Antibiotic Resistance Spreads through Diverse Species and Habitats, Part I

The public health threat amplifies as drug-resistant pathogens move freely through various environments and species

Shannon Weiman

Antibiotic-resistant bacteria continue to spread across the globe—directly affecting human and animal patients while also establishing reservoirs from which those strains continue to emerge long into the future, according to several researchers who spoke during the 2015 ICAAC conference held in San Diego last September. Their findings paint a broader and, in some ways, more alarming picture of the expanding influence and dynamic nature of antibiotic resistance than experts previously drew.

Antibiotic resistance genes and bacterial strains carrying them are infiltrating diverse environments, threatening to cause long-term havoc along with the current challenges for which they are so well known. The reservoirs for such strains in both terrestrial and aquatic habitats, as well as within wild and domesticated animals, foster the accumulation, spread, and reintroduction of such drug resistance into humans, posing serious long-term public health threats.

In particular, several types of pathogens that infect humans, including extended-spectrum β-lactamase producing Escherichia coli (ESBL-E. coli), carbapenem-resistant Enterobacteriaceae (CRE), and methicillin-resistant Staphylococcus aureus (MRSA), are highly adaptable and move freely among diverse animal hosts before they jump back into humans. Researchers are now documenting environmental excursions by these and other pathogens—monitoring environmental reservoirs that they occupy while also identifying how they are being transmitted from one host species to another. These tracking studies are part of a larger effort to predict and eventually prevent further spread of drug-resistant pathogenic strains into and within human populations.

Soil Microbes as a Source and Reservoir of Antibiotic Resistance

Soil environments are a likely source for novel antibiotic-resistance genes, according to Fiona Walsh of Maynooth University in Ireland, who spoke during the 2015 ICAAC session “Surrounded by the Enemy—the Environment and Foodstuffs as Sources or Reservoirs of Antimicrobial Resistance Threats.” Within soil, resident bacterial species constantly battle one another over turf, she says. Some species produce toxins or growth inhibitors that enable them to thwart and thus outcompete their neighbors. Humans co-opted some of those same compounds for use as antibiotics. Meanwhile, neighboring microbial species in soils developed the means to resist toxins from aggressor microbial species. Buried in soil, those resistance mechanisms are of little consequence to humans. However, serious public health consequences can arise when soil bacteria transmit their resistance genes to microbial species living within humans or animals (Fig. 1).

SUMMARY

➤ Antibiotic resistance genes and bacterial strains carrying them are infiltrating diverse terrestrial and aquatic environments, threatening to cause long-term havoc along with the challenges for which they are currently known.
➤ Soil environments are a likely source for novel antibiotic resistance genes, whereas water provides a more dynamic reservoir, accumulating and also dispersing bacteria carrying familiar antibiotic resistance factors.
➤ Wildlife species as well as domesticated animals are spreading multidrug resistant strains across vast geographic areas.
➤ Pets are reservoirs for antibiotic-resistant strains, acting as conduits between humans and the environment, and disseminating resistance factors into other animal species for further spread.
Walsh’s research focuses on uncovering the hidden presence of such resistance genes in soils from both rural and urban sources. She identifies novel resistance genes that act against the quinolone antibiotic naladixic acid, and tries to identify how each works before they exert any impact on public health. “If they ever emerge from the soil and transfer to human clinical pathogens, it will be important to characterize their activity,” she says.

There is precedent for such concern, says Dereje Dadi Gudeta of the University of Copenhagen, Denmark, who spoke during the session, “Emergence of Resistance: Environmental and Food Chain Threats.” Such an environmental transfer likely began the worldwide spread of the Klebsiella pneumoniae carbapenemase (KPC) from that bacterial species to diverse other gram-negative pathogens, including Salmonella enterica, E. coli, and Pseudomonas spp. Gudeta finds ancestral homologues of KPC in various Chromobacterium species, gram-negative bacteria found in both soil and water that only rarely infect mammals. “The recovery in three distinct Chromobacterium species of functional class A β-lactamases with up to 76% amino acid identity to KPC suggests that [these enzymes] may have evolved from possible ancestor genes resident on the chromosome of members of this genus,” he says. The ancestral genes then likely transferred to strains of other gram-negative bacteria that are

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**FIGURE 1**

Dissemination of antibiotics and antibiotic resistance within agriculture, community, hospital, wastewater treatment, and associated environments. Soil, water, and wild and domestic animals are all intertwined in the spread of resistance. (From Davies J and Davies D, Microbiol. Mol. Biol. Rev. 74:417–433, 201.)
common pathogens of humans when transposon Tn4401 was mobilized.

Soil microbes also harbor resistance genes that are commonly known for their clinical significance among human pathogens, including more than a dozen β-lactamases and various carbapenemases, according to Walsh. “We identified the multidrug-resistant (MDR) nature of soil bacteria by selecting them on one antibiotic,” she says. “In this group of soil bacteria, greater than 80% of isolates are resistant to 16–23 antibiotics.”

The vast majority of these strains carry efflux pump resistance mechanisms, Walsh continues. Where these genes originated is not known, but possibly they are native to soil bacterial populations or were acquired after mingling with human- and animal-associated microbial strains, which contaminate soil environments in various ways, including via wastewater, farm runoff, and manure, she says. “To minimize the risks to human health posed by resistance genes in the environment and the risk to the environment by waste containing resistance genes, we must identify the critical points of control that are resistance gene hotspots.”

Water Exposes Humans to Antibiotic-Resistant Bacteria

Water is a dynamic reservoir, accumulating and also dispersing antibiotic-resistance bacteria. In particular, municipal wastewater treatment facilities foster the mixing of bacterial strains shed from human populations, leading to rampant exchanges of resistance genes among microorganisms that congregate there, according to Amy R. Sapkota of the University of Maryland School of Public Health. “Water is a complex and diverse reservoir of antibiotic-resistant bacteria,” she says. “Municipal wastewater is the richest aquatic habitat in terms of known antibiotic resistance genes.”

Jill Hoelle of the Environmental Protection Agency (EPA) in Washington, D.C., concurs, noting that MDR bacteria are present in wastewater-treatment plants across the United States (US). While most E. coli stains in these facilities are antibiotic susceptible, those that do carry resistance are frequently MDR strains, she says. “Some of these organisms have become resistant to all or almost all antibiotics, including last-sort drugs [like] carbapenems.” A disconcerting 41% of such MDR E. coli strains are carbapenem resistant, belonging mostly to phylogenetic subgroups B2 and D that are known for causing invasive human infections. These strains were most prevalent in samples taken from urban wastewater treatment facilities, suggesting that such urban reservoirs may pose greater public health risks than rural agricultural sources.

While wastewater treatment plants are intended to remove such contaminants, they are not always successful, according to Sapkota. For example, MRSA strains can survive treatment systems that do not use chlorination and then can pose risks to public health. Specifically, those individuals who are commonly exposed to reclaimed water, such as spray irrigation workers, are more likely to carry MDR S. aureus in their nasal cavities than are others, and could become a source for further spread of such strains among human populations, she says. There are no federal regulations regarding reclaimed water testing or use, and few studies to indicate public health risks, she points out. Further, droughts tend to exacerbate the situation, increasing reclaimed water use on farms and gardens, with the unintended consequences of possibly introducing and disseminating MDR clinical strains into the environment and increasing human exposures to them.

In addition, wastewater from pharmaceutical plants can release by-products into the environment that select for antibiotic-resistant pathogens, according to Avemaria Obasi of the University of Lagos, Nigeria. “Wastewater effluent systems represent a protective niche for commensals and pathogens favoring the horizontal transfer of genes encoding for resistance factors,” she says. ESBL-producing K. pneumoniae and Pseudomonas aeruginosa strains contaminate wastewater from pharmaceutical plants in Nigeria, she finds. In the lab, these strains can pass their resistance genes to other opportunistic pathogens, including E. coli. One of the ESBL resistance genes that she identified in wastewater isolates is also found in clinical isolates from patients with urinary tract infections in the region, she notes. However, further analyses are needed to determine whether such transmissions are occurring and whether they are contributing to public health risks in Nigeria.
Wildlife Spreads MDR Strains across Vast Geographic Areas

MDR bacteria within water reservoirs also can be disseminated to wildlife, in effect generating animal reservoirs for those bacteria that further disperse resistant strains to geographically distant sites and increase the chances for humans to be exposed to them. For example, an MRSA strain found in wild boars and deer in Spain traces to a local water source, according to Mark Holmes of Cambridge University in England, who spoke during the session, “Animal-Human Transmission of Methicillin-Resistant Staphylococci.”

“Presence of MRSA in the river water highlights the potential role of water in the dissemination” of strains carrying resistance, says Holmes. Indeed, many antibiotic-resistant strains made their way into diverse animal species across the globe, including the Iberian wolf, lynx, red fox, beaver, Spanish slug, and numerous fish and bird species. “These are indicators of environmental pollution, which contributes to spread and expansion of antibiotic resistant bacteria in fragile niches,” says Andrea Endimiani of the University of Bern in Bern, Switzerland.

One major source of such “pollutants” is livestock farms, Endimiani continues, citing findings by Christina von Salviata of the University of Berlin, Germany, and her collaborators (Fig. 2). Some 25% of flies and 33% of mice on a pig farm in Germany carry ESBL- E. coli, according to von Salviata. Because such organisms are near the bottom of the food chain, they carry enormous potential to disseminate these MDR strains to larger mammals, birds of prey, and other predators that can further spread antibiotic resistance factors regionally and, eventually, globally. In addition, 75% of green plants surrounding the pig farm were contaminated with antibiotic-resistant microbial strains, potentially exposing any animals that eat such vegetation.

Thus, the pig farm that von Salviata surveyed was seeding multiple environmental reservoirs in its immediate vicinity and beyond, Endimiani says. As one remedy to these amplifying effects, he supports the One Health concept, in which the

FIGURE 2

Pig farms can be sources of antibiotic-resistant organisms for multiple environmental reservoirs in their immediate vicinity and beyond. (Image ©istockphoto/Suljo.)
combined effects of human, animal, environmental, food production, and veterinary health are recognized for being intertwined and dependent on one another. Our surveillance systems and public health efforts to control MDR bacteria need to take into account this interconnectedness, he says.

Furthermore, environmental contamination can be self-perpetuating, with frequent exchanges of resistant bacteria between wild animals and aquatic habitats seeding additional environmental reservoirs. Wild animals can carry resistant bacteria in their feces, contaminating fresh water sources even in apparently pristine places, according to Endimiani. For instance, 36% of rivers and lakes in Switzerland contain ESBL-producing Enterobacteriaceae, which in turn make their way into 19% of fish inhabiting those waters, he reports.

Wild birds in particular, especially migratory species, are of great concern for further disseminating antibiotic-resistant bacterial strains. “Migratory birds are the global spreaders,” says Endimiani, referring to Franklin’s Gulls, which travel each year from Canada to the southern tip of Chile and back, carry ESBL-E. coli, depositing them in their feces all along the way. “They retain, as gut carriers, human MDR bacteria and plasmids acquired from dumps in corresponding regions,” he says. “Thus bird migration could contribute to the dissemination of MDR E. coli over the globe.”

He also notes that cormorants and mallards carry ESBL E. coli with quinolone resistance genes across Europe, while migratory wild birds in Germany carry carbapenemase-producing S. enterica originating from Asiatic regions. MDR strains have even made their way into wild bird populations in remote and developing regions of South America, says Alice Batalha-Jesus of Universidade Federal de Rio de Janeiro, Brazil, who documents ESBL-E. coli in black vultures and tropical screech owls in Brazil.

Because of their scavenging activities, omnivorous diets, and close interactions with humans, gulls and crows are particularly prone to being colonized with MDR pathogens of humans, says Ivana Jamborova of the University of Veterinary and Pharmaceutical Sciences in Brno, Czech Republic. In the US, for example, 11% of fecal samples from crows contain AmpC-producing E. coli, while 2% contain ESBL E. coli, she adds. “We identified E. coli clones and plasmids previously documented in humans and food animals, most of which are potentially pathogenic for humans or animals. . . including E. coli strains of hyper-epidemic sequence type (ST) 131, ST405, and ST648. This constitutes alarming environmental contamination by MDR bacteria.” Their extensive interactions with humans and wild animals make these bird species efficient transmitters of such pathogenic strains across various human, animal, and environmental reservoirs, further amplifying this already serious public health threat.

**Companion Animals Carry MDR Strains between Humans and the Environment**

Dogs, cats, and other pets are yet another conduit for transmitting MDR microbial strains within and between species. The intimate interactions between pets and the humans who take care of them foster colonization with community strains, which can spread back to human populations, serving as sources for recurrent human infections. In addition, pets shed these resistant strains into the environment (Fig. 3).

“Pets and wild animals are important reservoirs...that contribute to the spread and expansion of MDR pathogens, particularly for CTX-M-1/15-producing E. coli and newly emerging OXA-48- and NDM-producing E. coli and K. pneumoniae,” says Endimiani, referring to various types of β-lactamases. While data is scarce on precise colonization rates, studies in Western Europe suggest that approximately 18% of pets are colonized with these types of drug-resistant strains of E. coli. In parts of Asia, these rates are higher—estimated at 24.5% for mainland China and a whopping 87–92% in Hong Kong.

“There is increasing recognition that companion animals may act as a reservoir for community-associated MDR pathogens,” says Thomas Gottlieb of the University of Sydney, Australia. While reports of CRE in cats, dogs, and horses are common across the globe, animals in Australia remained free of CRE until recently. The resistance gene IMP-4 was first detected in Acinetobacter baumannii in Hong Kong in 1997, and first recognized in Australia in 2002 during a clonal outbreak in several urban hospitals. It continues to spread within humans, based on isolates from hospitals across eastern Australia.

During a localized outbreak in a Sydney cat shelter, Gottlieb identified such animals as an
unexpected source of *S. enterica* strains carrying this resistance gene. While strains harboring IMP-4 and infecting humans were documented in Australia during the past decade, this recent finding is the first evidence of its spread to other animal species, he says. “No CREs had been reported in companion animals or livestock in Australia, either as pathogens or commensals.” The IMP-4 carbapenemase gene likely moved from human clinical *E. coli* and *E. cloacae* isolates, and then spread to a wide variety of other Enterobacteriaceae via highly promiscuous plasmids such as IncA/C, he adds. “The maintenance of IMP-4 in broad-range plasmids may contribute to diffusion and maintenance in different bacteria in the Asia-Pacific [region].”

Some cats are asymptomatic carriers of IMP-4-carrying *S. enterica*, making them difficult to track and facilitating further spread of the clone, according to Gottlieb. He says that implementing infection control measures and better controls over antibiotic use in veterinary hospitals and human clinics could help to minimize the unwitting spread of these MDR strains. Meanwhile, the feral nature of many cats and their predatory activities against wild birds and mammals make them an efficient bridge between humans and other wild animal species. For example, the IMP-4 resistance gene moved relatively recently into flocks of silver gulls within southeastern Australia, he found. The colonization of these wild birds likely will accelerate the spread of these MDR microbial strains throughout Australia. “Gulls and other birds can be an efficient means of spread between waste dumps, which may include hospital waste and MDR flora, and to the community,” he says.

Companion animals also serve as reservoirs of MRSA strains, which can colonize skin and nasal mucosa, and are easily passed between humans and dogs, according to Holmes of Cambridge University. For example, dog owners sometimes become chronically infected with MRSA because their pets reinfect them following antibiotic treatment, he says, citing findings from Farrin Manian of St. John’s Mercy Medical Center in St. Louis, Mo. Similarly, more than 8% of infections due to MRSA in an outpatient facility were traced to

![Companion animals also serve as ways for drug-resistant organisms to move between species, often serving as sources to recurrent human infections.](Image©istockphoto/sdominick.)
family pets serving as reservoirs for these pathogens, according to Vance Fowler of Duke University in Durham, N.C.

This path for MRSA transmissions is a two-way street, with humans sometimes infecting dogs, Holmes points out, describing the chain of transmission of a particular strain of pathogen, called ST22, involving staff and animals at a particular animal hospital. “Direct transmission occurred in both directions, substantiating the view that ST22 has a broad host range and behaves as a nosocomial pathogen within a veterinary health care setting just as it does within human hospitals,” he says.

Such strains can circulate among humans, dogs, cats, and even horses—sometimes merely colonizing their animal hosts and other times causing fulminant illnesses, Holmes continues. “A shared population of an important and globally disseminated lineage of MRSA can infect humans and companion animals without undergoing host adaptation. This study furthers the One Health view of infectious diseases that the pathogen pool of human and animal populations are intrinsically linked and provides evidence that antibiotic usage in animal medicine is shaping the population of a major human pathogen.”

Part 2 of this article will appear in the next issue of Microbe.

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