Antibiotic Resistance in Agricultural Systems

Antibiotic resistance (ABR) in health-care environments is a hot topic, garnering considerable media coverage. Public attention has focused on a diversity of issues related to ABR, from the development of superbugs like methicillin-resistant Staphylococcus aureus (MRSA) to speculation about the overuse of biocides like triclosan in hand soaps. A report published in 2000 by the World Health Organization (WHO) identified ABR as one of the most critical human health challenges of the 21st century. The report observed that more than two million Americans are infected each year with resistant pathogens and 14,000 die as a result.

Despite the seriousness of the problem, comparatively little attention has been devoted to environmental sources of ABR from bacteria that live in soil and water. Researchers are only now beginning to understand the multifaceted, complex relationships between common agricultural practices—such as using animal manure for fertilizer or reclaimed wastewater for irrigation—and the observed levels of ABR in the environment.
It’s important to remember that bacteria evolved ABR genes naturally, over millennia of evolution, in order to survive in a hostile environment. These genes—carried on small, closed loops of DNA called plasmids—typically express proteins that confer the ability to break down antibiotics or prevent their entry into bacterial cells.

This “background” level of ABR must be taken into account when measuring environmental levels. “ABR is a natural phenomenon,” says ASA, CSSA, and SSSA member Jean McLain, associate director of the Water Resources Research Center at the University of Arizona. “Unless we address the natural ABR when designing our research studies, we will not be able to accurately identify any agriculturally induced increases or decreases.”

ASA and SSSA member Lisa Durso, a research microbiologist at the USDA-ARS, Lincoln, NE, also emphasizes that ABR is not a new occurrence. “Genes that code for ABR have been found in ice cores from the time of the woolly mammoths,” she says. “Soil from a four-million-year-old cave in New Mexico contained bacteria that were naturally resistant to a suite of modern antibiotics, including recently developed antibiotics!”

Durso notes that the development of ABR as a result of evolutionary pressures is only part of the story. She explains that the genes that code for ABR can also be expressed following exposure to other kinds of environmental stress, even in the absence of any actual antibiotics. “The biology and ecology of natural ABR in soil is complex,” Durso says, “and there is still a lot that we don’t know about the specific mechanisms responsible for the development of resistance.”

Detection Methods and Standards

Methods to detect ABR bacteria are broadly classified into two types. The first type relies on culturing bacteria in the laboratory and evaluating their growth when exposed to a panel of antibiotics. A commonly used benchmark is the minimum concentration of the antibiotic that will inhibit the growth of 50% of the bacterial population—the MIC50 value.

Left: Dr. Annesly Netthisinghe, Western Kentucky University, and research technician Marty Haley, USDA-ARS, take soil samples to evaluate the effects of poultry litter application on microbial communities. Photo courtesy of Kim Cook. Center: Microbiologist Lisa Durso examines the results of a test to quantify bacteria in cattle fecal specimens and runoff from manure-amended soils. The number of yellow and glowing wells helps determine the number of bacteria present. Photo by Peggy Grab/USDA-ARS. Right: Methicillin-resistant Staphylococcus aureus bacteria (MRSA, yellow) being ingested by neutrophil (purplish blue). Photo courtesy of NIAID.
The second method to identify the resistance gene itself at the molecular level, says Durso, "is to measure the actual 'resistance' part of ABR. The actual resistance part of ABR can only be measured by cultivating and relying primarily on the screening of isolated bacterial strains. But this method is time-consuming and expensive, and they only provide information for strains that can be cultured. In the environment, it is estimated that less than 2% of bacteria can be cultured—the vast majority will not grow under laboratory conditions, making them challenging to study by conventional methods.

Durso explains that gene-based methods overcome these limitations. However, methods that detect bacterial DNA are only able to provide information on whether or not an ABR gene is present, not about whether or not it is active.

Molecular methods in widespread use include polymerase chain reaction (PCR), a highly sensitive method for detecting specific DNA sequences. A variation of PCR, called real-time PCR or quantitative PCR (qPCR), can detect both the genes present and quantify their relative amounts with a high degree of accuracy.

Although PCR is a relatively simple technique in recent years, rapid developments in technology have made so-called next-generation sequencing a favored method for studying ABR. The technique, also known as massively parallel sequencing, can determine the DNA sequence of an entire bacterial genome in a single day—a process that took weeks or even months just a decade ago.

While many ABR studies are examining individual species or small groups of species, molecular methods enable a big-picture approach. McLain cites a study that showed that there are the same kinds of ABR genes in cattle and chicken waste as there are in Antarctic sea ice and the Sargasso sea. "In other words, ABR occurs in both agricultural and non-agricultural environments.

The main stumbling block in measuring ABR, though, is that methods, analysis, and reporting all need to be standardized. "In agricultural settings, Durso says, "it is crucial that methods, analysis, and reporting all need to be standardized. In order to best target limited resources, we need to start sorting out baseline levels of resistance and determining which types of resistance (whether naturally occurring or caused by human activity) can be impacted by agricultural best management practices."

In order to do this, Durso maintains that developing community standards for reporting studies on ABR would make it easier to compare results between studies.

McLain's research focuses on ABR in recycled municipal wastewater that is used for groundwater replenishment in Arizona. The use of recycled wastewater has driven groundwater levels in the southwestern U.S., especially in drought-stricken regions like Arizona. McLain's research shows that there are the same kinds of ABR genes in recycled wastewater as there are in Antarctic sea ice and the Sargasso sea. "In other words, ABR occurs in both agricultural and non-agricultural environments.

When it comes to measuring ABR in agricultural settings, Durso says consider the levels of ABR genes in a single day—a process that took weeks a decade ago.
water unimpacted by recycling—in other words, the natural levels of ABR. “In far too many studies, natural levels of these genes are not assessed. As a result, the findings of the study could be heavily—and perhaps incorrectly—skewed against the use of recycled municipal wastewater.”

McLain recently published the results of a study conducted jointly with the USDA’s Arid-Land Agricultural Research Center in Maricopa, AZ. The study compared ABR patterns of Enterococcus in sediment isolated from water storage basins containing either reclaimed water or groundwater. The results revealed that high levels of resistance to certain antibiotics (including lincomycin, ciprofloxacin, and erythromycin—commonly used in clinical practice) existed in sediments regardless of the water source. Higher levels of ABR were also not detectable in reclaimed water sediments. In fact, the study showed that multiple-antibiotic resistance was lower in reclaimed water sediments compared with freshwater sediments. McLain cites the need for adequate controls in any studies that examine the impact of ABR in wastewater recycled for use in irrigation or groundwater replenishment.

As is the case for water, the development of ABR in soil can result from multiple, complex pathways. McLain acknowledges that concerns exist about using animal manure as fertilizers, which come from farming of poultry, meat, and fish treated with antibiotics. However, she points to studies showing that ABR can occur in meat products from antibiotic-free animals, “which suggests that limits on the agricultural use of antibiotics, though a good start, are not in themselves sufficient to prevent the transfer of ABR into the food chain.”

Such an overuse of antibiotics caused high levels to be shed in feces—up to 75% of the dose.” As these antibiotics enter the environment, they may kill susceptible bacteria in soil and water, potentially enriching the environment in ABR bacteria.

Another manure management practice under scrutiny is the use of poultry litter as fertilizer. “The value of poultry litter has increased as the cost of conventional fertilizer has increased, and it has even become more economically feasible
to transport poultry litter longer distances,” explains ASA, CSSA, and SSSA member Kim Cook, microbiologist at the USDA-ARS Food Animal Environmental Systems Research Unit, Bowling Green, KY. This increased use of poultry litter prompted Cook and her colleagues at Western Kentucky University to undertake a study, and an article from it has been submitted for publication to the Journal of Environmental Quality.

The study set out to determine the differences in microbial populations in soils with or without applied poultry litter and in systems with conventional-till or no-till management. Says Cook, “We evaluated concentrations of pathogens (Campylobacter and Salmonella), indicator organisms (E. coli and enterococci), and bacteria with genes that could make them resistant to tetracycline, sulfonamides, and/or streptomycin antibiotics.” The researchers used qPCR to measure the levels of ABR genes.

Cook found that application of poultry litter increased the concentrations of the ABR genes examined by several orders of magnitude over background. However, while ABR genes were detected throughout the study, concentrations had returned to near background levels by the end of each field season.

Cook explains, “The response to poultry litter application in the second field season was significantly reduced compared with the first year; peak concentrations of resistance genes were reached sooner, concentrations were significantly lower, and there were fewer days above background.”

Another study examined the occurrence of both antibiotics and ABR genes in swine manure used as fertilizer. Conducted by Shannon Bartelt-Hunt, Xu Li, and Daniel Snow at the University of Nebraska with John Gilley at the USDA-ARS, the study determined that whenever swine manure is used on cropland, there is potential to find antibiotics and ABR genes in runoff that typically occurs after rainfall. However, Bartelt-Hunt says, “The method used to apply the manure is a significant factor.” Methods that incorporate the manure into the soil, for example, “had lower concentrations of antibiotics and ABR genes in runoff compared with fields where manure was applied to the soil surface without mixing,” she says. The study also noted that the concentration of antibiotics and ABR genes measured after the first rainfall event diminished with each subsequent rainfall.

Changing Practices

Should current agricultural practices change to diminish the threat of ABR, despite lack of clarity about how ABR is related to agricultural use of antibiotics?

Durso says it is important to be aware that “any practice that impacts the kinds of bacteria present in a particular habitat will also, inherently, impact ABR.” For example, composting can change the number and types of bacteria present in manure and manure-impacted soils. It can also change the number and types of ABR bacteria. She believes that soil scientists are uniquely positioned to shed light on the problem of ABR, “because they understand the physical, chemical, biological, and spatial complexities of soil.”

Cook notes that her study did not find a consistent correlation between soil management practices and the concentration of ABR bacteria from applied poultry litter. However, she says other studies show that pre-treatment of litters and manure—such as composting, stockpiling, and digestion—reduces the concentration of ABR bacteria.

“When time and/or space do not permit pre-treatment, farmers should just be aware that poultry litter application may increase the level of ABR genes in soils even for extended periods of time,” Cook says. “More research is necessary to determine if this phenomenon has any effect on...”
overall levels of ABR in the farm and public sectors.”

McLain acknowledges the difficulty in drawing definitive conclusions regarding the connection between ABR and agricultural practices. She cites the example of using biosolids—the solid portion of the waste derived from sewage treatment—for agricultural fertilizer. Raw biosolids typically contain trace levels of antibiotics and ABR bacteria, but these are, in theory, reduced during composting before application onto farm fields.

But McLain asks, “Do some of the ABR and antibiotics remain in the biosolids after composting? Depending on the length and temperature of composting, yes, they do. And if they do survive, how do they affect the environment?”

She says that biosolids are widely used because they are a very rich source of carbon and nitrogen. She speculates that they may induce the native soil bacteria to flourish and outcompete any resistant microorganisms entering the system. “Our initial research suggests that this may be the case,” McLain says, “and thus the environmental benefits offered by biosolids truly confound the resistance story. We have a lot more research to do.”

Future Directions

What should the priorities be for future research into ABR in agricultural settings? “I’m going to sound like a broken record here, but first and foremost, we must address background resistance,” McLain says.

Durso agrees. “I think one big take-home message is that this is an extremely complex situation and not all resistance is created equal,” she says, also stressing the need for researchers to be more specific in the language used when discussing ABR. “There are literally hundreds of different kinds of resistances covered by the ABR umbrella. There are over 250 drugs listed by the WHO as antimicrobial agents of concern for human health. For each of those drugs, there are multiple ways that a bacterium can be resistant and suites of genes that code for each type of resistance.” And yet, even in the scientific community, it is common to measure one or two specific types of resistance and then discuss those results using the broader “ABR” language, according to Durso.

One point to consider, she adds, is that just because ABR is detected in an environmental sample does not necessarily mean it poses a human health threat. She explains that different scientific communities have different opinions about which types of resistance should be measured.
“The environmental community frequently looks at tetracycline resistance, for example, while the human public health and food safety communities are not as interested in tetracycline resistance, since tetracycline is not a frontline drug in the clinic.”

McLain believes that “it will be impossible to develop, implement, and evaluate mitigation strategies for the containment of ABR without first coming to a consensus on assessment methods that address the complexities of environmental samples and standards for quality control of detection and quantification assays.”

August Workshop on Antibiotic Resistance in Agroecosystems

The *Antibiotic Resistance in Agroecosystems: State of the Science* workshop will bring together environmental chemists and microbiologists working on issues associated with the occurrence and effects of antibiotics in agricultural ecosystems. The workshop will be held 5–8 Aug. 2014 at the Biosphere2 Conference Center, Oracle, AZ.

Participants will discuss, identify, and debate the best available methods to measure antibiotics, to track antibiotic-resistant (ABR) bacteria and ABR genes in environmental samples, including manure-impacted water and soil. They will discuss which antibiotics and resistance targets are most relevant in environmental samples and how to address the confounding factors of background and baseline resistance.

Specific objectives of the proposed meeting are to:

• Gather top experts to openly discuss, debate, and come to consensus on a suite of tools (including molecular and cultivation methods) to track occurrence of antibiotic compounds, ABR bacteria, and ABR genes resulting from the use of antibiotics in agricultural (animal and crop) production;

• Actively involve graduate and undergraduate students in the workshop and introduce them to the scientific processes of developing, validating, and implementing methods for detection of antibiotic compounds, ABR bacteria, and ABR genes in pre-harvest and manure-impacted samples; and

• Effectively disseminate conference results by facilitating publication of review articles and a reference guide to raise awareness and exchange the best research practices developed through this workshop with fellow scientists.

This conference is organized by SSSA members Jean McLain and Lisa Durso. *Save $100 by registering before 30 June. For more information: https://wrrc.arizona.edu/arasos-home.*