



Wildlife Is Overlooked in the Epidemiology of Medically Important Antibiotic-Resistant Bacteria

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ABSTRACT Wild animals foraging in the human-influenced environment are colonized by bacteria with clinically important antibiotic resistance. The occurrence of such bacteria in wildlife is influenced by various biological, ecological, and geographical factors which have not yet been fully understood. More research focusing on the human-animal-environmental interface and using novel approaches is required to understand the role of wild animals in the transmission of antibiotic resistance and to assess potential risks for the public health.

KEYWORDS antibiotics, carbapenemase, environment, landfill, transmission, wild animal

An increasing incidence of antimicrobial resistant (AMR) bacteria has been reported globally, presenting one of the greatest medical challenges of our time. For example, carbapenems are among the most important antibiotics utilized as drugs of last resort to treat life-threatening infections in humans caused by multidrug-resistant bacteria. Unfortunately, the clinical efficiency of carbapenems is threatened by carbapenemases, broad-spectrum beta-lactamases capable of rapid degradation of these reserved antibiotics. The emergence and global spread of carbapenemase-producing *Enterobacteriales* (CPE) is of great concern to public health. Although resistance to carbapenems is associated mainly with hospital-acquired infections, there is a growing body of evidence that CPE found their way outside hospitals. Only a few studies have documented CPE in food-producing animals, food, and companion animals (1). And surprisingly, carbapenemase producers have also been reported in wildlife (2). Current data indicate that multidrug-resistant bacteria can spill over from their anthropogenic sources into natural ecosystems, possibly creating secondary reservoirs in the environment where clinically important resistance can be maintained and from where it can spread further.

In this issue of Antimicrob Agents Chemother, Ahlstrom et al. (3) provide data on the first detection of CPE isolates in wildlife in the United States. By examining feces of almost 1,000 gulls from seven locations near solid waste sites in Alaska for CPE using antibiotic-supplemented medium, they obtained 7 *Escherichia coli* isolates positive for the carbapenemase genes *bla*_{KPC-2} or *bla*_{OXA-48}. Although the overall prevalence of CPE found in wild birds in this study was quite low (<1%), this observation is surprising since the surveillance of carbapenem resistance in Alaska initiated in 2013 has so far revealed only four imported cases of human infections by CPE. Three *bla*_{KPC-2}-positive *E. coli* isolates from gulls were assigned to sequence type 410 (ST410), a successful clone with reported interspecies transmission between wildlife, humans, and companion animals (4). Four highly related (only two single nucleotide polymorphisms [SNPs]) sequence type 38 (ST38) *E. coli* isolates with chromosomally integrated *bla*_{OXA-48} were found in the gulls as well. Of note, ST38 is considered a globally dispersed multidrug-

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resistant clonal group of extraintestinal pathogenic *E. coli* commonly associated with urinary tract infections and bacteremia in humans. This high-risk clone has been also found in wildlife (5). The authors made an interesting observation that the *bla*_{OXA-48}-positive isolates were obtained from temporally and spatially separated samples, suggesting the resistant strain was maintained in the gull population or in an environmental source often visited by gulls. Whether the gulls obtained CPE locally from anthropogenic sources, such as the nearby landfill, or brought them via migration remained a question that the authors may answer in their ongoing research in Alaska (6, 7).

During the past decade, clinically relevant AMR bacteria have been isolated from various wildlife species, mainly synanthropic birds, in all continents, including Antarctica. So what are the driving forces of the AMR bacteria in wildlife? We are of the opinion that the occurrence of antibiotic-resistant bacteria in wildlife is influenced by various factors which have not yet been fully understood. Current data indicate that the biology and ecology of the host, including the level of anthropogenic impact in the area where the wild animals live and feed, are among the key factors (8, 9). Some of the main anthropogenic sources of the AMR bacteria in wildlife already suggested were landfills, insufficiently treated wastewaters draining into rivers and lakes, and waste from intensively managed livestock farms (2, 8). Several studies have proven that the bacterial genotypes found in wildlife in terms of sequence types and clinically relevant antibiotic resistance determinants resemble those found in humans and animals (10). Other reports found only a limited overlap in this regard, indicating that the horizontal transfer of genes via mobile genetic elements might play a more central role (11). Interestingly, a recent large-scale study from the Chinese poultry production system revealed a high prevalence of NDM-beta-lactamase-producing bacteria coharboring the plasmid-mediated colistin resistance gene *mcr-1* in poultry and also in insects and wild birds (12). This study pointed out that frequent dissemination of resistant isolates to the farm environment is occurring and highlighted the importance of wildlife in further dissemination of high-risk bacteria that combine resistance to two groups of last-line antibiotics.

A number of papers, including the above-mentioned study by Ahlstrom et al. (3), suggested that the accumulation of human refuse on landfills is one of the major anthropogenic-induced drivers in the transmission of resistant bacteria into wildlife, especially to wild bird species, such as omnivorous gulls and corvids, that are increasingly foraging on waste in urban areas. The *E. coli* strains showing identical genotypes as those isolated from landfills and wastewaters were found in gulls (13), indicating that the transmission of AMR bacteria between anthropogenic waste and birds may occur frequently. Of note, in two recent reports, an alarming prevalence of CPE in gulls has been documented. The VIM-producing *E. coli* isolates were found in 19% of yellow-legged gulls that were living in close proximity to humans in France (14). Striking results were brought by a report from a breeding colony in Australia, where 40% of the chicks of silver gulls carried IMP-4-producing *Enterobacterales* of various species and sequence types in their guts (9). Local observations revealed that during the breeding season, gulls fed predominantly on anthropogenic waste as thousands of birds per hour were observed leaving a main waste depot located near the gull colony. Interestingly, over the last decades, environmental contamination by human refuse completely changed the feeding behavior of some wild animals. Gulls and corvids feed on artificial diet, as they tend to seek the highly nutritious food in cities, fisheries, and especially on landfills. A similar shift in feeding behavior has been observed in other wild bird species, such as herons, storks, ibises, and some birds of prey (black kites and vultures), in various parts of the world. Changes in feeding behavior are followed by changes in migration patterns in some birds. For example, white storks in Spain have become resident (not migrating to sub-Saharan Africa) and substantially dependent on the food sources from local landfills during winter.

What are the importance and the public health implications of bacteria with clinically relevant resistance mechanisms found in wildlife? Above all else, wildlife is a

generally overlooked part of the environment which is highly influenced by human activities and plays an important role in relation to antibiotic resistance in the One Health concept. Moreover, wild animals not only are useful sentinels mirroring the presence of the AMR bacteria in the contaminated environment in a particular area but also have been recognized as possible reservoirs, melting pots, vectors, and secondary sources of multidrug-resistant bacteria for humans and animals. Wild birds are ubiquitous, and their feces are freely dispersed into the environment, possibly contaminating surface waters and soils where crops are grown. However, at this stage, it is difficult to assess human health risks of the AMR bacteria in wildlife, as it requires active surveillance of clinically relevant resistant bacteria in the environment, including wildlife, which is currently very limited. The use of antibiotics and human to human transmission are clearly the driving forces in CPE dissemination. However, the environment can be a significant part in the overall transmission scenario. Wildlife may play an important role in such a scenario, especially in countries with poor hygiene standards and sanitation, with insufficient wastewater treatment and waste management, where animals and humans live close together, and where clinically important multidrug-resistant bacteria are widely present in the environment (15). However, data from these areas on the occurrence of the AMR bacteria in wildlife are largely missing.

A particular concern regarding the role of wild migrating birds in the dispersal of AMR bacteria is their capacity for long-range movements across borders or continents and from regions with high levels of AMR bacterial contamination to less affected areas. The *E. coli*-producing extended-spectrum beta-lactamases have been described in migratory birds in remote areas with low human exposure (16). In contrast, a study on gulls migrating between Canada and Chile did not support the above-mentioned hypothesis, while suggesting that the AMR bacteria were obtained locally rather than transferred via migration (17). Therefore, the role of globally moving species in the dissemination of AMR bacteria over long distances remains unclear. To resolve this, it is crucial to understand whether wild animals are just temporary carriers of the AMR bacteria or if the resistant bacteria obtained from the contaminated environment can be maintained in their gut for a long time, giving more opportunities for further transmission to other individuals in the population or through the environment to other species. Studies with longitudinal observations will surely provide useful information on the dynamics of AMR bacteria in the gut of wildlife species. Innovative approaches that have recently been used to study AMR bacterial dissemination via the movement of wildlife, such as satellite telemetry tracking devices tagged on landfill-foraging gulls in Alaska, may help to understand spatiotemporal distribution pattern of wild birds, together with the degree of their association with anthropogenic sources and further dispersal potential of AMR bacteria (7).

Despite the ongoing research in this field, there are still many questions that need to be answered to better understand the implications of AMR bacteria in wildlife. Most reports of AMR bacteria in wildlife are survey based, snapshots, or small scale which enables us to only speculate on possible sources and routes of the AMR bacterial dissemination. Well-designed and conducted studies focusing on the human-animal-environmental interface that aim to clearly identify sources and their ranges of the AMR bacteria for wildlife, transmission pathways, directions, and nets are urgently needed. The areas where strong AMR bacterial presence and diverse anthropogenic inputs possibly clouding our observations may not be suitable in this regard. While research in areas with lower and well-defined anthropogenic sources as those studies being conducted by Ahlstrom and colleagues in Alaska may answer many essential questions. In the One Health concept, technologies such as whole-genome sequencing based on selectively obtained AMR bacterial isolates are highly useful for identifying shared bacterial strains, their similarity on the level of SNPs, antibiotic resistance genes, and mobile genetic elements (6). Nevertheless, the genotyping of selected AMR bacteria enables us to see only a small portion of the problem. Identifying the abundance of AMR bacterial genes and mobile genetic elements in the gut of wild animals by other approaches, such as metagenomics, could give us a more complete picture in future.

Another issue that has not gotten attention in the current literature is the combined impact on wildlife of antibiotics and other compounds, such as heavy metals and biocides, present in the environment. Antibiotics or their active residues are present in urine and feces of treated humans and animals or in effluents from drug manufacturers, resulting in their spread into the environment. Do these antibiotics ingested by wild animals have selective pressure on their gut microbiota? Also, the fate of the AMR bacteria in the environment and wildlife is still unclear. Could AMR bacteria and mobile genetic elements carrying the resistance genes further evolve after their transfer to the environment? There are some hints suggesting this phenomenon may exist (2).

As nearly all environments and wildlife around the globe are influenced by anthropogenic activities and the occurrence of heightened AMR bacteria, a worldwide One Health collaborative approach is certainly needed. Unfortunately, current national and international programs are generally biased toward human and domestic animal AMR bacterial surveillance and the environment has only limited attention. One of the initiatives in this regard is a recently approved networking project, “Wildlife, agriculture soils, wastewater environments, and antimicrobial resistance—what is known, needed, and feasible for global environmental surveillance (WAWES),” supported by the Joint Programming Initiative on Antimicrobial Resistance. Within this project, an international consortium of experts with a shared objective was built to explore and propose a way of performing global comparative surveillance of AMR bacteria in the environment, with the special focus on wildlife.

In conclusion, the spread of AMR bacteria in wildlife deserves considerable attention. More research focusing on the human-animal-environmental interface and using novel or combined approaches is clearly required to understand the role of wild birds and other animals in the transmission of clinically relevant resistant bacteria and the AMR bacterial genes and to estimate the level of potential hazard to public health. The intervention to minimize transmission of AMR bacteria from intensive livestock and human population into the environment is clearly crucial. Better waste management will help in this regard. An organized standardized surveillance of AMR bacteria in the environment and wildlife, which is currently missing, is needed to understand the extent of the problem on a global scale and to propose possible solutions.

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