

Antibiotic resistance surveillance tools in Puerto Rican watersheds after Hurricane Maria

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Civil engineering doctoral student Benjamin Davis takes water samples from the Rio Marin River in Patillas, Puerto Rico, in early 2018. Credit: Virginia Tech

When Hurricane Maria made landfall, devastating Dominica, St. Croix, and Puerto Rico in September 2017, flooding and power outages wreaked havoc on the debilitated land, resulting in the contamination of

waterways with untreated human waste and pathogenic microorganisms.

Six months after the deadly Category 5 hurricane, Virginia Tech civil and environmental engineering Professor Amy Pruden led a team of Virginia Tech researchers, including Maria Virginia Riquelme and William Rhoads, then post-doctoral researchers, who packed their bags and lab supplies and headed to Puerto Rico.

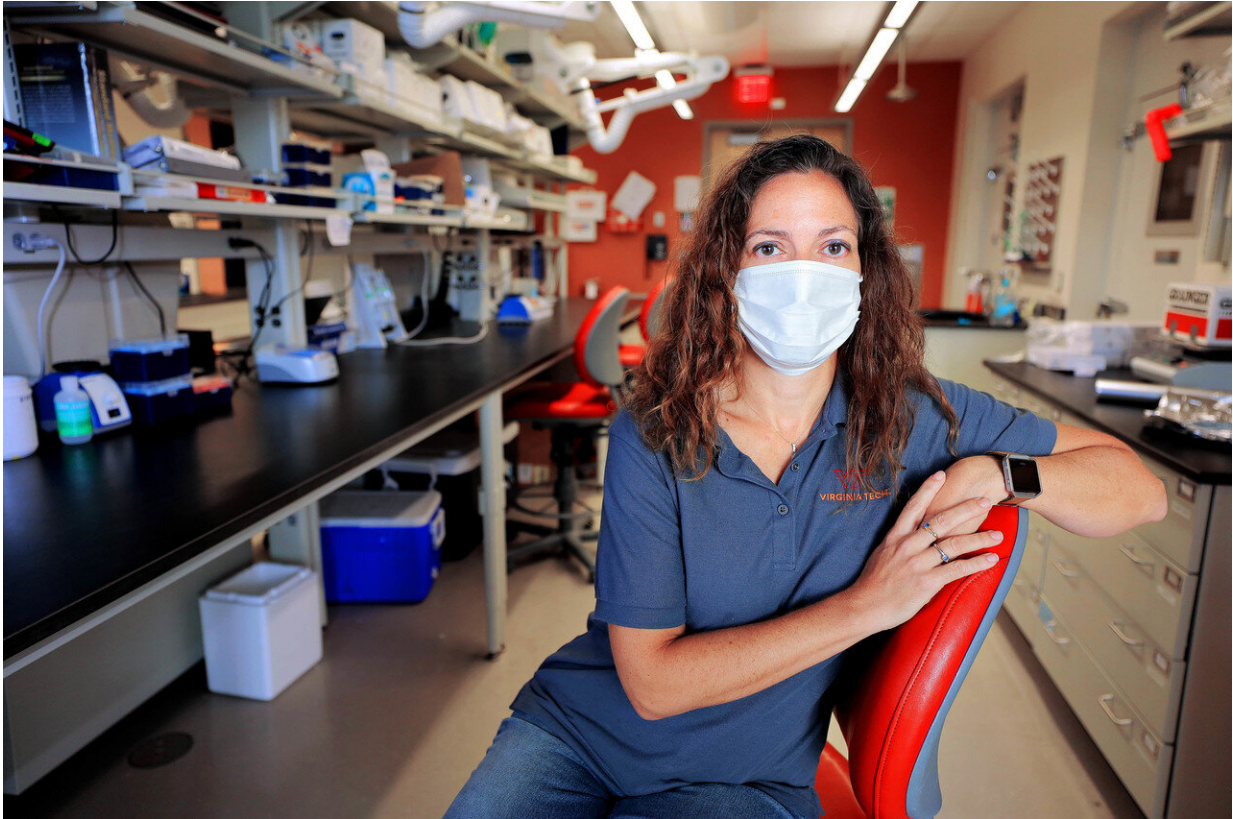
The island territory of the United States located in the northeast of the Caribbean Sea had been devastated, plunging its 3.4 million inhabitants into crisis. The mass destruction presented a critical opportunity for the researchers to study how wastewater infrastructure damage might contribute to the spread of antibiotic resistance—a growing global public health threat.

In a study published in American Chemical Society's *Journal of Environmental Science & Technology*, Virginia Tech researchers and international collaborators have further developed an innovative antibiotic resistance surveillance approach by applying DNA sequencing techniques to detect the spread of disease in watersheds impacted by large-scale storms.

"This study is a critical step toward establishing a unified and comprehensive surveillance approach for antibiotic resistance in watersheds," said Pruden, the W. Thomas Rice Professor of Civil and Environmental Engineering. "Ideally, it can be applied as a baseline to track disturbances and public health concerns associated with future storms."

Over the past decade, Pruden, a microbiologist and environmental engineer, has worked with her students using next-generation DNA sequencing, a specialty of Pruden's, to examine *Legionella* strains as they operate before, during, after, and outside of Legionnaires' disease

outbreaks in various towns and cities across the country, including Flint, Michigan.



Amy Pruden, Virginia Tech researcher. Credit: Virginia Tech

With RAPID funding from the National Science Foundation and collaborating with principal investigator Christina Bendoragoda, research scientist at the University of Washington with expertise in watershed modeling and geospatial analysis, Virginia Tech researchers teamed up with Graciela Ramirez Toro, professor and director of the Centro de Educación, Conservación e Interpretación Ambiental, and her research group at the local Interamerican University in San German, Puerto Rico.

Together, they identified three sampling sites in watersheds with distinct land-use patterns and levels of wastewater input that were ideal for tracking down geospatial patterns in occurrence of bacterial genes that cause antibiotic resistance.

Pruden's doctoral student and first author of the paper Benjamin Davis used a method called shotgun metagenomic DNA sequencing to detect antibiotic resistance genes in river water samples from three watersheds, including samples collected by hiking to far upstream pristine reaches of the watersheds and downstream of three wastewater treatment plants. Metagenomics is the study of genetic material recovered directly from environmental samples.

Analysis of the data revealed that two anthropogenic antibiotic resistance markers—DNA sequences associated with human impacts to the watershed—correlated with a distinct set of antibiotic resistance genes, relative to those that correlated specifically with human fecal markers.

A clear demarcation of wastewater treatment plant influence on the antibiotic resistance gene profiles was apparent and levels were elevated downstream of wastewater treatment plants, resulting in a high diversity of genes impacting resistance to clinically important antibiotics, such as beta lactams and aminoglycosides, in the watershed samples. Some of the beta lactam resistance genes detected were associated with deadly antibiotic-resistant infections in the region and showed evidence of being able to jump across bacterial strains. Beta lactam resistance genes were also noted to be more accurately predicted by anthropogenic antibiotic resistance markers than human fecal markers.

Although baseline levels of antibiotic resistance genes in Puerto Rican watersheds prior to Hurricane Maria are unknown, surveillance methodologies like these could be used to assess future impacts of major storms on the spread of antibiotic resistance, the researchers said.

Many international communities will likely not have access to sophisticated metagenomic-based monitoring tools in the near future, but the identification of single gene targets, such as the anthropogenic antibiotic resistance markers, make watershed surveillance of antibiotic resistance much more accessible. And such genes can be quantified directly by quantitative polymerase chain reaction, yielding cost-effective, rapid results in less than a day.

More information: "Demonstrating an Integrated Antibiotic Resistance Gene Surveillance Approach in Puerto Rican Watersheds Post-Hurricane Maria" *Environmental Science & Technology* (2020). pubs.acs.org/doi/abs/10.1021/acs.est.0c05567

Provided by Virginia Tech

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