

Spreading of antimicrobial resistance in agro-ecosystems: urgent need of assessment

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One of medicine's greatest achievements in the first half of the previous century was the discovery and commercial production of antibiotics. However, excessive use of antimicrobials in health care, agriculture, aquaculture, horticulture and industrial sectors impacted the expression, selection, persistence and transfer of antimicrobial resistance within the bacterial community. The World Health Organization (WHO), Geneva, Switzerland has identified antimicrobial resistance (AMR) as among the 10 threats to global health¹. AMR is considered an emerging contaminant with the potential to harm the environment and human health if not properly monitored. Therefore, in order to achieve the goals of sustainable development, interdisciplinary efforts have become imperative. It necessitates urgent multisectoral action in order to achieve the sustainable development goals. According to a report released by CDC, USA, every year more than 2.8 million people are infected with antibiotic-resistant infections in the US alone leading to 35,000 deaths². According to WHO, by 2050 resistant diseases could cause 10 million deaths each year if no proper action is taken. The pattern of antibiotic resistance varies between different regions and countries, corresponding to antibiotic production and consumption rates³. Overall, AMR threatens global health, food security, livelihoods and treatment costs.

Some of the commonly known routes through which AMR reaches the agro-ecosystem include the use of sludge from sewage treatment plants (STPs) as manure to improve soil fertility, the use of STPs-treated water for irrigation purposes, the use of poultry waste as fertilizers, surface run-off from landfill/open dumping sites, etc. Agro-ecosystems are one of the major drivers of dissemination of AMR genes and in terms of amplification, they are more likely to support the global spread and persistence of AMR in the environment. In many countries, the largest use of antimicrobials is in agriculture, and not in the medical sector. Various antimicrobial agents are used as livestock feed supplements to protect them from diseases, are released in their excretory waste, and increasing the spread of AMR genes. Application of antibiotic-contaminated manure from food

animals to supplement nutrients in the soil benefitting soil health and crop productivity results in an increase in transmission of antibiotics and antibiotic resistance genes (ARG) in the soil as these antimicrobials and their metabolites present in animal manure may seep into the soil and reach the agro-ecosystems. In a comprehensive study, eight sulphonamides, seven fluoroquinolones and four tetracyclines were detected in manure from large-scale animal feedlots in China⁴. On farmland application of antibiotic-contaminated manure, 49 antibiotics – mainly chlortetracycline, tetracycline, doxycycline, enrofloxacin, oxytetracycline, ciprofloxacin and norfloxacin – were detected in the soil⁵. Agricultural lands with livestock manure have increased levels of both dissolved organic matter (DOM) and antibiotics. DOM in different manure sources regulates the fate of antibiotic residues⁶. Heavy metals also contribute to the dissemination of AMR. Heavy metals, particularly copper and zinc have been recovered from manure as they are used as food additives in animal husbandry and are considered as drivers of antibiotic resistance⁷. Even if the use of antibiotics is decreased or abandoned by animal producers, detectable ARGs may persist for decades in agricultural lands and as a result of their tenacity in the agro-ecosystem, plant crops become exposed to the ARGs, further posing risks to human health⁸.

Extensive use of antimicrobials for prophylaxis and therapeutic purposes is also prevalent in commercial poultry management. Coccidiosis causes morbidity and death in poultry, ruining the industry, causing economic loss. Therefore, to control avian coccidiosis in modern poultry production, anticoccidial drugs are being used. These drugs are further transmitted to the environment causing addition to AMR. Thus, several antibiotic resistant bacteria (ARBs) and ARGs are detected in the poultry environment⁹. The resistance genes also develop after the application of various antimicrobial agents for plant protection from various diseases and directly reach the agro-ecosystems. The most commonly used disinfecting agent in water treatment plants, viz. chlorine confer resistance to tetracycline, nalidixic acid and chloramphenicol¹⁰. The digested effluents (digestate) from biogas

reactors are used as fertilizers or soil conditioners. These digestives contain more AMR genes than the input resources. Horizontal transfer of resistance genes occurs from the plant materials to soil microbes¹¹. Another source for the occurrence and spread of AMRs is the waste water treatment plants (WWTPs). Water after treatment from WWTPs is applied to agricultural fields for irrigation, thus helping spread of AMR genes. Several ARGs harbouring microbial communities have been characterized in hospital wastewater due to the wide utilization of antibiotics in hospitals. Some of them are tet(M), tet(S), tet(Q), tet(O), tet(W) and mec(A). The wastewater from hospitals released in agricultural fields without proper treatment adds further to the occurrence and spread of AMR compounds in agro-ecosystems¹².

Agro-ecosystems thus act as a vehicle of AMR transmission contaminating the soil and water. They are also the end-points in the dissemination, selection, spread and emergence of AMR in the environment. There is a reduction in the effectiveness of medicines due to AMR, making diseases and infections difficult or impossible to treat, leading to increased mortality and prolonged illness in people and animals. It is also associated with production loss in agriculture. Antibiotics in the soil can alter the microbial community structure and functions, ammonification, N-mineralization, nitrification, decomposition processes, etc. They could therefore affect access of crop plants to nutrients for efficient plant growth¹³. These pollutants from agro-ecosystems can be transferred to the food chain, increasing the risk of human exposure and leading to clinical problems of resistant disease in human medicine, thereby leading universal health crisis. It is, therefore, imperative to have a full understanding of the persistence and prevalence of antibiotic resistance within the agricultural ecosystem and its dissemination in the environment. AMR can be transmitted to humans by a resistant pathogen of agricultural origin, by consuming pathogen-contaminated food or water, or by direct contact with livestock. Human-to-human transmission may also occur¹⁴.

The occurrence of AMRs is already well defined from the clinical perspectives but

there are lack of current knowledge to define the types and the levels of AMR occurring in the agro-ecosystems. Thus, emphasizing the current global scenario, it is imperative to utilize the available knowledge in relation to AMR and agro-ecosystems, and identify their role as source and sink of AMR. The sources mentioned here are major factors responsible for making agro-ecosystems a hotspot for AMR genes, and increasing the global challenge of AMR. Effective interventions to control AMR need to be addressed in an integrated manner, as they have inter-connections with human health. In the present scenario, there is limited knowledge about the dissemination of AMR within agro-ecosystems and to humans through the food web and the risks posed by agricultural release of antimicrobial agents. Future research must focus on developing novel strategies to better understand the diversity of AMR and its varying mechanisms of persistence and dissemination, its long-term effects on the agro-ecosystems and the development of novel strategies to combat the problem of AMR transmission in agro-ecosystems. To reduce the use of antimicrobials in food and agriculture, awareness and access to resources are necessary. For example, an alternative way could be developed to combat coccidiosis instead of traditional antibiotics, such as vaccination consisting of attenuated *Eimeria* species, or using natural products like phytogenic feed additives, probiotics and synbiotics. Scientifically tested plants with anticoccidial properties and their phytochemicals must be considered to suppress of *Eimeria* sp.¹⁵. More effective strategies need to be developed for the quantification of antibiotics discharged into the agro-ecosystems and the estimation of human exposure to antibiotics, ARBs and ARGs quantitatively. Government regulations should be implemented for the prudent use of antibiotics and better waste management. There is a need for greater investment focusing on global AMR surveillance and research programmes to develop effective control strategies for AMR mitigation. AMR-harboring bacteria enter into various crop plants as endophytes and are transmitted through the food chain to other organisms, including humans and animals¹⁶. Soil enriched with AMR-harboring bacteria through run-off reaches various

aquatic environments, thus contaminating and posing threat to aquatic life^{17,18}. There is still a question regarding how the addition of these AMR genes to the soil affects soil fertility and native microbiota.

Some suggestive measures to reduce AMR contamination in the environment are as follows: (i) Source-wise assessment for the prevalence of AMR genes harbouring bacteria in response to agricultural practices and crop management will be useful in knowing the effective route of AMR transmission and AMR harbouring microbe enrichment to the environment. (ii) Proper surveillance at every level is of utmost importance. (iii) There is a need to include additional steps in sewage treatment in STPs to control the AMR bacterial population in sludge and treated wastewater. (iv) The use of chemical fertilizers and pesticides should be minimized by employing nano-fertilizers. (v) Areas dependent on STP-treated water for irrigation purposes must be marked and monitored continuously to ensure that the water does not contaminate other sources through surface run-off. (vi) To understand the potential spread of AMR through agro-ecosystems, proper monitoring of AMR levels in manure is recommended.

In conclusion, due to advancements in instrumentation, and the application of techniques like HPLC, LC-MS, HR-MS and ICP-MS, it is possible to determine the quality and quantity of parent antimicrobial agents and their residues/metabolites in the environment. This will help determine the extent of contamination of AMR-enriching compounds in the respective environments. Due to advancements in molecular biology tools and techniques, knowledge about the structure and function of microbial communities (culture-dependent and independent) in the environment can be gained. Therefore, the appropriate application of genomics, proteomics and metabolomics-based approaches will lead to the development of a microbiome database that will contribute to the formulation of AMR mitigation strategies in a sustainable manner in order to maintain the agro-ecosystems and prevent the spreading of AMR in the environment.

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ACKNOWLEDGEMENTS. S.K.D. thanks the Ministry of Human Resource and Development, Government of India for financial assistance to work on agroecosystems under the Institution of Eminence scheme of Banaras Hindu University, Varanasi (R/Dev/D/IOE/Incentive/2022-23/47663; IOE scheme no. 6031).

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