# CRISPR-based method enhances detection of antibiotic resistance in wastewater

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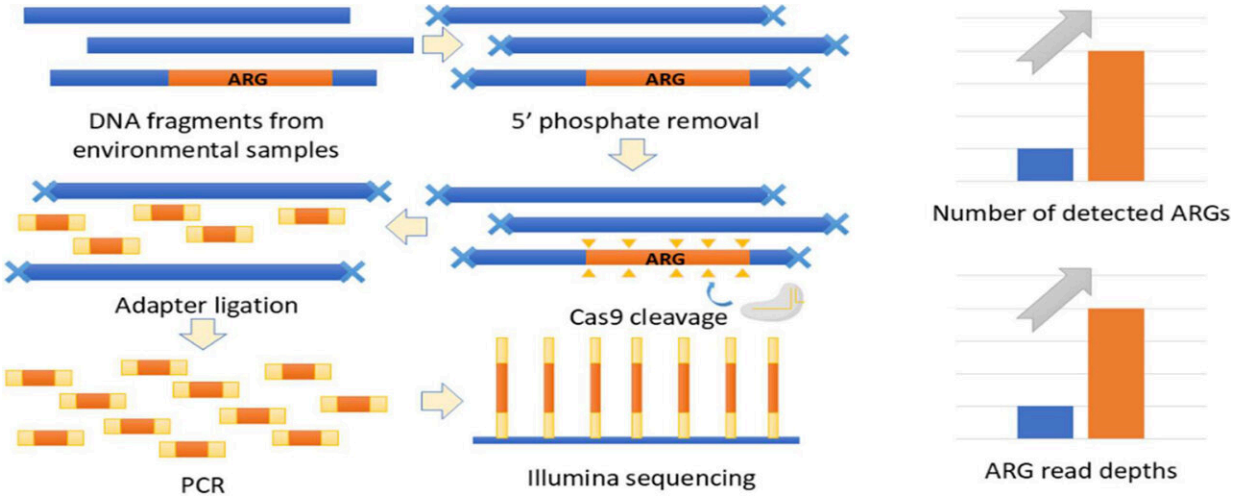


Credit: *Water Research* (2024). DOI: 10.1016/j.watres.2024.123056

Antibiotic resistance is a global concern that threatens our ability to prevent and treat bacterial infections in humans and animals. To better monitor the emergence and spread of resistance, researchers at the Carl R. Woese Institute for Genomic Biology have developed a CRISPR-enriched metagenomics method for the enhanced surveillance of antibiotic resistance genes (ARGs) in wastewater.

The research is published in the journal *Water Research*.

While antibiotics are powerful modern tools for combating infections, bacteria change and adapt over time in response to antibiotic exposure, therefore decreasing effectiveness. Widespread overuse and misuse of antibiotics in the health care and food industries further accelerate this problem.

Beyond direct exposure to antibiotics, resistance is also passed between different bacteria through the transfer of small pieces of bacterial DNA called antibiotic resistance genes. There are over 5,000 identified ARGs, and these genes can be found in clinical samples, as well as bodies of water, originating from hospitals, farms, and sewage systems.

"ARGs can reduce the life-saving power of drugs used to treat bacterial infections," said Helen Nguyen (IGOH), a professor of civil and environmental engineering at the University of Illinois Urbana-Champaign. "Wastewater detection of ARGs with clinical significance allows public health authorities and physicians to anticipate what is circulating in communities."

Wastewater contains numerous different ARGs mixed together with genetic material from various sources, including humans, viruses, and bacteria. Because ARGs only make up a tiny percentage of the total DNA content, uncovering them in wastewater samples requires sensitive detection methods. The most common technique is quantitative polymerase chain reaction (qPCR). This method uses RNA guides called primers to identify the specific DNA sequences of known ARGs, which are then amplified for detection.

"qPCR is a sensitive method that many people in public health are well-trained to do, but it requires primary design and validation, which is very time-consuming," said Yuqing Mao, a doctoral student in civil and environmental engineering who was the first author of the paper. "Since qPCR is used to pull out targeted gene sequences, all the other genetic

material in the sample remains completely unknown."

The second method, metagenomics, is not as sensitive as qPCR, but captures a more complete story of the genetic information contained in a sample. Metagenomics involves breaking all the sample DNA into millions of smaller fragments which are simultaneously sequenced using next generation sequencing technologies. Computational algorithms piece together the full DNA sequences for comparison against databases to determine their identities.

"ARGs make up less than 1%—probably even closer to 0.1%—of DNA in the sample. Using standard metagenomics methods, 99.9% of the DNA detected is not associated with ARGs," Mao said.

To enrich the amount of ARG-associated fragments in the samples, Mao, Nguyen, and their collaborator, Joanna Shisler, who is affiliated with the Department of Microbiology at Illinois, leveraged the CRISPR-Cas9 system—a highly effective tool for gene editing.

The DNA is fragmented in random locations when using standard metagenomics methods, but the incorporation of CRIPSR-Cas9 allows for targeted fragmentation within ARGs. By designing a pool of 6,010 different guide RNAs that could specifically bind to DNA at different sites found in ARGs, the Cas9 protein could be directed to cut at these locations.

"Our new CRISPR method increases the abundance of ARG fragments in the sample, which increases their chances of being read and detected. CRISPR also has better potential for multiplexed assays than something like PCR because the molecular interaction is simple and straightforward for CRISPR," Mao said.

Their new method lowered the detection limit of ARGs by an order of

magnitude, from $10^{-4}$ to $10^{-5}$, compared to standard metagenomics, and found 1,189 more ARGs and 61 more ARG families that are low in abundance in wastewater samples.

As a sixth-year graduate student, Mao built this project from the ground up—overcoming scientific obstacles and learning many new techniques along the way. She said, "The first time I got the sequencing results, I never expected how much more sensitive it would be compared to the regular method—it detected many more ARGs than we thought it would. After finishing the project, I feel like I have grown up."

But while this work is wrapped up, Mao and Nguyen are already pursuing multiple new directions, including expanding the applications of their CRISPR-Cas9 metagenomic method to a broader range of environmental samples and using their results to guide the design of new qPCR primers.

**More information:** Yuqing Mao et al, Enhanced detection for antibiotic resistance genes in wastewater samples using a CRISPR-enriched metagenomic method, *Water Research* (2024). DOI: 10.1016/j.watres.2024.123056

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