



From environment to clinic: the role of pesticides in antimicrobial resistance

Jeadran N. Malagón-Rojas,¹ Eliana L. Parra Barrera,² and Luisa Lagos²

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ABSTRACT

Antimicrobial resistance (AMR) in pathogens has been associated mainly with excessive use of antibiotics. Most studies of resistance have focused on clinical pathogens; however, microorganisms are exposed to numerous anthropogenic substances. Few studies have sought to determine the effects of chemical substances on microorganisms. Exposure to these substances may contribute to increased rates of AMR. Understanding microorganism communities in natural environments and AMR mechanisms under the effects of anthropogenic substances, such as pesticides, is important to addressing the current crisis of antimicrobial resistance. This report draws attention to molecules, rather than antibiotics, that are commonly used in agrochemicals and may be involved in developing AMR in non-clinical environments, such as soil. This report examines pesticides as mediators for the appearance of AMR, and as a route for antibiotic resistance genes and antimicrobial resistant bacteria to the anthropic environment. Available evidence suggests that the natural environment may be a key dissemination route for antibiotic-resistant genes. Understanding the inter-relationship of soil, water, and pesticides is fundamental to raising awareness of the need for environmental monitoring programs and overcoming the current crisis of AMR.

Keywords

Drug resistance, microbial; pesticides.

New mechanisms of antimicrobial resistance (AMR) are emerging and spreading globally, threatening our ability to treat infectious diseases. In response, the World Health Organization WHO has developed the Global Action Plan on Antimicrobial Resistance as a global framework for national action to combat antimicrobial resistance (1).

Simultaneously, there has been worldwide overuse of pesticides (2): from 1990 – 2016, average pesticide used per area of cropland increased from 1.54 to 2.63 (kg/ha). Recent evidence shows that AMR can occur in natural environments—a result of multiple interactions between livestock and the soil and water polluted by chemicals commonly used for agriculture. The link between glyphosate and AMR as an emergent outcome has been reviewed (3). Resistant bacteria originating in an altered environment could become a major problem for public health (4). The role of pesticides and resistance in gram-negative bacteria has been shown (5, 6).

Considering the non-clinical aspects that spread AMR, there was a need for a comprehensive and interdisciplinary approach. Accordingly, the One Health strategy acknowledges that human health is interrelated with animals and the environment (7). One Health takes a collaborative, multi-sectoral, and transdisciplinary approach, working at the local, regional, national, and global levels (7). In spite of this holistic approach to raise awareness and more adequately address the problem of AMR, it is necessary to elucidate and investigate the links between the anthropogenic activities that use non-antibiotic chemicals and AMR mechanisms in the environment.

Current evidence on antimicrobial-resistant bacteria (ARB) is mainly focused on clinical antimicrobial use and the role of non-antibiotic molecules. More studies are needed to examine associations with environmental components. Understanding all the ways that resistance mechanisms operate is important. This report aimed to underscore the growing ARB problem as a

¹ Facultad de Medicina, Universidad El Bosque, Bogotá, Colombia.

² Grupo Salud Ambiental y Laboral, Instituto Nacional de Salud, Bogotá, Colombia. ✉ Luisa Lagos, llagos@ins.gov.co

consequence of anthropic activities that underlie and heighten this public health crisis.

Pesticides as triggers: Resistance developed beyond the clinical environment

Soil microbiota serves as an early contributor to AMR and a reservoir of genes for resistance to clinical pathogens. Metagenomics studies have identified an exchange of antibiotic resistance genes (ARG) between environmental bacteria and clinical pathogens (5). Multidrug-resistant (MDR) soil bacteria also contain genes for resistance to the main classes of antibiotics, such as aminoglycosides, amphenicols, β -lactams, sulfonamides, and tetracycline (5).

An association between pesticides and AMR has been suggested by some studies (6). Some strains of pesticide-degrading bacteria isolated from soils exposed to pesticides were resistant to five commonly-used antibiotics. It has been suggested that such resistance could be conferred by a plasmid that contributes to cross resistance via an unspecific organophosphorus hydrolase that also degrades antibiotic derivatives (6). This is a case of cross resistance, where the natural selection process could be involved in MDR derived from horizontal gene transfer (6).

Screening AMR profiles in soil microbiota from organic and non-organic farming showed genes for resistance to clinical antibiotics in both types of soils; the antimicrobial resistance of the cultivable isolates, including clinically relevant ones, was mediated by efflux pumps (8). Nevertheless, considering that sub-lethal chronic exposure of *E. coli* to glyphosate-based herbicides is not mutagenic (9), epigenetic effects of such pesticides are still likely.

A series of studies have evaluated changes in the Minimum Inhibitory Concentration after simultaneous exposure to herbicides and antibiotics (10). A sub-lethal effect of biocides is related with an adaptive multiple-antibiotic resistance phenotype, associated with an increase in the expression of efflux pumps. For *E. coli* and *Salmonella enterica* Serovar Typhimurium, it was observed that contact with three herbicides, the dicamba (Kamba500[®], Nufarm, Otahuhu, New Zealand), 2, 4-dichlorophenoxyacetic acid (2,4-D), and glyphosate (Roundup[®], Monsanto, Melbourne, Australia) induced different changes in the response to antibiotics: for *S. enterica* Serovar Typhimurium, exposure to Kamba[®] and 2, 4-D increased tolerance to ampicillin, chloramphenicol, ciprofloxacin, and tetracycline; for *E. coli*, a similar response was observed, except without an increased tolerance to ampicillin. In addition, for both species, exposure to Roundup[®] increased tolerance to ciprofloxacin and kanamycin. Since an efflux pump inhibitor restored *E. coli* susceptibility to antibiotics, a transport mechanism was suggested to be involved in the susceptibility change (10). To further explore this response, new studies were planned: short-term evolution experiments using either the natural variability within a monoculture of *E. coli* or *S. Typhimurium*, or isogenic strains only differing in their level of AMR. Resistance evolution was evaluated in short-term evolution assays with different combinations of antibiotics and commercial herbicide (11). The mutation rate for *E. coli* and *S. enterica* increased while continuously exposed to Roundup[®] and ciprofloxacin, resulting in a higher number of resistant variants, accelerating resistance evolution under these conditions (11). The effect of other common biocide formulations based on either atrazine, copper, or

pyrethrins were tested on *E. coli*. The efflux pump AcrAB-TolC showed a relationship between copper in combination with the antibiotic tetracycline. This was caused by both the induction of the adaptive efflux system and by chelation of the antibiotic by copper. An adaptive response was observed, demonstrating that the experimental environment can accelerate the evolution and the resistance response (12).

Studies with *P. aeruginosa* isolated from agricultural soils have shown that exposure to herbicides can induce a decrease in the susceptibility to aztreonam, an antimicrobial commonly used to treat these infections. Nevertheless, the resistance mechanism could not be determined because resistant strains did not have either β -lactamase encoding genes nor increased expression of MexAB-OprM, an efflux pump previously involved in *P. aeruginosa* resistance (13). Therefore, it follows that pesticides used in agricultural soils are strong selective pressures for resistant strains in natural and agricultural environments.

From environment to clinic: a pathway for resistant bacteria

AMR has been stated to be a critical functional trait of pesticide-degrading microorganisms. Microbial degradation of pesticides may constitute “hot spots” for pesticide-degraders, promoting AMR by different molecular mechanisms, including genetic changes (4). In this vein, elucidation of the origin of AMR has driven a focus on the pre-existence of an environmental resistome, with the soil as an important reservoir for the evolution and spread of diverse resistance mechanisms where horizontal gene transfer could be playing a major role (14).

Although the bacterial composition of soils from conventional and organic farming are similar (8), the community dynamics of such complex environments, interacting with other phyla and chemical mixes from anthropogenic activities, can lead to resistance. This could be a result of not only agrichemicals use, but also of the complex interactions of a highly diverse antibiotic-producing microbiota. The β -lactamases enzymes that hydrolyze the β -lactam class of antibiotics have been found in soils with and without anthropogenic activity. ARG levels in agricultural soils increased from 1940 – 2010, such that the presence of broad-spectrum β -lactam ARG genes (bla_{TEM} and bla_{SHV}) was 15 times higher. Additionally, there is evidence of the exchange of ARG between environmental bacteria and clinical pathogens. That is, MDR soil bacteria have genes similar to human pathogens, such as *A. baumannii*, *C. diphtheriae*, *E. coli*, *E. cloacae*, *K. pneumoniae*, *P. aeruginosa*, *S. typhimurium*, and *S. typhi* (5).

ARB in soils can be dispersed by run-off to other environments, including to potable water sources (15). Run-off is associated with the proliferation, prevalence, and persistence of ARB and ARG in aquatic environments (15). Additionally, it has been observed that physicochemical water parameters are able to modify bacterial community structure, as well as ARG profiles (16). Surface water is another media harboring and propagating ARB, originating from several mechanisms in different areas influenced by anthropogenic activities.

Aerosols are another important source of contamination, especially in polluted urban areas. Microbial aerosols in the air are more diverse than water surface communities, and allow bacterial transport from polluted aquatic environments to

nearby areas (17). ARG within the soil-plant system suggests a potential route of ARG transfer to the human microbiome via the food chain (18).

Prospects for future studies and action policies

AMR affects human, animal, plant, and environmental health. In order to improve public health outcomes worldwide, One Health has been established as the framework for developing strategies, programs, and policies (7). Although AMR has been frequently associated with medical management, pesticides should be a high priority in the One Health framework. Most research has not focused on the diverse range of interactions between natural bacteria populations and agrochemical use in the environment. Broad identification of ARG is done almost entirely in pathogenic bacteria, but research should be focused on aquatic and soil microorganisms as well. Because measures against AMR are primarily undertaken in the human health sector, the environment is unrepresented on national, multi-sectoral AMR committees, allowing significant gaps in data, studies, and control strategies to persist (19). The link between chemical use in agriculture and ARB in humans must be prioritized for combating the antibiotic-resistance epidemic (20).

AMR burden varies by geographical location, and while it is important to determine epidemiology on the local and institutional level, it may be imperative to also identify the substances in the environment that increase resistance. Studies of such bacteria communities in polluted soils, especially pesticide use in areas with poor sanitary conditions, should be of special interest. Studies of farming systems are essential to determining optimal use and regulation of antimicrobials. Metagenomics now allow the study of soil and water bacteria populations in their natural habitat; more such studies will generate new insights into the adaptive bacterial evolution, mechanisms of resistance, and gene transfer. This new methodology may elucidate the combined effects caused by mixtures of emergent contaminants being used, released, and transported to natural environments.

The actions recommended here are in agreement with activities stated in the WHO Global Action Plan on Antimicrobial Resistance (1). The consequences and effects of pesticides on microorganisms remain unknown, but overuse and AMR have increased in the last decades. Therefore, the policies in clinical

and agricultural settings should consider this emergent problem, especially in countries that do not regulate chemical use. In short, public health policies must be integrated with environmental and farming policies.

Conclusions and recommendations

AMR can be acquired in the natural environment when antibiotics and agrochemicals used by both clinical and agricultural activities contaminate ecosystems, changing the bacterial community structure and environmental resistomes. The mixed effects of pesticide use, as well as the effects of pesticides combined with other emerging contaminants, can induce cross-resistance and must be considered.

AMR can occur with or without antibiotic molecules and can originate in remote places, far from clinic locations. ARG transfer from environmental bacteria to pathogenic bacteria is a key determinant for antibiotic resistance. Specifically, evaluating soils in the flood plains of contaminated rivers is essential to determining where AMR is originating and how pesticides impact the exchange of resistant genes. Many molecules used by the farming industry, are not tested for sublethal effects.

The relevance of pesticides to AMR development is alarming. More evidence of pesticides as agents disturbing bacterial antibiotic susceptibility and generating transient adaptive responses is imperative. The role of pesticides, not only as toxins, but as pathways to AMR must be further evaluated to address the current crisis of antibiotic resistance and to raise awareness of the need for environmental monitoring and regulation.

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Del medioambiente al consultorio: la función de los plaguicidas en la resistencia a los antimicrobianos

RESUMEN

La resistencia a los antimicrobianos (RAM) en los agentes patógenos ha sido relacionada principalmente con el uso excesivo de los antibióticos. La mayoría de los estudios sobre la resistencia se han centrado en los agentes patógenos de importancia clínica; sin embargo, los microorganismos están expuestos a numerosas sustancias antropogénicas. Son muy pocos los estudios dedicados a determinar los efectos que producen las sustancias químicas en los microorganismos. La exposición a estas sustancias puede contribuir a los niveles mayores de la RAM. Comprender las comunidades de microorganismos en el medioambiente natural y los mecanismos de la RAM por los efectos de las sustancias antropogénicas, como los plaguicidas, resulta importante para abordar la actual crisis de la resistencia a los antimicrobianos. En este informe se hace énfasis en las moléculas, en lugar de los antibióticos, que se usan generalmente en los productos agroquímicos y que podrían contribuir a que se desarrolle la RAM en entornos no clínicos, como el suelo. Asimismo, se analiza el papel de los plaguicidas como mediadores en la aparición de la RAM, así como la manera en la que los genes y los microorganismos resistentes a los antibióticos se trasladan hacia el entorno antrópico. La evidencia indica que el entorno natural podría ser una vía clave de propagación para los genes resistentes a los antibióticos. Comprender la interrelación entre el suelo, el agua y los plaguicidas es fundamental para crear conciencia en cuanto a la necesidad de establecer programas de seguimiento ambiental y así poder superar la crisis actual de la RAM.

Palabras clave

Farmacoresistencia microbiana; plaguicidas.

Do meio ambiente à clínica: o papel dos pesticidas na resistência antimicrobiana

RESUMO

A resistência antimicrobiana dos patógenos está principalmente associada ao uso excessivo de antibióticos. Os estudos sobre resistência se concentram, na sua maioria, nos patógenos de importância clínica. Porém, os microrganismos são expostos a inúmeras substâncias antropogênicas e existem poucos estudos sobre os efeitos que os compostos químicos causam nos microrganismos. A exposição a estes compostos pode contribuir para o aumento da resistência antimicrobiana. Entender as comunidades de microrganismos em ambiente natural e os mecanismos de resistência antimicrobiana por ação das substâncias antropogênicas, como os pesticidas, é essencial no combate à atual crise de resistência antimicrobiana. Este informe destaca os compostos ativos (exceto antibióticos) normalmente usados nos produtos agroquímicos que podem contribuir para o surgimento de resistência antimicrobiana em ambientes não clínicos, como o solo. Examina-se o papel dos pesticidas como mediadores da resistência antimicrobiana e as vias pelas quais os genes que conferem resistência e as bactérias resistentes aos antimicrobianos alcançam o ambiente antrópico. As evidências indicam que o ambiente natural pode ser uma das principais vias de disseminação dos genes que conferem resistência aos antibióticos. Entender a inter-relação entre solo, água e pesticidas é fundamental para conscientizar sobre a necessidade de programas de monitoramento ambiental e combater a atual crise de resistência antimicrobiana.

Palavras-chave Resistência microbiana a medicamentos; praguicidas.
