production is intensifying in response to growing demand, and population growth and rapid urbanization are producing more pollution. Together these trends suggest that processes driving the proliferation of antibiotic resistance will continue for the foreseeable future, unless concerted and globally coordinated action intervenes. Hopefully, these trends will provoke us to better manage the issue and enable policies that take the critical roles of the natural environment into consideration.

Precautionary measures might include reducing the overall release of antibiotics, and of co-selecting compounds, into the environment by more controlled and judicious use as well as by tackling critical hotspots such as hospitals, drug manufacturing sites, wastewater treatment plants, and agricultural sources through improvements in sewage and wastewater management. Further precautions include ending the use of antibiotics as growth promoters in animal husbandry; minimizing the use of household and personal care products containing antimicrobial substances; and encouraging technological innovations that ensure newly developed antibiotics break down rapidly after providing their beneficial effects.

Responsible policies must be informed by fundamental research into the contribution of antimicrobial and co-selecting chemical contamination in the natural environment to overall levels of antimicrobial resistance, as well as the evolution and transmission of resistance. For instance, research on the fate of antibiotic residues upon contact with soil helps regulatory bodies understand which antibiotics remain bioactive—capable of exerting selective pressure—and therefore require more attention.²³ Similarly, gaining insights into the capacity of antimicrobials to select for resistance in aquatic environments can help us design more effective regulatory measures and wastewater management strategies based on the selection effects, rather than on discharge concentration. Communication of the findings to a wider audience is vitally important in increasing awareness of the issue among the public, policymakers and community leaders.

Welcoming Citizen Engagement to Monitor Antimicrobials in the Environment

To reduce further antimicrobial resistance, researchers need to understand how bacteria encounter antimicrobials and co-selecting compounds in diverse environments and how such exposure unfolds for the emergence and spread of resistance. Many challenges—such as time, resource and data limitations—impede our ability to answer such fundamental questions.

Recruiting help from civil society could complement the professional scientific and technical workforce, and including their contributions will make them part of the solution as well as build awareness. Involving various stakeholders across sectors could address data gaps and offer opportunities to gain new insights. It can help scientists to detect hotspots of antimicrobial pollution, map out patterns and identify intervention strategies.

For instance, online tools could motivate farmers to enter data on the types and amounts of antibiotics they are using and provide information regarding how antibiotic-contaminated wastewater is disposed. Interested consumers can enter data on their antibiotic use, disposal of expired drugs, or use of household products with antimicrobial properties. High school students can collect soil and water samples, or even fecal samples from indicator species of animals, for analysis in projects guided by scientists.^{40,41} Campaigns could be devised that host dedicated hackathon events, attracting programmers to help develop new tools such as phone apps for chemical identification and statistical analysis on concentrations and trends in timing.

When antibiotic treatment fails because of resistance, the response is to use more antibiotics. This has led to overuse and perennial demand for new antibiotics to replace those that are no longer effective. When medical doctors and veterinarians are confronted with infected patients who would benefit from antibiotic treatment, concern over antimicrobial resistance in the natural environment is not their priority concern. Nevertheless, viable antibiotics, co-selecting compounds and resistant bacteria must be prevented from entering the natural environment where they may foster emergence of new resistance genes. Without prevention, we will be at direct and significant risk of exposure to environmental reservoirs of antimicrobial resistant pathogens.

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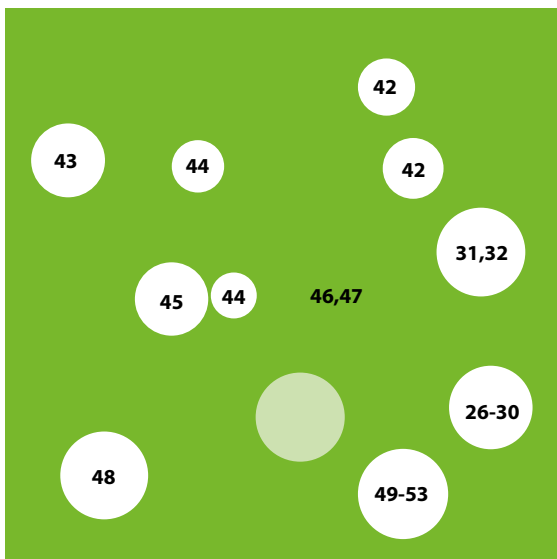
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Methicillin-resistant *Staphylococcus aureus* bacteria being enveloped by a human white blood cell
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