A water sample students will test for antibiotic-resistant DNA.

Searching for KILLER DNA

Faculty and students dive into research
that identifies antibiotic-resistant genes in water

BY ALEJANDRA NAVARRO • PHOTOGRAPHS BY FRANK POOLE

ON THE EDGE OF THE THAMES RIVER IN NORWICH, CONN., near her home, graduate student Sapna Patel hunts for trouble. She is searching for DNA—specific pieces of genetic material with coding that can turn ordinary bacteria into antibiotic-resistant "super germs."

Patel scoops river water into a plastic cup and stares at the cloudy sample that may contain traces of the microbial troublemaker. Each year, antibiotic-resistant germs cause life-threatening infections in nearly 2 million Americans and kill approximately 90,000 people, according to a Centers for Disease Control 2006 report. They are strong enough to resist many common antibiotics once used as a first line of defense against bacterial infections.

News organizations have placed much attention on methicillin-resistant Staphylococcus aureus, a bacterial infection known as MRSA, which has become the fourth-largest killer in the United States, surpassing the AIDS virus and taking 19,000 lives annually. Recently, hospitals have seen a rise in cases of the potentially deadly intestinal superbug, clostridium difficile (C. difficile).

Like a copy machine, PCR equipment duplicates DNA to identify it.

"It's very scary," says Patel. She encountered a patient with MRSA while working at a nursing home during her undergraduate years, and witnessed how quickly the germ can attack the body. She understands the importance of finding a way to stop the spread of these germs and is eager to help find one.

She is part of a research project spearheaded by Lisa Cuchara, associate professor of biomedical sciences. This year, Cuchara, Patel and senior Lindsey Maglio are searching for the presence and prevalence of 14 strains of antibiotic-resistant DNA in the lakes, ponds, rivers and streams in New England. Kristen Wolfe, an adjunct biology professor, is assisting with the project.

Cuchara hopes that within a year, she will have preliminary findings that can help her get funding to continue the research. "We will understand better how the DNA got in the water," Cuchara explains. Eventually, this research could help to determine an acceptable limit for the amount of antibiotic-resistant DNA in bodies of water.

Her work parallels studies currently being done by researchers in other corners of the country, and her findings could validate or contradict their conclusions. She hopes to unearth details about this critical problem that may promote collaborative investigations or entice larger institutions and organizations, such as the Centers for Disease Control, to launch more extensive studies.

Her goal is to provide not only a foundation for future research, but also a foundation for future researchers, in particular, her students. Quinnipiac is a teaching institution, but research plays an important role in students' education across disciplines. It is particularly important for students in the sciences.

Cuchara's project, which is expected to last several years, is in its infancy. She began collecting water samples last summer with health sciences graduate student Padma Vijayan and senior Jessica Dunlap. Vijayan of Monroe, Conn., completed an independent research project related to Cuchara's research. Dunlap was one of five science students selected for a Summer Undergraduate Research Fellowship, where students conduct research under the supervision of a professor during the summer.

Students compete for a spot in the eight-week research program. In addition, the SURF students meet regularly to share their research methods and findings on their respective projects.

"If they are not aware of what tools are out there to help them answer questions and don't have experience in evaluating the results and the ethical consequences of their research findings, then we really haven't taught them anything," explains Professor Joan Bombace, chair of psychobiology. She also directs the Interdisciplinary Research program and oversees students in the SURF program.

From this experience, Patel is gaining skills that will help her get into medical school after graduation. Patel, who earned a BS in biology and biochemistry at Eastern Connecticut State University, says she would like to conduct clinical studies at a university hospital. She has found the research so interesting, she hopes to find a related topic that she could develop into a master's thesis.

Associate Professor Lisa Cuchara, and graduate students Sapna Patel, Padma Vijayan and senior Lindsey Maglio prepare DNA samples for testing.

Cuchara's philosophy is that "education is not the filling of a pail, but the lighting of a fire," which applies to both her teaching and research. "If you can follow a Jell-O recipe, you can perform gel electrophoresis with DNA," she says of the process used to separate DNA by size. "The trick is to inspire someone to want to learn how to investigate the questions and then work with them to analyze and understand the results that are obtained."

Cuchara wants her students to get "their brain cells engaged" and their hands dirty. That's exactly what they are doing both in the field and in the lab.

Cuchara's team is seeking genes that are resistant to tetracycline, sulfonamide, ampicillin and chloramphenicol. Eventually, the professor plans to expand her project to search for genes that are resistant to other antibiotics, such as methicillin (to which MRSA is resistant), and evaluate samples of soil, as well as water. In the lab, students are extracting the DNA from water samples and amplifying the DNA via PCR (polymerase chain reaction). This process enables researchers to produce millions of copies of a specific DNA sequence to detect antibiotic-resistant genes.

Vijayan appreciated working in the lab with Cuchara. "When I began this project, I had the theoretical knowledge, but I now have learned more hands-on techniques in the lab," says Vijayan, who plans to become an environmental scientist.

Conducting a research project is a lot more work than Maglio expected, but worth it. The team has had to develop protocols, or procedures to follow, and a plan outlining how the project will unfold.

"It's more intricate and has a lot of different pieces to it," she said. For Maglio, the experience is good preparation for graduate school.

"It's a privilege to be able to work on something that really could have an influence on some serious problems," she says. "It's definite-

FINDING MRSA FASTER

Heather Miller MHS '08 of Guilford, Conn., discovered an easier and faster way to find the strain type of methicillin-resistant Staphylococcus aureus, the deadly antibiotic-resistant bacteria known as MRSA. By identifying the different strains, hospitals can prevent these "super bugs" from spreading and becoming stronger.

She conducted her research while interning at the Veteran's Administration Hospital in West Haven, Conn., where she now works. She teamed with VA personnel and biology professor Ken Kaloustian. The study became her master's thesis, and in October, she presented her findings at the Interscience Conference on Antibicrobial Agents and Chemotherapy in Washington, D.C.

Miller tested the accuracy of new equipment that automatically extracts DNA (or RNA) from a sample, as opposed to the traditional manual extraction process that takes twice as long to test half the samples. Once DNA is isolated, hospital labs can amplify it (make millions of copies) and identify the strain by comparing DNA fragments to an existing library of DNA. This enables the staff to distinguish patients with different strains of the bacteria and determine if the germ is spreading; for example, a health care worker unaware that he or she is carrying the bacteria.

Miller says, "If you have a Typhoid Mary in your hospital, you want to find that person and make sure he or she is safe and gets treatment."

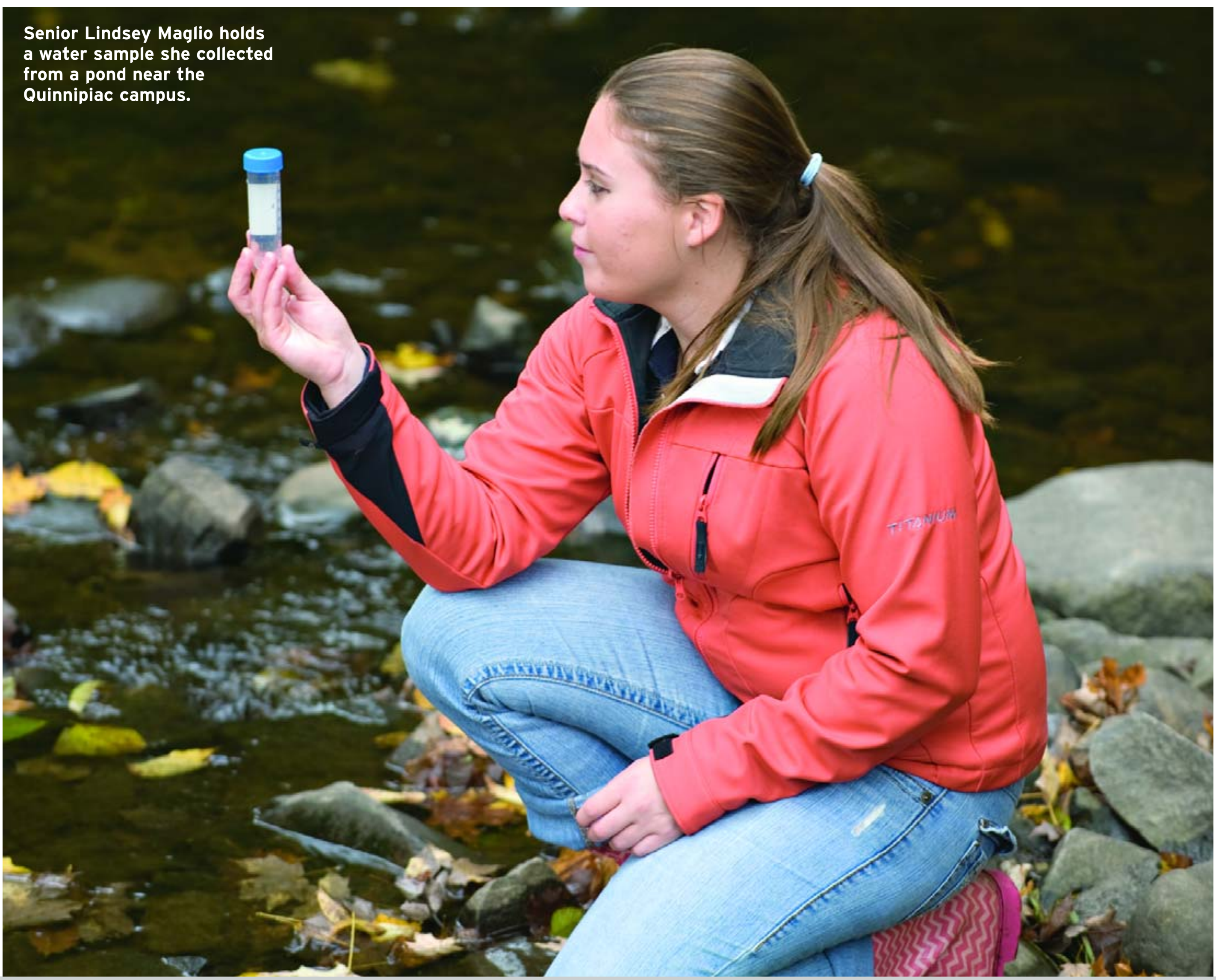
“Science doesn’t always work out the way you think it will. The challenge—and the possibility of finding something new—is the part of science that fascinates me.” —SAPNA PATEL

ly eye-opening and it’s very cool to be working on it.”

So far, water samples have been collected from several rivers and lakes, including Sleeping Giant Creek, Todd’s Pond, Quinnipiac River, Mill River, Thames River, Sackett Point and various other places in New England.

Cuchara is paying close attention to waterways near farms and ranches that breed livestock, such as chickens and cows. Seventy-five percent of all antibiotics are used for livestock production to treat sick animals or prevent disease outbreaks. These medications also are used as a cheap and easy way to grow larger animals. The antibiotics, however, pass through animals in excrement that eventually seeps into nearby bodies of water. There are five times as many domestic food animals as there are people in America, and their daily fecal excretion can be 400 times greater than that of humans, Dr. Stuart B. Levy says in his book, *The Antibiotic Paradox: How the Misuse of Antibiotics Destroys Their Curative Powers.*

The European Union has tried to address the problem by banning antibiotic use in animals, but the United States is still grappling with



Senior Lindsey Maglio holds a water sample she collected from a pond near the Quinnipiac campus.

the issue. Cuchara hopes that her research will support the hypothesis that correlates antibiotic use in animals and the presence of antibiotic-resistant DNA in water.

"Perhaps this will change the practices in agriculture or change the conditions for animals," Cuchara says. "It will get people thinking about what we should be doing about it."

Part of the problem is the proliferation of antibiotics use in both humans and animals. In 1929, Alexander Fleming discovered penicillin when he noticed that fungi on a petri dish created a protected area where bacteria would not grow. By the 1940s, penicillin was available and crushing bacterial infections. Three decades later, doctors were so confident about the power of antibiotics, they began prescribing them more frequently, and for ailments other than bacterial infections, such as the flu virus. Antibiotics don't work on these viruses.

The World Health Organization points to several societal factors that have contributed to the spread of these disease-causing "super germs," including overcrowding in urban areas; a growing geriatric population in hospitals and nursing homes where these germs are present and spread easily among people with weak immune systems; and the growth of global trade and travel.

When people use antibiotics, about 95 percent of the drugs are flushed from the body and end up in the sewage system. People also flush unused drugs down the toilet, contributing to this DNA pollution. It's not surprising that studies have found numerous pharmaceuticals in well water and city water systems across the country.

To exacerbate the problem, if a person has an infection caused by bacteria that is resistant to a specific antibiotic, such as tetracycline, that antibiotic obviously will not kill the resistant bacteria. The tetracycline, however, will kill other bacteria—including good bacteria that keep the body healthy. The demise of weaker bacteria only gives resistant bacteria room to grow, and possibly, exposure to other types of resistant genes.

Today, hospitals must rely on more powerful antibiotics—some of which were once considered the last line of defense. Bacteria have proved to be formidable opponents, quickly gaining genes resistant to new antibiotics as they are developed and used.

There are a few ways bacteria become resistant to antibiotics, according to the Food and Drug Administration. First, they can naturally mutate to become immune to antibiotics. In addition, bacteria can share their antibiotic-resistant DNA, passing it along like a secret war map between soldiers. The two don't even need to be the same type of bacteria for this exchange to take place. Bacteria also can capture DNA floating in water or buried in soil, left behind by dead bacteria. They adopt the traits on that bit of DNA. That is what interests Cuchara most.

"DNA stays in the water long after the bacteria have died," she explains. Most water filtration systems don't eliminate DNA, and some water treatment plants have conditions that could encourage the spread of these genes between bacteria.

The bottom line is researchers have a lot to learn about these "super bugs" and their prevalence in water. Cuchara's team is eager to dive in.

"Finding the results might be challenging. Science doesn't always work out the way you think it will," Patel admits, adding, "The challenge—and the possibility of finding something new—is the part of science that fascinates me."

THE DRIVE FOR PURITY

The rise in antibiotic-resistant bacteria, or "super germs," has researchers looking into the overuse of pharmaceuticals to keep humans and animals—specifically those that contribute to our food supplies—disease free.

The origins of this desire for purity are of particular interest to Kathy J. Cooke, a professor of history and director of the University's honors program. Cooke is researching the rise of purity as an American ideal during the late 19th and early 20th centuries in her project, "The Drive for Purity: The Emergence of an American Icon."

She was awarded a prestigious National Science Foundation grant of $122,778, which enables her to spend a full calendar year studying the societal, scientific and religious influences of the demand for purity. She hopes to write a book based on her research.

"My working hypothesis is that purity as an American ideal was primarily driven by public health innovation," Cooke explains. Public health activists began advocating for pure water, milk and food to fight disease. The movement eventually spread to animal breeding, agriculture, eugenics, religion and the environment. Religion, however, may have influenced these early health policies, says Cooke, who grew up on a dairy farm in Michigan.

Cooke began her research studying purity in breeding, including agricultural breeding and human reproduction. As she continues her project, she will investigate the public health origins of the purity movement, and later look into Christian fundamentalism and the social purity movement.

Cooke points out that religious folks—from the Puritans in the early American colonies to the Christian fundamentalists of the 1900s—valued purity. "Was it a social drive that influenced purity in religion or was it religion that influenced social purity?" she asks.

Purity remains a focal point in American culture—playing a role in social interactions, laws, ethics and science—but sometimes with harmful results, Cooke says.

As people demand purity in one area of life, they inadvertently may take away the purity from another area.

For example, people turn to antibiotics and antibacterial soaps and gels to keep bacteria at bay, but this is overkill, and actually contributes to the development of resistant strains of bacteria. Furthermore, the body needs certain bacteria to stay healthy. "The idea that all bacteria are bad has leached into the American psyche," Cooke says. "It has its roots in this idea of purity."