

ANTIMICROBIAL RESISTANCE

Wastewater: An unexpected highway for antibiotic resistance

Scientists have shown that extracellular DNA (exDNA) in wastewater and connecting river systems is an overlooked but important reservoir of mobile, high-risk resistance genes that influence how antimicrobial resistance persists and spreads. Jorisna Bonthuys reports.



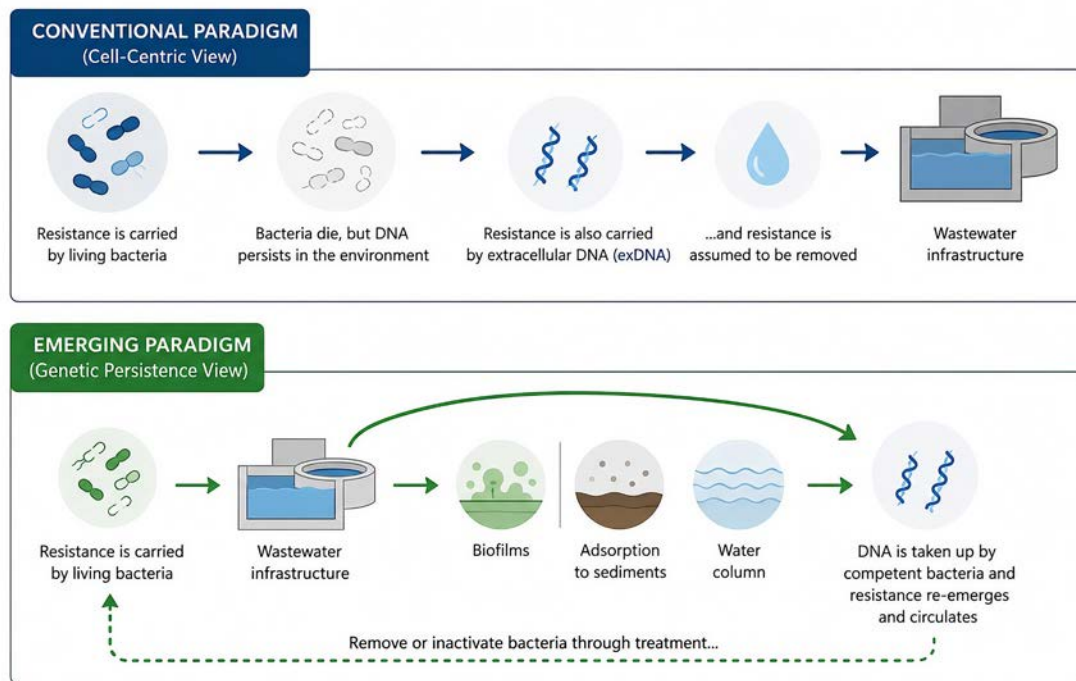
South Africa has more than 950 municipal wastewater treatment plants, yet only 13% comply with the minimum water discharge standards. Moreover, while the country's older wastewater treatment plants can remove harmful chemicals and eliminate bacteria, they were not designed to remove exDNA – genetic material released from bacteria during the treatment process. Despite such shortcomings, these facilities remain vital tools for public health monitoring because of their diverse microbial communities, dynamic ecosystems and key role in the sanitation system.

A study led by researchers from the DSTI-NRF Research Chair in African Microbiome Innovation at Stellenbosch University (SU) investigated how antimicrobial-resistant genes (ARGs) behave in wastewater treatment plants and the rivers connected to them. The team focused on nine wastewater treatment plants of different sizes and with different treatment technologies in the

City of Tshwane.

The findings were published in the journal *Cell Reports* in an article titled 'Persistence of high-risk antimicrobial resistance genes in extracellular DNA along an urban wastewater-river continuum'. The study revealed that wastewater and river systems are dynamic reservoirs of antimicrobial resistance (AMR), the ability of disease-causing microbes to withstand antimicrobial treatments.

AMR is already considered a major threat to health and development. South Africa, with its complex burden of infections and non-communicable diseases, has high rates of antibiotic resistance, which is a growing concern. The findings also indicate that exDNA is an overlooked yet important reservoir of genetic resistance that warrants greater attention from scientists, wastewater engineers, and public health officials.



From a cell-centric to a genetic persistence view of antibiotic resistance, highlighting the role of extracellular DNA (exDNA) in the persistence and circulation of resistance genes in wastewater environments.

"The results underscore the need to understand how AMR spreads through ecosystems and into human populations," says Dr John Paul (JP) Makumbi. Makumbi, a medical microbiologist and the paper's first author, is conducting postdoctoral research on this topic at SU.

Novel research approach

Wastewater-based epidemiology – using sewage to monitor community-level health trends, much as it has been used to track polio – has been in use for many years, initially concentrated in the Global North but now well established in African settings, including a growing number of South African studies. Increasingly, these efforts also draw on the One Health approach, which recognises the interconnection between people, animals, plants and their shared environment. This approach will also be important in tackling rising AMR threats, Dr Makumbi believes.

Most AMR-related research examines resistance genes within living bacteria. This gap is especially clear in low- and middle-income countries, where the high cost of tools to study environmental AMR has limited research. Much less research has examined resistance genes that are free in the environment and not associated with living organisms. This study aims to fill that gap.

The researchers sampled key points in 2023. At each sampling site, they collected raw influent and treated effluent. They filtered the samples through fine membranes to remove intact bacterial cells, leaving only free-floating genetic material dissolved in the filtrate.

From this, exDNA was extracted, purified and sequenced using shotgun metagenomics, a technique that reads genetic sequences directly without requiring prior cultivation of any

organism. The team screened the extracted genetic material for resistance genes and traced their likely taxonomic origins. The researchers then compared the effects of single-stage activated sludge processes (ASP-only) with combined ASP-biofilter systems on antimicrobial-resistant genes. ASP is a biological process for developing an activated mass of microorganisms capable of aerobically stabilising waste.

The treatment plants they focused on varied in scale and technology, serving catchment populations from a few thousand to over a million people. Four plants relied solely on the standard ASP process, the primary biological treatment method for municipal and industrial wastewater in South Africa, while five combined ASP with an additional biofilter stage.

What turned up in the sampling

As expected, resistance genes were more abundant in wastewater than in river water: raw influent carried 196 different resistance genes and effluent carried 187, compared with 84 upstream and 94 downstream in the rivers. Only six genes were unique to rivers, suggesting a shared resistome between treatment plants and the waterways they discharge into. The term resistome refers to the complete collection of antibiotic resistance genes and their precursors within a given microbial community.

The study also shows exDNA not only harbours high-risk antimicrobial resistance genes but also serves as an ecologically distinct AMR reservoir dominated by a few key groups. This free-floating genetic material is found outside cells in environments such as soil, aquatic ecosystems, and human tissues. The researchers found genetic material from two major bacterial groups, commonly known to exhibit high-risk resistance profiles. Both the *Pseudomonadota* and *Bacteroidota* groups are commonly associated with multidrug-resistant behaviour.

JP Makumbi



Researchers collect water samples at a wastewater treatment facility in an urban centre in South Africa to investigate how antimicrobial resistance genes move through water systems

Across all samples, the team identified 561 individual exDNA-associated resistance genes, representing 127 distinct genes and spanning 12 broad classes of antibiotics. Several of these are frontline weapons against serious infections; carbapenems and cephalosporins, in particular, are considered last-resort options when other antibiotics have failed.

"We found exDNA carrying resistant genes in the effluent," Dr Makumbi points out. "Sewage brings together microbes from many sources, including hospitals, abattoirs, homes and street runoff, thereby increasing opportunities for horizontal gene transfer and the emergence of novel resistance genes."

Even after conventional treatment killed bacteria carrying resistance genes, these genes persisted, floating free in the water and intact enough to be picked up by other living bacteria or viruses.

Upstream ecosystems are also key drivers of resistance gene spread in their own right, independent of any treatment plant's influence, the findings show. "This means that river systems function less like passive channels and more like hotspots where the cumulative signature of multiple pollution sources builds up," Dr Makumbi says.

Bacteria are notorious for sharing genes – and not only with each other while alive, Dr Makumbi explains: "They [bacteria] don't just share these genes from one live bacterial cell to another but can also pick up genes from DNA that has been

JP Makumbi



Researchers sampling downstream river water to assess the real-world impact of wastewater discharge on freshwater systems in a large city in South Africa.

released from a dying cell and then incorporate it into their own genome [the complete set of genetic instructions found in an organism]."

This process, known as horizontal gene transfer, allows organisms within or between species to acquire new traits – including antibiotic resistance – directly from their environment. "Bacteria naturally produce antibiotics, and the same bacteria also produce resistance genes that help them survive while killing their competition," Makumbi explains. That is the natural loop. The fact, however, remains that AMR is also a clinical problem we cannot avoid."

In wastewater treatment plants, this process [of sharing genes] is accelerated by overexposure to a toxic mix of untreated effluent from abattoirs, hospitals and industry, raw sewage and other pollutants. "Higher levels of ARGs in river samples likely result from the combined effects of these releases and the shared pool of resistance genes between treatment plants and river environments," Dr Makumbi notes. "This means a wastewater treatment plant can act as a superspreader of antimicrobial-resistant genes, shaping the genetic landscape of wastewater and freshwater environments."

The paper reports that multi-stage systems combining ASP with biofiltration did outperform ASP-only plants, reducing resistance genes across several antibiotic classes.

An old pipeline facing new threats

The study's findings highlight the need to rethink wastewater treatment plant design and develop smarter strategies that specifically target exDNA removal.

"We should protect our wastewater treatment plants by pretreating effluent from high-risk sources such as abattoirs, hospitals and industry before it enters the system," Makumbi says. "The same logic applies to treating effluent before it reaches the environment. Everyone will benefit from less polluted water entering our rivers."

Historically, many wastewater treatment plants were built to remove pathogens and harmful chemicals — not to specifically target and destroy free-floating DNA.

Some plants in South Africa and elsewhere are beginning to adopt more advanced approaches, such as ultraviolet treatment, to reduce the load of resistance genes. But as Makumbi notes, "progress remains slow".

"We need to consider the full pipeline of resistance when tackling antimicrobial resistance," Makumbi says. "This means that we will need to incorporate techniques like shotgun metagenomics within our routine surveillance to see what is happening within our systems. It doesn't have to be in daily surveillance, but to have some integration of some of these techniques, for example, some long-term studies, especially through government agencies that may have the resources to do this."

Although combining ASP and biofiltration showed slightly better efficacy at lowering ARG abundance, the presence of high-risk ARG classes makes it clear that ongoing breakthroughs in treatment technology are vital.

Biological treatment alone, the study suggests, may not be enough to address exDNA persistence. Strategies that disrupt genetic material in the first place could further reduce the risk these genes pose once they're released.

The stakes extend well beyond academic interest in microbial ecology. Treated wastewater is increasingly reused for irrigation, industrial processes, and, in some regions, even for potable use — a trend likely to grow as water scarcity intensifies.

"In water-scarce countries, there is a move to ensure that effluent quality supports reuse for certain purposes as a water conservation effort," Dr Makumbi notes. "If exDNA carrying high-risk resistance genes passes largely unaffected through treatment, residual genes in treated effluent present a route for reintroducing them into the human population. We need to consider the full pipeline of [AMR] resistance and follow a One Health approach when tackling antimicrobial resistance."

Prof Thulani Makhalanyane, who holds the DSTI-NRF Research Chair in African Microbiome Innovation and leads the broader research programme on microbes at SU, frames the challenge in a regional and global context: "Future wars will in part be based on water security and AMR. This is especially true in Africa, where ageing infrastructure, poor management, and an inability to sufficiently integrate science into policymaking are compounding the problem. We hope that the findings from this study will add to the call for action to ensure water security."

Next steps

At this stage, the exact mechanisms by which ARGs survive and move within microbiomes and between ecosystems remain unclear. More work is also needed to quantify transformation rates in natural settings, evaluate wastewater treatment technologies that remove exDNA, and trace gene flow in wastewater treatment facilities and connected rivers.

With this in mind, Dr Makumbi will expand his research efforts to the Western Cape. He will be investigating antimicrobial resistance genes in exDNA along urban wastewater systems and rivers in the City of Cape Town and elsewhere.

"If we are to slow the advance of antimicrobial resistance, we must broaden our surveillance and mitigation strategies to account for the mobility of genetic material itself," Dr Makumbi says. "Incorporating exDNA surveillance into routine and long-term monitoring programmes will give a fuller picture of AMR risk and help evaluate treatment effectiveness."

"That means treating not only microbes but their DNA, rethinking wastewater infrastructure, and aligning environmental management with clinical stewardship. The persistence of high-risk antimicrobial resistance genes in exDNA along wastewater-river continuums is a warning, and the time to act is now," he concludes.

To view the original article, visit: <https://www.sciencedirect.com/science/article/pii/S2211124726002068>

More about antimicrobial resistance

- The World Health Organization (WHO) defines antimicrobial resistance (AMR) as instances when bacteria, viruses, fungi and parasites do not respond to antimicrobials.
- AMR is a major global health and development threat. Studies show that almost 5 million deaths per year can be linked to AMR — more than HIV/AIDS and malaria combined. It is projected that by 2050, the figure will rise to 10 million per year, at a cost of US\$100 trillion to the global economy.
- The misuse and overuse of antibiotics, for example, taking them when they are not needed or taking the wrong type, remains one of the main drivers of AMR. Lack of adequate access to appropriate new and existing vaccines, diagnostics and medicines also contributes to the crisis.
- Most human deaths from antimicrobial resistance occur in sub-Saharan Africa.
- One in six laboratory-confirmed bacterial infections worldwide was resistant to antibiotics in 2023.
- Experts warn that antimicrobial resistance in farm animals, if unchecked, could lead to a pandemic.
- AMR affects all countries, regardless of income, and spreads across borders. People living in low-resource settings and vulnerable populations, such as the very young and very old, are especially impacted.
- Between 2018 and 2023, antibiotic resistance rose in over 40% of the pathogen-antibiotic combinations monitored, with an average annual increase of 5–15%.

Sources: WHO, The Lancet and Science