New Mexico Water Resources Research Institute
New Mexico State University, MSC 3167
PO Box 30001, Las Cruces, NM 88003-8001
(575) 646-4337 Fax 646-6418
http://wrri.nmsu.edu

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Contact: Cathy Ortega Klett 575-646-4337

Tracking Antibiotic-Resistant Microbes

Story by Will Keener, New Mexico Water Resources Research Institute (attached photos)

To address growing concerns about antibiotic-resistant microbes in the natural environment, a method is needed to understand the pathways of these organisms from their sources. New Mexico State University researchers have reported a successful first step, developing a way to link sources of antibiotics and antibiotic resistance in the community to antibiotic-resistant microorganisms found in wastewater treatment plants and their effluent.

"Our long-term goal is to identify and quantify the sources that are contributing most to the problem," says Jesus Sigala, a graduate student in NMSU's Plant and Environmental Sciences Department. "If a single source proves to be the dominant contributor, some type of direct pre-treatment might be a required and recommended option. If diverse sources are found to contribute to the antibiotic resistance in the effluent, then improved treatment options at the wastewater treatment plant will be needed. We still have a lot to learn, but options are available."

Jesus Sigala prepares culture plates for use with wastewater samples. He is now continuing his research as a graduate student.

Working with Adrian Unc, Assistant Professor in the Plant and Environmental Sciences Department, Sigala submitted a first report on the research last year. The project, funded in part by the New Mexico Water Resources Research Institute, showed several promising results:

The primary clarifier (foreground) at the Las Cruces Wastewater Treatment Plant was one of several places where wastewater samples were taken for the study.

- The method used in the tests was able to discriminate between some community wastewater sources.

- The method discriminated resistant bacteria from a spectrum of other bacteria found in a wastewater treatment plant.

- Two antibiotic types were found to be especially helpful in identifying hospital wastewater sources.

To conduct the study, Sigala and Unc sampled four wastewater source streams, each serving a distinct area, in the Las Cruces, New Mexico, wastewater system in 2008. They compared those to other samples taken during the same period at the city's water treatment plant after different treatment stages. Generally, wastewater may originate in residential or industrial areas or it may include agricultural runoff, and medical sources.

Beginning in 2008, the researchers took samples at four "lift stations," where city wastewater is pumped to higher elevation so that it can then drain to the treatment plant. Three samples taken over two days were mixed to get a composite – more representative – sample. The project made use of wastewater treatment plant samples that are taken routinely for lab testing. "We took the same samples at the different treatment stages and from the effluent," Sigala says.

With the composite samples, the process of growing the bacteria on agar plates began. The plates used two types of agar, one simulating a nutrient-rich environment, the other a nutrient-poor one. The plates were then treated with four distinct antibiotic types to identify the resistant strains. Other "control plates," without antibiotic amendment showed the diversity of organisms found in the samples.

In a laboratory providing special protection to researchers and samples, DNA from the resistant microbes was extracted using a commercially available kit. Next, the amount of DNA was measured using an ultraviolet light measuring system. "You need to know how much you have before the next step," says Sigala. In that step a special enzyme is used to replicate the DNA. "Without this reaction there would not be enough DNA. You need to amplify it for later steps," Sigala explains.

A process called DGGE, for denaturing gradient gel electrophoresis, separates the amplified DNA according to organism type. DNA strands are connected by bases that vary in the strength of their hydrogen bonds. Under electrical current and in the presence of certain chemicals, the DNA strands migrate and settle at distinct locations in the gel, depending on the order and type of their bases. The gels are then stained and scanned, to better see different bands or patterns, called "fingerprints." A software program interprets the fingerprints, including the number and location of the bands, and provides a data matrix for researchers to analyze.

Statistical analysis of the many factors and variables is conducted on the data to help sort out the bewildering array of possibilities. "We selected four distinct types of antibiotics, including some older types and a new antibiotic. We saw that resistance was detected all across the sources," Sigala says. "With older antibiotics you see increases in resistance."

To improve the approach, the researchers hope to develop a fingerprint comparison protocol for each potential source and be able to evaluate it against data from the water treatment plant. In the initial project, the research showed that erythromycin-resistant and cefaclor-resistant microbes are most similar to hospital sources. “If we were able to develop a source-tracking protocol, we would possibly recommend those two antibiotics as source discriminators,” says Sigala.

Other data showed that some effluent fingerprints don’t coincide with any source. The next step is to extend sampling periods throughout the year and cover all eleven lift stations in the city. Work started on that with sampling in February. Sigala, who earned his bachelor’s degree in 2009, is continuing the project for his master’s degree project.

Last November, Sigala and Professor Unc traveled to Leeds, England, where the graduating senior presented his research results at the 14th European Biosolids and Organic Resources Conference. “I got some questions and some suggestions that helped me,” Sigala says of the experience.

“Our proposed protocol will be further verified using more complex molecular methodologies such as gene microchips and pyrosequencing,” says Unc. Sigala will collaborate with another graduate student and the team will take advantage of expertise developed at other universities. “Successful completion of this project will result in a significant increase in our understanding of the health risks associated with wastewater collection and treatment, and will offer a novel and rapid method for evaluating the significance of individual sources of antibiotic resistance to the system,” says Unc. “Eventually we hope to verify our approach in other wastewater treatment systems within and outside New Mexico.”

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Will Keener is a writer with the New Mexico Water Resources Research Institute. The institute is a nonprofit organization that funds water-related projects at all of New Mexico’s universities.