Municipal Wastewater Treatment: A Novel Opportunity to Slow the Proliferation of Antibiotic-Resistant Bacteria?

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Located on the Mississippi River in St. Paul, the Metropolitan Wastewater Treatment Facility is the largest treatment plant in Minnesota, averaging 180 million gallons of wastewater daily from 62 communities and 800 industries.

The discovery of antibiotics and their subsequent application to clinical medicine is one of the outstanding scientific achievements of the twentieth century. The tale of how antibiotics were discovered is one of scientific legend: Sir Alexander Fleming astutely recognized that a contaminated Petri dish actually contained a bacteria-killing mold. For his discovery of penicillin, Fleming shared the 1945 Nobel Prize in physiology/medicine with Sir Howard Florey and Ernst B. Chain.

The unique feature of penicillin (and other antibiotics) is not merely that it kills bacteria—there are many compounds that have such a capability—but that it specifically affects bacteria. This key feature is absolutely critical for the medical application of antibiotic therapy. Antibiotics administered to humans are lethal to disease-causing bacteria but do not impact the patient. This is possible because antibiotics act on features of the bacterial cell that are absent in humans. For example, penicillin prevents the formation of new bacterial wall materials; human cells do not even contain a cell wall.

During the last half-century, antibiotics have become pervasive in human medicine. Since the discovery of penicillin, a plethora of new antibiotics, semi-synthetic antibiotics, and synthetic antibiotics (antibacterials) have been discovered or developed (Table 1). These new drugs target different features of bacterial physiology, thus expanding the range of bacterial species that can be successfully treated with antibiotics. Antibiotics are also used extensively in agriculture and for other non-medical purposes. Low doses of antibiotics are often included in animal feed to promote growth and increase weight gain, as well as prevent the onset of

Table 1. Major Classes of Antibiotics and Antibacterials, and Representative Drugs in Each Class

Class	Representative Drug(s)
β-lactams	Penicillin, Amoxicillin, Methicillin
Aminoglycosides	Streptomycin, Neomycin, Kanamycin, Gentamicin
Macrolides	Tylosin, Erythromycin
Ketolides	Telithromycin
Tetracycline	Tetracycline, Oxytetracycline
Lincosamides	Clindamycin
Ansamycins	Rifampin
Glycopeptides	Vancomycin
Quinolones/fluoroquinolones	Nalidixic acid, Ciprofloxacin
Sulfonamides	Sulfamethoxazole

disease. Although reliable estimates are difficult to obtain, most scientists believe that approximately 70% of all antibiotics are used for agricultural purposes.

In this article, we report on a research project that investigated the role of municipal wastewater treatment facilities in the spread or control of antibiotic-resistant bacteria. The project was supported by a grant from CURA's Faculty Interactive Research Program, as well as grants from the Undergraduate Research Opportunity Program at the University of Minnesota. We hypothesized that the disinfection processes most treatment facilities use would adequately inactivate antibiotic-resistant bacteria in wastewater. However, our research suggests that treatment facilities, which are primarily designed to protect water quality, do not adequately prevent resistant bacteria from being released into the environment. We conclude that relatively simple changes in the design, operation, and regulation of municipal wastewater treatment facilities could substantially reduce the release of these bacteria and, we hope, slow the proliferation of antibiotic resistance among bacteria appearing in clinical patients.

A Brief History of Antibiotic Resistance

Antibiotic-resistant bacteria were discovered soon after the medical use of penicillin began. At the time, the development of resistant bacteria was largely viewed as inconsequential. If a patient had an infection that a resistant bacterium caused, then an alternative antibiotic was always available for effective treatment. However, some foresighted scientists warned of the pending problem of antibiotic resistance. In his Nobel acceptance speech, Alexander Fleming himself cautioned doctors about the danger of giving an "underdosage" of penicillin, noting: "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body . . . Moral: If you use penicillin, use enough."

The pioneering work of Stuart Levy in the 1970s was also informative. Levy was concerned that antibiotic use in agriculture at subtherapeutic concentrations could lead to the proliferation of antibiotic resistance. His research demonstrated that tetracycline-resistant bacteria were present in the droppings of chickens within one week after tetracycline was included in their feed. More alarming, however, the bacteria in chickens that were fed only tetracycline became resistant to multiple antibiotics within two weeks. Finally, multiple-antibiotic-resistant bacteria dominated the fecal material of farmers working with these chickens within five months, even though the farmers had received no antibiotics during the study.

It was not until the 1980s, however, when a multiple-drug-resistant form of tuberculosis emerged, that scientists became concerned about antibiotic resistance. Multiple-drug resistance soon appeared among other pathogens, particularly among nosocomial (hospital-acquired) infections. Today, 40% to 60% of nosocomial *Staphylococcus aureus* infections are methicillin resistant. The problem of antibiotic-resistant bacteria is particularly significant for immunodeficient patients, who are susceptible to a broader array of pathogens, many of which are multiple-drug resistant.

Many believe that the problem is linked to excessive antibiotic use in hospitals, making them a "hot spot" for resistant bacteria. Unfortunately, mounting evidence refutes this perspective. Community-acquired methicillin-resistant *Staphylococcus aureus* is becoming far more prevalent, particularly at public gymnasiums, where insufficiently sanitized towels are prevalent.

The Development of Antibiotic Resistance in Bacteria

The simplest method by which bacteria become resistant to antibiotics is via a *point mutation* of the deoxyribonucleic acid (DNA) within their genome. Point mutations are typically lethal to the bacterium or have no effect, but on rare occasions these mutations are beneficial (from the bacterium's perspective) and allow the organism to become resistant to antibiotics. Point mutations, however, are not the major concern with respect to antibiotic resistance. This form of bacterial evolution is slow and random, and it is unlikely that bacteria could rapidly achieve resistance to multiple antibiotics via point mutations alone.

Ultimately, the proliferation of antibiotic resistance is caused by the propagation of specific genes that allow bacteria to defy the lethal effects of antibiotics. These *antibiotic resistance genes* are probably not new, but likely result from millions of years of evolution, during which time bacteria have developed many mechanisms to survive the dangers that the world thrusts upon them. Certainly, many of these genes were specifically developed to counteract antibiotics, which are, after all, naturally occurring compounds. Many antibiotic resistance genes, however, likely are subtle adaptations of genes that provide protection against other toxic compounds. For example, there is a strong correlation between genes that encode for resistance to heavy metals and antibiotic resistance genes.

The existence of antibiotic resistance genes, however, is insufficient to explain the global proliferation of resistance. Bacteria also harbor other genes that are specifically designed to help bacteria rapidly evolve—genes designated as *evolution genes* by 1978 Nobel Prize winner Werner Arber. Evolution genes allow bacteria to rapidly develop new genes (usually by manipulating preexisting genes) and to spread them throughout the bacteria population. The evolution genes that allow *lateral gene transfer* are perhaps the most important class of evolution genes with respect to antibiotic resistance. Lateral gene transfer is the exchange of genetic material between different bacteria; it allows bacteria to share their abilities to resist antibiotics. This is believed to be the principal mechanism by which similar resistance genes are found throughout the world among many different species of bacteria.

During the last 20 years, scientists have also recognized the importance of *integrons*, another type of evolution gene. Integrons are responsible for integrating resistance genes into the genomes of bacteria, and then controlling the expression of these resistance genes. Because of this unique ability, integrons can be viewed as a genetic "luggage rack" in which different genes can be kept until they are needed. Integrons are a key component in the development of multiple-antibiotic-resistant bacteria because they allow bacteria to easily accumulate numerous genes.

Responding to Antibiotic Resistance

Although scientists have known about antibiotic-resistant bacteria for almost as long as they have known about antibiotics, the assumption was that new antibiotics would be discovered or developed faster than bacteria could become resistant. The discovery of new antibiotics, however, has slowed substantially since the 1960s. In fact, most "new" antibiotics are merely subtle modifications of previously existing ones and have little impact on bacteria that are already resistant.

During the last decade, therefore, there has been a considerable effort to restrict antibiotic use to only those applications where antibiotics are appropriate. Physicians are now reminded to avoid prescribing antibiotics for viral infections such as influenza and the common cold. Likewise, patients are carefully instructed to follow prescription guidelines so that enough of the drug is administered to limit the development of resistant bacteria. There is also increasing pressure to limit or eliminate non-medical use of antibiotics and antibacterials. As noted above, a substantial fraction of all antibiotics are used in agriculture at subtherapeutic concentrations. Although the United States appears to be far from prohibiting this practice, the European Union is banning subtherapeutic antibiotic use in agriculture in 2006. Although more controversial, many scientists—led by the Alliance for the Prudent Use of Antibiotics—are recommending the elimination of triclosan and other antibacterials from liquid hand soap, toothpaste, and other common household items.

A New Paradigm: Resistance Control?

The current situation with respect to antibiotic resistance is bad and the future is bleak. The discovery of new drugs has slowed to a trickle—a problem that will only worsen as pharmaceutical companies devote a greater fraction of their research and development budgets to less essential drugs (e.g., Botox, Viagra). Simultaneously, the ever-increasing use and misuse of antibacterials in common household products can only exacerbate the problem.

From our perspective, current efforts to reduce the spread of antibiotic resistance are an excellent first step. Certainly, our historically indiscriminate use of antibiotics needs to end. The more important issue is to identify novel approaches to limit the spread of antibiotic resistance. Our intention in undertaking this research, therefore, was to take a different approach to solving the problem of antibiotic resistance. We started by asking some simple yet fundamental questions about the proliferation of antibiotic-resistant bacteria.

First, where do the majority of antibiotic-resistant bacteria originate? Certainly, many bacteria are naturally resistant, but the majority of antibiotic-resistant bacteria result from antibiotic use. Therefore, people and animals taking antibiotics are most likely the primary source of antibiotic-resistant bacteria.

Second, how do resistant bacteria spread throughout the world after they originate inside a person? Humans actually contain about 10 times more bacterial cells in their bodies than they do human cells. The overwhelming majority of these bacterial cells reside in our gastrointestinal tracts, and most are released from the body during defecation.

Having asked and answered these two simple questions, we then inferred that municipal wastewater treatment plants, which handle virtually all human toilet waste in large municipalities (in rural areas, septic systems are more commonly used), would be critical in reducing the spread of antibiotic resistance. We hypothesized that municipal wastewater treatment facilities could adequately control the release of antibiotic-resistant bacteria to the world.

Municipal Wastewater Treatment Facilities: How Do They Work?

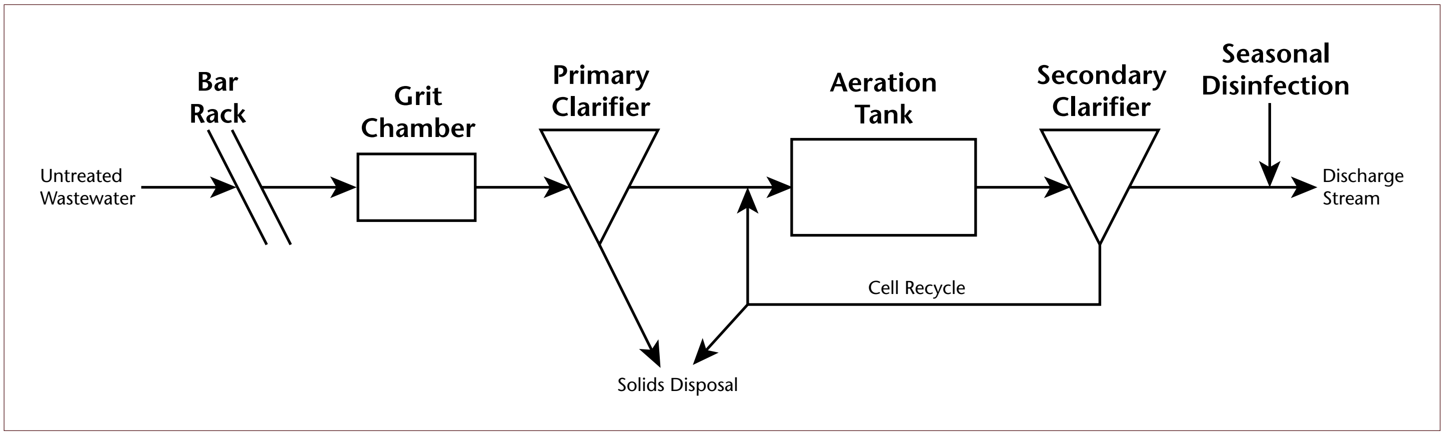
Municipal wastewater treatment facilities are primarily designed and operated to protect the environment. Municipal wastewater treatment facilities remove readily biodegradable compounds from sewage. Although there is relatively little in human sewage that is toxic, these biodegradable compounds are of environmental concern because if they were released untreated, they would biodegrade in the environment, resulting in oxygen depletion leading to septic conditions. Municipal wastewater treatment facilities, therefore, allow surface waters to maintain high dissolved oxygen levels, improving their aesthetic and recreational use value, as well as their ability to support healthy populations of fish and other aquatic fauna.

Although all municipal wastewater treatment facilities are unique, most are similar in design and involve a common series of unit operations (Figure 1). The first few unit operations, called primary treatment, are designed to remove particles from the wastewater. The bar rack removes large particles (greater than 1 inch), whereas the grit chamber removes sand and other dense, rapid-settling particles. The primary clarifier is a quiescent settling zone that allows organic particles to settle or float so that they can be removed. These primary treatment operations account for about 50% of the treatment that occurs.

The next unit operation, the aeration tank, is designed to remove dissolved organic compounds (which are readily biodegradable) from the wastewater by creating conditions favorable for the growth of bacteria. The tank works by bubbling air through the wastewater, allowing bacteria to metabolize pollutants that are present. Because these bacteria grow in excessive quantities, they must be removed from the wastewater. This is accomplished by the next unit operation, which is a quiescent settling chamber called the secondary clarifier. The combination of the aeration tank and the secondary clarifier is called the activated sludge process, which is the most common technology for the secondary treatment of wastewater.

Following primary and secondary treatment, the quality of municipal wastewater is quite good—not yet potable (i.e., safe to drink), but often

Figure 1. Schematic Diagram of a Conventional Municipal Wastewater Treatment Process



Note: Individual unit operations are labeled in bold lettering.

as good as or better than the quality of many lakes and rivers. This treated wastewater, however, still contains pathogenic bacteria that could make people sick if they accidentally ingested the water. Municipal wastewater treatment facilities, therefore, perform a final treatment step in which the treated wastewater is disinfected to help reduce the number of disease-causing microbes. Disinfection is required only when recreational use of the receiving stream is a reasonable expectation. In Minnesota, for example, wastewater treatment facilities usually disinfect their wastewater only from April to November.

In addition to treating the wastewater, municipal wastewater treatment facilities must deal with the solid residues that the primary and secondary clarifiers collect. These solid residues are readily biodegradable organic materials that are most commonly treated by a process called *anaerobic digestion*. The conventional anaerobic digestion process, which largely mimics our gastrointestinal tracts (hence the “digestion” nomenclature), is kept free of oxygen and operated at 98.6°F. Following digestion,

Photo courtesy of Timothy LaPara

An aeration tank at a municipal wastewater treatment plant. The tank removes dissolved organic compounds by bubbling air through the wastewater, creating favorable conditions for the growth of bacteria that are capable of metabolizing pollutants.

the treated wastewater solids are either applied to farmland as a fertilizer and soil conditioner, or sent to a landfill for disposal. The former alternative is preferred as a "sustainable" practice, whereas landfill space is finite.

Because anaerobic digestors operate at conditions similar to the human body, they are not particularly good at eliminating human pathogens. Numerous alternative treatment technologies, therefore, have been developed to better treat wastewater solids. All of these alternative treatment technologies are more expensive, however, and thus municipalities do not frequently use them. Perhaps the most attractive treatment alternative is thermophilic anaerobic digestion, which operates almost identically to conventional anaerobic digestion, except that it operates at sufficiently high temperatures (greater than 110°F) to kill most human pathogens.

Methodology and Analysis

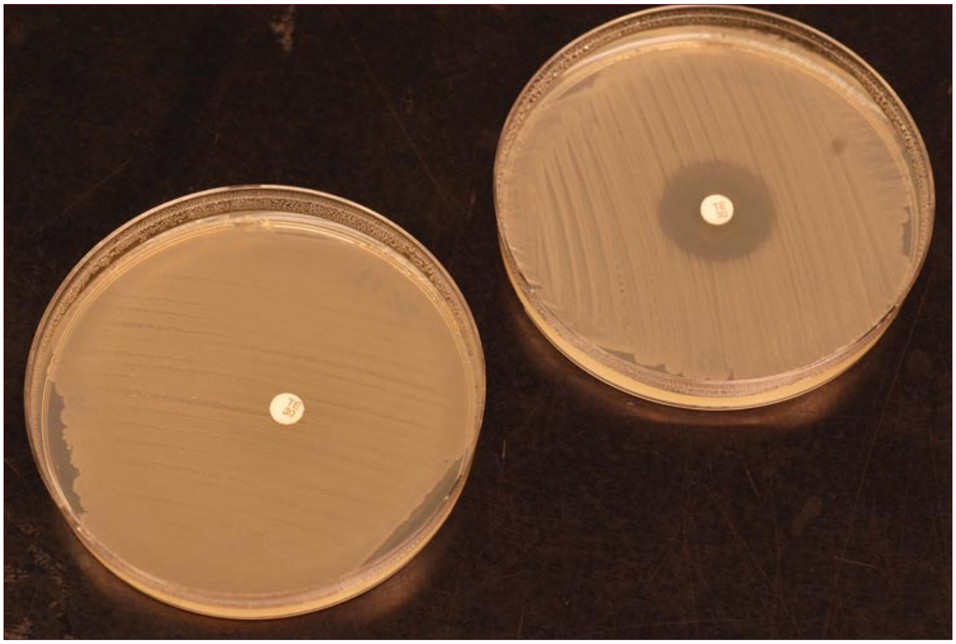
The first goal of our project was to determine the extent to which municipal wastewater treatment facilities prevent the release of antibiotic-resistant bacteria. There are two potential paths by which antibiotic-resistant bacteria can escape a municipal wastewater treatment facility. The most obvious is in the treated wastewater. Our research, therefore, investigated the importance of secondary clarification and disinfection in preventing the release of antibiotic-resistant bacteria from the aeration tank. Resistant bacteria could also be released in the solids collected during primary treatment and from the secondary clarifier. Our research, therefore, compared the effectiveness of two variations of conventional anaerobic digestion and thermophilic anaerobic digestion at destroying resistant bacteria.

We investigated the efficacy of wastewater disinfection at the Metropolitan Wastewater Treatment Facility in St. Paul. This facility is very large, treating an average of 180 million gallons of sewage each day. Typically, the quality of treatment from the Metropolitan plant is top-notch, and the facility regularly wins state and national awards for operational excellence. Throughout the year, we quantified about 100,000 (10^5) tetracycline-resistant bacteria per milliliter of water in the aeration tanks at the Metropolitan plant. From the treated wastewater, we quantified about 300 tetracycline-resistant bacteria per milliliter in the winter (i.e., when disinfection was not performed) and about 30 tetracycline-resistant bacteria per milliliter during the summer (i.e., during the disinfection period). That is, about 99.6% and 99.97% of the resistant bacteria in the aeration tanks are removed in the winter and summer, respectively. Although this removal efficiency might seem sufficient, 30 bacteria per milliliter translates to more than 10 trillion (10^{13}) tetracycline-resistant bacteria released each day from this treatment facility into our waterways.

We also investigated the efficacy of anaerobic digestion at the Western Lake Superior Sanitary District (thermophilic process) and the Empire Wastewater Treatment Facility (conventional process), which are located in Duluth and Farmington, respectively. Both of these plants have also earned awards for operational excellence. We again detected about 100,000 (10^5) tetracycline-resistant bacteria per milliliter in the waste stream entering the anaerobic digestors at each of these treatment facilities. However, we were unable to detect any tetracycline-resistant bacteria in the waste stream leaving the anaerobic digestors at these two treatment facilities, in part because the research method we used is unable to detect levels of tetracycline-resistant bacteria below 1,000 (10^3) per milliliter of sludge solids. However, this suggests that both anaerobic digestion processes were able to inactivate at least 99% of antibiotic-resistant bacteria. We are currently attempting to develop an alternative technique to measure the efficiencies by which these anaerobic digestors inactivate antibiotic-resistant bacteria.

The second goal of our research was to characterize the antibiotic-resistant bacteria in sewage. From the three treatment facilities, we isolated and identified 173 bacterial strains that were resistant to tetracycline. All of these bacterial strains were pathogenic (disease-causing—e.g., *Shigella* or *Klebsiella* spp.), possibly pathogenic (e.g., *Escherichia coli*), or non-pathogenic but related to pathogens (e.g., *Citrobacter* spp.). In more than 50% of these bacteria, we also detected at least one gene encoding for tetracycline resistance.

Based on these initial data, we then studied 14 different tetracycline-resistant bacterial strains in more detail. All 14 of these strains contained an integron and were resistant to at least three different antibiotics (we tested resistance to amoxicillin, ampicillin, chlortetracycline, enrofloxacin, erythromycin, sulfamethoxazole, trimethoprim, and tylosin). We also tested these bacteria for lateral gene transfer. Although this work is still ongoing, many of these bacterial strains are capable of



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Bacteria growing on petri dishes that include disks treated with the antibiotic tetracycline. The bacteria growing on the left petri dish were obtained from treated wastewater and are resistant to tetracycline, as shown by the ability of the microbes to grow near the white disk. The bacteria growing on the right petri dish are a tetracycline-sensitive strain of* E. coli*. The circular ring around the disk shows that these microbes cannot grow in the presence of tetracycline.

exchanging with other bacteria a gene encoding for tetracycline resistance.

Following our work on tetracycline-resistant bacteria, we isolated an additional 65 different bacteria that were resistant to ciprofloxacin. Ciprofloxacin is a relatively new antibiotic and there is not much known about bacterial resistance to it. Once again, we found that all of these bacterial strains were pathogenic, possibly pathogenic, or related to pathogens. We then focused our efforts on 11 of these strains, all of which were resistant to at least four different antibiotics. About half of these strains contained an integron or a gene encoding for resistance to tetracycline. Although this work is also ongoing, our analysis revealed that several of these strains were capable of laterally exchanging genes encoding for resistance to ciprofloxacin.

Conclusion and Policy Recommendations

Our research has demonstrated that extremely high numbers of antibiotic-resistant bacteria are released from municipal wastewater treatment plants, even when disinfection is performed. Our original hypothesis was that disinfection would adequately inactivate antibiotic-resistant bacteria in treated municipal wastewater, and that an outcome of our work would be to encourage the implementation of year-round disinfection. Instead, we learned that although a 99% inactivation looks encouraging, 1% of a very large number (10^{15}, or 1 quadrillion) still represents a very large number (10^{13}, or 10 trillion) of antibiotic-resistant bacteria that are released from the Metropolitan Wastewater Treatment Facility each day.

The bacteria that we studied were all pathogens or related to pathogens and all were resistant to multiple antibiotics. A substantial fraction of these bacteria (greater than 50%) harbored genes encoding for tetracycline resistance. These bacteria frequently harbored integrons (genes that allow bacteria to accumulate multiple genes for antibiotic resistance) and some of them were capable of transferring their resistance to other bacteria. The frequency of lateral gene transfer of ciprofloxacin resistance, which occurred in more than 40% of the strains we studied, is particularly worrisome because this trait is typically very rare (less than 1%) among clinical strains of ciprofloxacin-resistant *E. coli*. Simply put, the bacteria that we detected in municipal wastewater are some of the most resistant bacteria ever studied. There is a substantial need, therefore, to prevent these organisms from reaching the environment.

At first glance, the most obvious solution to the problem of antibiotic-resistant bacteria in treated municipal wastewater would be to require more stringent disinfection. The majority of municipal wastewater is disinfected using chlorine, which poses a security risk (chlorine gas is very dangerous) and generates disinfection by-products that are known or suspected carcinogens. Although we recommend a policy shift to include year-round wastewater disinfection, we do not recommend that more stringent disinfection regulations be imposed because of these unwanted consequences.

Instead, we recommend that wastewater effluents be passed through a sand filter prior to disinfection. Sand filters can physically remove antibiotic-resistant bacteria from treated wastewater, but without the use of potentially dangerous chemicals. At the present time, sand filters are rarely used in wastewater treatment, but they are commonly used at drinking water treatment facilities, so the technology is well-developed and well-understood. Additional research is needed, however, to optimize the removal/inactivation of antibiotic-resistant bacteria by our proposed combination of sand filtration and effluent disinfection.

Although our research on the fate of antibiotic-resistant bacteria in anaerobic digestors was inconclusive due to the limitations of our research method, we suspect that our ongoing research will demonstrate that thermophilic anaerobic digestors achieve substantially better inactivation efficiencies than conventional technologies. This ongoing research is particularly pertinent because of a recent shift in policy that emphasizes the application of treated wastewater solids to land rather than putting these residues into landfills—that is, the “environmental friendly” practice of applying wastewater solids to land may have unexpected and undesirable consequences in terms of the proliferation of antibiotic-resistant bacteria.

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