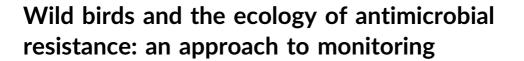
COMMENTARY





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Abstract

Tackling the global threat of antimicrobial resistance (AMR) requires joint efforts according to the principles of the One Health approach. In this context, wildlife, and especially wild birds, are recognized as an important bridge between environment, humans and livestock in perpetuating AMR. Over the last decades, important progress has been made in understanding the role of wild birds as carriers of antibiotic-resistant bacteria and their genes (ARGs) within ecosystems; however, there are still many knowledge gaps regarding transmission sources and routes. This commentary summarizes studies from recent years focusing on AMR in wild birds, highlighting the most frequently found zoonotic bacteria harboring ARGs and the possible exchange scenarios between humans, livestock, and wild birds. We emphasize the need to standardize and optimize a wild bird monitoring approach for AMR surveillance that includes non-invasive sampling methods, cultureindependent techniques for identification of ARGs, database integration and implementation, and machine learning technology. This multidisciplinary perspective, which could involve veterinarians, biologists, ornithologists, conservationists, and managers, may represent part of the solution, not only for wildlife conservation but also for global health, considering that the goal is to reverse the route of AMR.

KEYWORDS

AMR monitoring, cross-disciplinary approach, environmental health, transmission routes, wild birds, wildlife research and management

Antimicrobial resistance (AMR) refers to the property of bacteria that become less susceptible to an antimicrobial agent (Tang et al. 2023). Bacteria can gain AMR by overexpressing or duplicating some genes, undergoing chromosomal mutation, or obtaining resistance genes from other bacteria by horizontal gene transfer. Although AMR is a natural phenomenon, anthropogenic pressures, such as human wastewater systems or animal husbandry facilities, may increase the development, multiplicity, and quantity of antibiotic-resistant bacteria and genes in the environment (Berendonk et al. 2015). Inadequate treatment of waste from humans and livestock containing antimicrobial drugs leads to the environmental spreading of antibiotic-resistant bacteria and antimicrobial resistance genes (ARGs), thus resulting in one of the major threats to public health worldwide. The prevalence of multidrug-resistant (MDR) bacteria is globally increasing (Yuan et al. 2016). Some recent investigations outlined the emergence of MDR bacterial pathogens from different origins, including humans, birds, livestock, and water organisms, which leads to the need for routine antimicrobial susceptibility testing and improved screening of emerging MDR strains (Yuan et al. 2016).

Transmission is a cardinal element in antimicrobial resistance spread; identifying the sources and detecting the transmission routes is fundamental to address the antimicrobial resistance issue. Almost every ecosystem in the biosphere can play a role, in different degrees, in the origin, evolution, and spread of AMR (Larsson et al. 2018). The environment may act as a reservoir for ARGs, collectively constituting the antibiotic resistome, and promotes horizontal gene transfer and the sharing of resistance genes as a bacterial adaptation response (Samreen et al. 2021). Wildlife is considered an environmental reservoir and potential melting pot of AMR, contributing to its transmission across different ecosystems. The antimicrobial-resistant genes harbored by pathogens, causing problems in medical settings, might spread among the bacteria present in the microbiomes of ubiquitous animals that could be in turn bioreactors for antimicrobial-resistant genes among human pathogens (Lagerstrom and Hadly 2021, Laborda et al. 2022). Importantly, 61% of human pathogens are zoonotic (Taylor et al. 2001), 60.3% of emerging infectious diseases are zoonoses and, of these, 71.8% originate from wildlife (Jones et al. 2008).

Wild sedentary and migratory birds are important reservoirs of zoonotic pathogens (Benskin et al. 2009). The anthropization of ecosystems has forced many wild birds to adapt their biology to human presence and the possibility of contact with humans has increased (Martín-Maldonado et al. 2022). The transmission of resistant microorganisms between wildlife and humans is currently being documented (Laborda et al. 2022). The isolation of antimicrobial-resistant bacteria (AMRB) in wild birds results from a spill-over phenomenon through environmental pollution with human or domestic animal-origin strains (Benskin et al. 2009). The prevalence of AMRB and ARGs in wildlife is higher in areas with a significant human presence, indicating that AMR in wildlife is an important indicator of anthropic pollution (Marcelino et al. 2019, Laborda et al. 2022, Martín-Maldonado et al. 2022). Wild birds can also be involved in the dissemination of ARGs through horizontal gene transfer among different bacteria, which can later disseminate among different hosts (Laborda et al. 2022).

Sedentary birds could move along short distances and the transfer will take place locally (Laborda et al. 2022). Among sedentary birds, synanthropic species live in contact with humans and depend on human resources for nutrition and habitat because urban areas provide resources to feed and reproduce (Martín-Maldonado et al. 2022).

Migratory birds are in the spotlight for their ability to carry bacteria, viruses, parasites, and fungi over long distances through their routes between countries and continents, twice a year (Giorgio et al. 2018, Elsohaby et al. 2021). Migratory birds, via their migration routes and during migration stopovers, may interact with sedentary birds, which in turn can interact with ornamental, domestic, or poultry species. This establishes a possible avian

circulation of several antimicrobial-resistance pathogens. This behavior introduces a high risk of spreading diseases beyond local outbreaks to become a global health issue (Giorgio et al. 2018, Şahan Yapicier et al. 2022).

The scientific community has investigated the role of wild birds with regard to AMR worldwide, but data are still lacking (Vittecoq et al. 2016, Martín-Maldonado et al. 2022). The role of migratory birds in the spread of MDR bacteria needs to be understood, considering the effect that AMR has on the economy and global health (Yuan et al. 2016, Elsohaby et al. 2021). We recognize that fully understanding the role that each component (i.e., domestic animals, human populations, wildlife, and environmental reservoirs) plays in the maintenance and the dispersal of AMR within bacterial populations, must be prioritized.

Our objective is to emphasize the importance and usefulness of a holistic approach to stem the silent pandemic of antimicrobial resistance through the monitoring of wild birds. We argue for a synchronous work among different professionals, researchers, and managers, who can together monitor antimicrobial resistance trends. It could represent an integrated system to predict the phenomenon and help target interventions. With this surveillance strategy of AMR monitoring, we could acquire additional information about zoonotic antimicrobial-resistant bacteria circulating among wild birds, how wild birds acquire antimicrobial-resistant bacteria, and which exchanges of antimicrobial-resistant bacteria occur between humans, domestic animals, and wildlife.

ANTIMICROBIAL-RESISTANT PATHOGENS CARRIED BY WILD BIRDS

Wild birds could carry in their microbiome AMRB that is recognized as a public health concern (Russo et al. 2022). Many recent studies could give a clear idea of which zoonotic bacterial species are the most frequently found in wild birds and which possible resistance determinants they harbor. For example, gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, and extended-spectrum beta-lactamase (ESBL)-producing gram-negative bacteria could be some of the main key indicator pathogens to delineate the evolution of AMR within a variety of environments and wildlife (Zhanel et al. 2008, Laborda et al. 2022).

Golden staph (*Staphylococcus aureus*) is a commensal bacteria that could be an opportunistic pathogen of humans and animals (Rossi et al. 2020). In particular, MRSA is a life-threatening pathogen in humans and its presence in animals represents a public health issue (Becker 2021). Often MRSA is multidrug-resistant, especially towards most beta-lactam antibiotics, and birds (e.g., Ciconiidae family) seem to have a significantly higher prevalence of golden staph when compared to other wild animals (Abdullahi et al. 2021). The occurrence of golden staph in wild birds has 18% of phenotypic and 64% of genotypic methicillin resistance, and strong biofilm formation (90%; Tareen and Zahra 2023).

Escherichia coli is the most commonly targeted bacteria in AMR studies and its resistance to carbapenems in wild birds and other wild animals has been largely identified (Vittecoq et al. 2016). *Escherichia coli* sequence type 38 is the most frequently reported carbapenem-resistant clone in wild birds (especially in birds from Laridae, Anatidae, Gruidae and Ciconidae families), and is argued to be exchanged intercontinentally between humans, wild birds and the environment (Ahlstrom et al. 2023*b*). Recent data arising from whole-genome sequencing and phylogenomic analysis revealed the presence of *E. coli* clones (ST295 and ST388) producing CTX-M-55 and CTX-M-1 ESBLs from migratory and resident Franklin's gulls (*Leucophaeus pipixcan*) in Chilean Patagonia; the same clones were also detected from the environment, companion animals, and livestock in the United States, which indicates a possible trans-hemispheric spread of international clones of ESBL-producing bacteria considered by the World Health Organization to be critical priority threats (Fuentes-Castillo et al. 2023).

Furthermore, fecal samples of wild birds collected along the Jinjiang River in Chengdu, China had a great prevalence of antimicrobial-resistant *E. coli* (59.4% to tetracycline, 65.3% to levofloxacin, 49.0% to ampicillin and 54.0% to nalidixic acid; Lin et al. 2022). Another study conducted in different regions of China showed that *E. coli* isolates from migratory birds exhibited high resistance to β -lactams (43.7%) and tetracycline (22.6%), and 15.3% of them were MDR (Yuan et al. 2016).

Multidrug resistance patterns were found in 44.8% of *E. coli* isolated from chicks of Canarian Egyptian vulture (*Neophron percnopterus majorensis*) in a supplementary feeding station in Fuerteventura (Suárez-Pérez et al. 2023). In Spain, the importance of supplementary feeding stations for Eurasian griffon vultures (*Gyps fulvus*) in the dissemination of AMRB from pig carcasses was also confirmed (Sevilla et al. 2020). Landfills and farm animal carcasses are significant points for the dissemination of AMR or particles of antibiotics and other drugs into ecosystems, sometimes resulting in problems for biodiversity (Sharma et al. 2018, Sevilla et al. 2020).

In Malaysia, isolated *E. coli* strains showed resistance to all the antibiotics tested, with 100% MDR *E. coli* from cloacal swabs of wild birds in Orang Asli villages and 44.4% from wild birds in Malay villages (Mohamed et al. 2022). The researchers also reported the presence of the eae gene in 12.1% of the *E. coli* isolates from the Malay villages, of which 71.4% identified as MDR. The latter finding could be attributed to various factors, including the feeding habits of these birds. These groups of birds in Malay villages fed on human garbage that was likely contaminated by bacteria that carry the eaeA and other ARGs or might become infected by contact with animal farms (Mohamed et al. 2022). The presence of commensal *E. coli* with resistance to third-generation cephalosporins, fluoroquinolones, and colistin was reported in samples from wild ducks and geese in Germany (Plaza-Rodríguez et al. 2021). In Alaska, the isolation from gull (*Larus* spp.) fecal samples of \geq 1 resistant *E. coli* from 16% of the collected samples and 58 different ARGs was documented (Ahlstrom et al. 2021).

Salmonella bacteria (Salmonella spp.) are a considerable cause of foodborne disease worldwide and can be found mostly in the intestinal tract of poultry and wild birds. During the last 20 years, a lot of clones of Salmonella showed increasing multi-resistance at global scale. Salmonella enterica serovar Typhi, the cause of typhoid fever, has a genomic element that carries resistance to 5 antimicrobials and could spread horizontally among serotypes; it is widely detected in birds (Cizek et al. 2007, Fuentes-Castillo et al. 2023). In the United States the presence Salmonella enterica in wild birds was documented: Typhimurium in this study was the dominant serovar and all the MDR strains were isolated from waterbirds or raptors, with 75% carrying resistance plasmids (Fu et al. 2022). Wild birds could also carry other zoonotic serovars such as Infantis (Card et al. 2023).

The enteric pathogens *E. coli* and *Salmonella* species were also isolated from 22 different wild bird species in 4 provinces of Turkey by Şahan Yapicier et al. (2022): *E. coli* isolates had the greatest AMR patterns for lincomycin (100%), penicillin, kanamycin, tetracycline, oxytetracycline, and doxycycline, whereas *Salmonella* serotypes were resistant to lincomycin, nalidixic acid, and penicillin (Şahan Yapicier et al. 2022).

Enterococcus bacteria (*Enterococcus* spp.) are opportunistic pathogens, able to acquire and transfer antimicrobial-resistant genes. In Central Italy, *Enterococcus* strains were isolated from the feces of wild birds of different species, with 99.02% of them classified as multidrug-resistant (Cagnoli et al. 2022).

Wild birds have also been identified as a relevant reservoir of Campylobacter bacteria (*Campylobacter* spp.) pathogens associated with human diseases (Olvera-Ramírez et al. 2023). In central Spain, *Campylobacter jejuni, Campylobacter coli*, and *Campylobacter lari* were isolated from cloacal swabs, aseptically collected from 4 families of birds of prey (Accipitridae, Falconidae, Strigidae, Tytonidae) that had been admitted to a wildlife rescue center, on the day of admission prior to any treatment or housing in the hospitalization cage. Resistance profiles to nalidixic acid (69.9%), ciprofloxacin (69.9%), and tetracycline (55.6%), and a low resistance to streptomycin (6.7%) were detected from the isolates.

Moellerella wisconsensis is a potentially zoonotic pathogen that was sporadically isolated from animals and humans. In Greece, a prevalence of 0.9% was reported in the examined wild birds, showing resistance to third-generation cephalosporins, tetracyclines, aminoglycosides, and trimethoprim-sulfamethoxazole (Thanasakopoulou et al. 2022). Although *Moellerella wisconsensis* is a rare clinical isolate, those findings underlined the potential role of wild birds in both the spread and dissemination of the gene blaCTX-M-1, which is one of the main genes encoding ESBL and is commonly located on bacterial plasmids that can spread easily between and within bacteria (Wibisono et al. 2020).

In Spain, ARGs were detected in fecal samples of 4 waterbird species wintering in southwest Spain. Genes conferring resistance to the most common classes of antibiotics were detected with higher presence in lesser black-backed gulls (*Larus fuscus*) and white storks (*Ciconia ciconia*) than in graylag geese (*Anser anser*) and common cranes (*Grus grus*; Jarma et al. 2021).

Taken together, these results highlight the complex distribution of AMR in natural environments and suggest that biological and anthropogenic factors play an important role in determining the emergence and persistence of AMR in wildlife (Johnson and VanderWaal 2020). Both sedentary and migratory wild birds could act as vectors of AMRB and ARGs, at short or long distances.

ROUTES OF ANTIMICROBIAL RESISTANCE

The whole environment is involved in the persistence and spread of AMR and AMRB are ubiquitous in natural ecosystems, and it is very difficult to trace the exchanges of AMRB and associated genes between humans, animals, and the environment, and to identify how resistant genes emerge and spread (Vittecoq et al. 2016, Laborda et al. 2022). Many ARGs are shared in the wild bird fecal and environmental resistome, with the blaTEM gene as a molecular marker highlighting the remarkable interconnectivity of ARGs between the microbiomes of wild birds and their habitats (Luo et al. 2022). The blaTEM gene is most commonly found within the genes encoding ESBL in community and livestock environments (Abrar et al. 2019, Effendi et al. 2022).

Health care facilities are important contributors to the emergence, evolution, and spread of antibiotic resistance, but other ecosystems are also involved in such dissemination. Selection for resistance in the environment and human or animal exposure to resistant bacteria are interlinked events (Murray et al. 2021). More and more studies have shown that identical or near identical strains, belonging to the same clonal complex, are circulating in wildlife, humans, and domestic animals (Monecke et al. 2013). This information did not determine how and in which direction the exchanges occurred. The source of AMRB in wild birds has not been determined yet, but it is likely to be related to direct contact with infected individuals, their tissues, or their feces, even though ARGs are ancient elements that evolved before the clinical use of antibiotics (Martinez 2008). Although resistance genes could be found in natural environments regardless of human impact, their presence has been reported to be higher in birds living in the ponds of a wastewater treatment facility, so contact with human waste likely influences the acquisition of ARGs by avian wildlife (Marcelino et al. 2019). The ESBL-producing E. coli in Swedish wild gulls were similar to human isolates, which was likely a result of anthropic pollution (Atterby et al. 2017). Once antibiotic resistance is present in the wild, wildlife can contribute to its transmission across disparate ecosystems (Laborda et al. 2022). There are several wild birds that share their habitat with humans because of increased food resources and preferred temperatures; for example, storks breed in urban areas and gulls use ports, coastal towns, beaches with a high human density, urban areas close to cultivated land, and urban and natural parks (Martín-Vélez et al. 2021, Xu et al. 2022). Sharing the same space could lead to an increased similarity of bacterial community composition among individuals. Studies regarding the microbiota communities show that cohabitation enhances transmission of microorganisms between livestock and wild animals, and the predominant presence of resistant microorganisms against medically important antibiotics occurs at the wildlife-livestock interface (Lee et al. 2022; Figure 1).

Climate change has induced ethological changes or possible alterations in the migration routes of various species and has modified the distribution of wildlife all around the world, including vectors involved in the dispersal of infectious diseases. These changes have led to inestimable losses of ecosystems for birds (Ahlstrom et al. 2021, Agache et al. 2022). Moreover, humans can come into direct contact with wild birds when they trap, hunt, or treat them as veterinarians and the risk of AMR transmission linked to these practices might be assessed as very strong (Vittecoq et al. 2016). Despite the significant work wild animals rescue centers do, they may contribute to the entry of AMR into ecosystems by releasing animals before antibiotics completely eliminate a pathogen and before the waiting time for administered antibiotics is completed (Baros Jorquera et al. 2021). These waiting times after antibiotics are administered, well-established for livestock farming, are insufficiently studied for wild species but should be a crucial measure before reintroducing animals into their environment (Benavides et al. 2024).

Wild birds could also have direct contacts with livestock undergoing antibiotic treatments. It has been suggested that vultures and other scavengers feeding on livestock carcasses might ingest active antibiotics that may

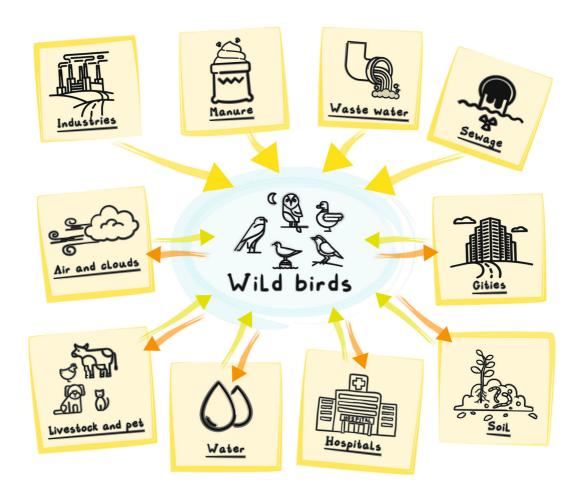


FIGURE 1 Transmission pathways of antimicrobial resistance in the wild bird-livestock-human interface.

be present in such carcasses (Blanco and Bautista 2020). Indirect contact could also pose a risk of AMR transmission into the food chain, affecting all the ecosystem (Blanco and Bautista 2020). Wild birds could be a source of antimicrobial-resistant microorganism colonization in livestock as well (Cizek et al. 2007, Navarro-gonzalez et al. 2020). Identical pathogen strains of *E. coli* were shared between free-ranging cattle and wild geese in California, USA, suggesting an identical environmental source of contamination and also potential interspecies transmission (Navarro-Gonzalez et al. 2020). Furthermore, black-headed gulls (*Larus ridibundus*), infected with resistant strains of *Salmonella*, represent a risk for contamination of surface waters and livestock feeds and dissemination of these strains among farm animals and humans (Cizek et al. 2007).

Soil could be a big hot spot of antimicrobial-resistant microorganisms and associated genes (Han et al. 2022). It could get contaminated from feces or urine deposition, manure use, or effluent flows (Singer et al. 2016, Peng et al. 2017, Han et al. 2022). Agricultural land cover could be an important predictor for the prevalence of any ARGs and the common practice of spreading animal manure or biosolids on agricultural soils as fertilizer could contribute to this relationship (Peng et al. 2017). These materials could be reservoirs for residual antibiotic compounds, AMRB, and ARGs, which might persist in the soil long after application and leach into rivers, lakes, or other aquatic ecosystems (Singer et al. 2016, Nappier et al. 2020).

Water seemed to be a major transmission media for AMRB (Taylor et al. 2011) that could persist in this environment, especially with the higher temperatures related to climate change and the presence of

sub-therapeutic concentrations of antibiotics in aquatic systems increasing AMR (Murray et al. 2021). The exchange of resistance genes between environmental microorganisms and human pathogens could occur in aquatic systems where birds stop or feed. In contaminated waters, birds could come into contact with antimicrobial-resistant microorganisms and ingest them or simply carry them on their feathers. For example, in Spain a goose population wintered mainly in the marsh of Doñana National Park, where the edges received discharges from urban wastewaters and could get contaminated (Paredes et al. 2021). Wastewater treatment plants are places with a large load of clinically relevant AMRB (Pärnänen et al. 2019) where birds could feed and come in contact with ARGs. Wastewater from livestock acts as an environmental reservoir and propagation site of ARGs to other clinically important human pathogens (Tymensen et al. 2018). Whatever the source of the water contamination, it generally increases when close to human activity areas (Pruden et al. 2012). Even in areas not closely associated with human activity, antimicrobics and associated resistance genes can be found. Water serves as an important vehicle for ARMB and free ARGs, traveling to other regions through rivers as reported in Costa Rica where detection of antibiotics and other drugs were found in rivers (Vargas-Villalobos et al. 2024). Furthermore, the use of antimicrobials in aquaculture has an impact on the spread of AMR, but its role is not entirely clarified. Surprisingly, to complete the cycle, ARGs have been detected in clouds and air at high altitude, confirming that the atmosphere represents a major route of their long distance spreading, with clouds as a selective environment for the most efficient stress response phenotypes (Rossi et al. 2023).

The exchange of AMRB among the various environments has been demonstrated using a combination of phenotypic, genomic, and animal telemetry approaches (Abdullahi et al. 2021). For example, gulls can acquire AMRB from anthropogenic sources and, later on, allow AMRB intercontinental spread via migratory movements (Ahlstrom et al. 2021). Moreover, ruddy turnstones (*Arenaria interpres*) travel between breeding areas in Siberia to non-breeding sites in Australia via East Asia as a migratory route, potentially acquiring and spreading several ARGs along the way (Marcelino et al. 2019).

A WILD BIRD MONITORING APPROACH FOR AMR SURVEILLANCE

To fully understand and mitigate AMR, it is important to consider the role of the natural environment as part of the One Health perspective that includes wildlife in its modeling (Jessup and Radcliffe 2023). It is essential to increase the knowledge about the effects of antibiotic and human exposure to wildlife and to the entire environment, and in particular how much they contribute to the spread of AMR. Overall, despite their importance for pandemic prevention, wildlife and environment are seldom considered in health security plans; monitoring wildlife health should be emphasized in the One Health approach to prevent and mitigate known and novel disease risks (Machalaba et al. 2021). Zoonosis surveillance is an important measure to control possible emerging diseases. Epidemiological surveillance with a focus on native fauna is a way to identify possible threats to humans and animals that are concealed in reservoirs or yet unknown (Duarte et al. 2019). The specific determinants and spatial distribution of resistant bacteria and ARGs in the environment remain incompletely understood. In particular, information regarding the importance of anthropogenic sources of AMR is lacking (Miller et al. 2020). Important progress has been made in the understanding of the role played by wild birds in AMR dynamics, but studies focusing on migratory birds that track hotspots and transmission routes of AMR remain scarce (Laborda et al. 2022, Martín-Maldonado et al. 2022). More research into the movements of migratory birds would help to identify potential sources for the ARGs they harbor and how resistant organisms move and spread. Restricting the use of antibiotics is not enough to tackle the AMR problem (Organization for Economic Cooperation and Development 2022); knowing the transmission routes and obtaining more data could make the difference.

Obtaining data by sampling wild birds could seem difficult, as it requires considerable logistical efforts, but this is not completely true. Bird ringing camps and wild animal rescue centers (only if sampling is carried out at arrival) are fundamental components of epidemiological surveillance work (Mazzamuto et al. 2022). There are easy,

economic, atraumatic, and non-invasive methods for sampling birds without interfering in their welfare, both in wildlife rescue centers and in bird ringing camps. For field studies, the collection kit is formed by a flat-bottomed paper bag, a weigh boat (tray), a vinyl-coated hardware cloth fencing (grate), a clothespin, and a 10% bleach solution (to clean the tray and grate). In the paper bag, a sterile tray is placed under a small grate, on which the birds, captured during the ringing camp, could be placed until they defecate; after the bird is removed from the bag, the tray is extracted and the fecal sample is moved to a collection tube and frozen or preserved (Knutie and Gotanda 2018). In wild animal rescue centers, the sampling kit includes a plastic storage box, a plastic tray, a vinyl coated hardware cloth, and a 10% bleach solution; the feces collection is performed from the tray after placing the bird in the box and waiting a few minutes for defecation (Borrelli et al. 2020). From a diagnostic-methodological perspective, several techniques have been developed to detect AMR in different environments to better understand the evolution and spread of this public health challenge. Classical cultural microbiology techniques are not always sufficient to analyze non-culturable bacterial species that can also carry and transfer AMR genes. Quantitative polymerase chain reaction (qPCR) and culture followed by sequencing and, overall, whole-genome sequencing (WGS) may be effective approaches for characterizing AMR genes harbored by wild birds (Ahlstrom et al. 2023a). In particular, metagenomics is a good matrix to investigate AMR and detect early global emergence events (Munch et al. 2022). Functional metagenomics has determined entirely sequence-novel resistance determinants, facilitating the characterization of resistomes from various environments. There are several ARGs that have emerged clinically and circulated globally through the environment and human and animal populations such as mph(E), msr(E), tet(A), tet(C), tet(W), sul1, and sul2 (Crofts et al. 2017, Munk et al. 2022). In addition, metatranscriptomics have been used to assess the diversity and abundance of ARGs in the microbiome of waterbirds in Australia and penguins in Antarctica, highlighting unique antibiotic resistance genes with ubiquitous resistance to tetracyclines and phenicols (Marcelino et al. 2019). We suggest that all these methods should be standardized to approach the AMR problem with a multidisciplinary perspective including the role of veterinarians, biologists, conservationists, and ornithologists considering the One Health strategy.

The strong evidence that the health of humans, animals, and ecosystems are interconnected stresses the importance of finding a solution to AMR with a new holistic approach where conservationist methods meet those of epidemiological monitoring (Figure 2). Monitoring and evaluation of data are integral components of an iterative, research-based approach to bird management and conservation and a new AMR surveillance strategy. Therefore, researchers and wildlife managers have the common goal of conserving wildlife and the habitats on which they depend, and can investigate and monitor levels of AMR that significantly affect the world economy and human and animal health, through collaborations that yield rewards to global health.

Wild birds represent indicators of environmental AMR and are bridge hosts that can potentially facilitate the persistence of AMR in the habitats they occupy (Ahlstrom et al. 2023*a*). The microbiomes of wild birds frequently harbor human bacterial pathogens, including AMRB and even the clones of concern at medical settings (Oteo et al. 2018, Stępień-Pyśniak et al. 2019, Dec et al. 2020). Wild birds appear to be useful sentinels for AMR surveillance both in natural and urban ecosystems because of their adaptive capacity in anthropogenic areas. Transmission routes are unknown, although direct contact, soil, and water seem to be of major importance in AMR circulation. There is evidence that migratory birds are potential reservoirs and spreaders of MDR bacteria (Yuan et al. 2016, Giorgio et al. 2018, Elsohaby et al. 2021, Jarma et al. 2021, Zhang et al. 2021, Card et al. 2023). Wild birds might provide a link between the 2 reservoirs, humans and livestock, creating a bridge for the transfer of AMR among apparently disconnected ecosystems, even between different continents (Yuan et al. 2016). They could carry ARGs that are frequently located in the same plasmids that cause the spread of AMR among human populations and livestock (Atterby et al. 2017, Tinoco Torres et al. 2020, Zeballos-Gross et al. 2021, Zhang et al. 2021). In the One Health perspective, future research should prioritize investigations into the routes and mechanisms of transmission, especially at wild birds-livestock interfaces (Hassell et al. 2017, Martelli et al. 2023).

The presence of AMR in wildlife is often interpreted as an anthropogenic impact such as inadequate management of antibiotic residues of human or animal origin (Laborda et al. 2022). But considering the ability of

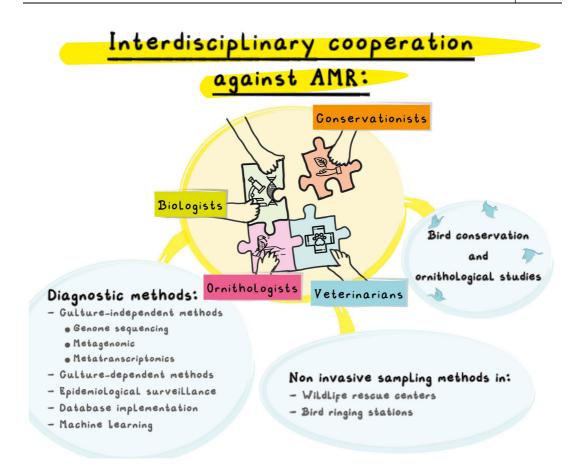


FIGURE 2 Holistic and cross-disciplinary approach underpinning wild bird monitoring to explore and tackle antimicrobial resistance (AMR).

birds to move over long distances, a model for monitoring resistant bacteria in wild birds, as reservoirs in the dispersal of resistant bacteria, could be useful even far from areas of high anthropogenic activity.

Investigation of AMR in the environment and wildlife still receives little attention, and only very recently understanding the selection and proliferation of environmental AMR became a collective priority among agencies charged with human, animal, and environmental health. Conversely, surveillance of AMR in humans and domestic animals in some countries has been ongoing for decades. It is important to increase surveillance of the resistome in hotspots in which there is high resistance gene evolution and transfer between organisms, including agricultural settings and wastewater and sewage systems where wild birds get contaminated (Crofts et al. 2017). Surely better management of anthropogenic wastes and sewage is a key way to avoid the access of wild birds to antimicrobials and prevent transmission (Martín-Maldonado et al. 2022).

To get comparable results, surveillance methods should be improved and standardized, such as genomic monitoring to timely identify antibiotic ARGs and their diffusion in the environment. There are also several new sequenceindependent methods of resistance determination that should be further explored, such as matrix-assisted laser desorption ionization-time of flight mass spectrometry, fluorescence *in situ* hybridization, and microfluidics-based techniques (Boolchandani et al. 2019). These methods could represent the starting points for new public AMRB and ARG databases that should be routinely updated with data from wildlife and the environment and for implementing machine learning, as a potential technology for AMR prediction (Tang et al. 2022).

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To prevent epidemic animal-borne infectious diseases, we should strengthen the monitoring of pathogens in wild animals, enhance the detection of emerging infectious diseases, improve the identification of the AMR risks, survey the vector arthropods, and study the migratory routes of wild birds (Qin et al. 2021). This holistic and integrated wild bird monitoring solution could be a new approach to epidemiological monitoring of AMR.

There are no nationally agreed or European guidelines for the use of antimicrobials in wildlife with the goal of limiting the spread of AMR. In many cases, knowledge of the pharmacokinetics of antimicrobials in wildlife species such as wild birds remains scarce. Several antimicrobials are used off-label in wildlife, and doses, frequency, duration, and routes of administration may be based on results from other domestic species. Recommendations for the correct use of antimicrobics in wildlife should be based on current knowledge and standardized practices are needed. Nothing in nature is lost and the goal is to reverse the route of AMR to change the fate of our ecosystem through forward-looking cooperation between different experts and ever closer synergies between wildlife managers and researchers.

MANAGEMENT IMPLICATIONS

The adoption of a monitoring system and process for detecting AMR in wildlife can be useful for animal and public health. We recommend that managers and scientists carefully consider the priority of health monitoring of migratory and resident wild birds through non-invasive sampling systems. Health monitoring methods could be taught in wildlife courses at colleges and universities to future wildlife scientists and managers. We also recommend that management studies or programs should focus on the broader ecological, social, and economic context of AMR ecology, wild bird conservation, and One Health. Wildlife health monitoring methods should be quickly evaluated to identify their relative strengths and weaknesses, including consideration of possible costs.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no known competing financial interests or personal relationships that could have influenced the work in this paper.

ETHICS STATEMENT

All procedures recommended in this study are in accordance with ethical standards to stress the animals as little as possible.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

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