The complex relationship between cyanobacteria and antibiotics/antimicrobial resistance in the environment: an emerging factor in the One Health vision on antimicrobial resistance

Maura Manganelli,[1] Emanuela Testai,[1] Geoffrey A. Codd[2,3]

[1]Environment and Health Department, Istituto Superiore di Sanità (ISS), Rome, Italy; [2]University of Stirling, School of Natural Sciences, Stirling, UK; [3]University of Dundee, School of Life Sciences, Dundee, UK

ABSTRACT

In 2015, the World Health Organization (WHO) declared Antimicrobial Resistance (AMR) as one of the most critical health issues. It proposed, with the Food and Agriculture Organization (FAO) and OIE (World Organization for Animal Health), to address this by a One Health approach, recognizing the connection between humans, animals, and environmental health. Currently, a hypothesis is developing that cyanobacteria and cyanotoxins may contribute to AMR in water. Recent research appears to suggest: i) an impact of cyanotoxins on antibiotic-resistance gene transfer between bacteria; ii) a role of cyanobacteria as a reservoir of AMR. Finally, cyanotoxin production appears to be stimulated by cyanobacterial exposure to antibiotics. These findings strengthen the importance of considering the environment in its complexity.

Corresponding author: Maura Manganelli, Environment and Health Department, Istituto Superiore di Sanità (ISS), viale Regina Elena 299, 00161 Rome, Italy.
E-mail: maura.manganelli@iss.it

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Antimicrobial resistance in the environment

In 2015, the World Health Organization (WHO) declared Antimicrobial Resistance (AMR) to be one of the greatest threats to public health and promoted a Global Action Plan to combat AMR with a One Health approach (WHO, 2015). The OIE (World Organization for Animal Health) and the FAO (Food and Agriculture Organization) subsequently joined the WHO in providing practical and legal tools to set up National Action Plans to reduce the use of antibiotics, promote their surveillance, and carry out further research (e.g., WHO, FAO, OIE, 2016). In 2023, the European Council published a recommendation to member states in an effort to harmonize AMR monitoring in all countries. In order to combat antimicrobial resistance from a One Health perspective, considering the environment as part of the problem, member states have to carry out several actions, including the implementation of i) measures to prevent, monitor, and reduce the spread of AMR in the environment and ii) a surveillance system of AMR also in food, in wastewater, and in the environment (EU, 2023). Based on previously existing programs, a wide variation in the occurrence of AMR in the different European countries emerged, based on bacterial species, antimicrobial groups, and geographical location (European Center for Disease Prevention and Control, ECDC, 2022). The highest percentage of resistance to the main classes of antibiotics used in hospitals has been reported in the southern countries. In particular, carbapenem-resistant Gram-negative pathogens represent a serious problem as they cause healthcare-associated infections, which are a significant threat to public health (ECDC 2022).

The One Health concept recognizes a tight connection between humans, animals and environmental health: diseases are transmitted from animals to humans and vice versa also via the environment. In the last decade, the environmental setting has been acknowledged to influence the diffusion and transmission of Antibiotic-Resistant Bacteria (ARB) and Antibiotic-Resistance Genes (ARGs) through various mechanisms (for a recent review, see Rzymski *et al.*, 2024). From the environment, both ARB and ARGs can return to humans mainly through exposure to water and food, but studies to exclude transmission via the air and other routes of exposure are still too limited (Stanton *et al.*, 2022). The accumulation of antibiotics and other contaminants, ARGs, and ARB, in aquatic environments released from domestic, municipal (including hospital), and industrial wastewater, as well as from animal farming and aquaculture plants, can adversely affect environmental health, altering ecosystems. Antibiotics can change not only the composition and size of the recipient bacterial community but can also stimulate the proliferation of new ARB through selective pressures (Baquero *et al.*, 2008). This can also happen at concentrations >100-fold lower than the minimal inhibitory concentrations of susceptible species (European Food Safety Authority, EFSA, 2021), that are similar to the low (ng/L) environmental concentrations. The few data available from the field indicate widespread dissemination of certain antibiotics at a global level; for example, surveys carried out in Northern Italy revealed substantial antibiotic concentrations downstream of Wastewater Treatment Plant (WWTP) effluents (Castiglioni *et al.*, 2018).

Selection in the aquatic environment can, therefore, be a likely event, particularly in hotspots such as wastewater, where multiple selectors are present at the same time at significant concentrations (Karkman *et al.*, 2018). Research is needed to standardize AB resistance testing for environmental bacteria, harmonize data for the development of useful databases, and develop AMR risk assessment. This should consider the risk of AMR emergence in the environment and should quantify the sub-inhibitory antibiotic concentrations that promote it in different scenarios. It should also evaluate the probability of the spread and transmission of antibiotic resistance from diffuse sources (Di Cesare *et al.*, 2024) and hotspots, including WWTPs (Hanna *et al.*, 2023), to downstream environments and finally to pathogenic bacteria, in addition to considering traditional eco-toxicological endpoints (Berendonk *et al.*, 2015). Some progress has been made, with respect to minimal selective antibiotic concentration and the risk of AMR emergence in the environment (Murray *et al.*, 2021) and in humans (EFSA, 2021). However, no other potential reservoirs other than bacteria in the environment have been considered until recently.

The role of cyanobacteria in antimicrobial resistance

Cyanobacteria are photosynthetic prokaryotes, many of which produce potent cyanotoxins, naturally occurring in almost every terrestrial and aquatic habitat (Svircev *et al.*, 2019; Chorus and Welker, 2021), including WWTPs (Romanis *et al.*, 2021). Their presence in water bodies is dramatically increasing due to human demands on water resources, eutrophication, and climate change (Huisman *et al.*, 2018). Risks to human health due to cyanotoxin exposure via drinking water, contaminated food, and recreational activities have been extensively reviewed (Buratti *et al.*, 2017; Chorus and Welker, 2021). Many acute poisonings of wild animals and livestock, after drinking from environmental water sources have been recorded (Svircev *et al.*, 2019). Animals appear to be attracted by cyanobacteria in water even when clean water is accessible and can readily drink sufficient volumes of water containing cyanobacteria blooms, or ingest shoreline scums and mats containing cyanobacteria, which can present a lethal oral dose of cyanotoxins (Wood *et al.*, 2016). Furthermore, some cyanobacteria are used as human and animal food supplements for their high content of vitamins and antioxidant compounds (Chorus and Welker 2021).

The complex and diverse relationships between cyanobacteria and antibiotics, ARB and ARG began to be considered only recently in a small number of papers (Figure 1). Cyanobacterial populations are closely associated with a wide range of species-specific bacterial communities, for which they can provide photosynthetically-produced organic matter and oxygen. Indirectly, they can thus increase ARB populations present in the environment during the development of cyanobacterial blooms (Zhang *et al.*, 2020). A more direct role of cyanobacteria as an ARG natural reservoir and source has been demonstrated in Lake Taihu, China. Wang *et al.* (2020) found ARGs in 8 cyanobacterial strains isolated from the lake and then, by laboratory experiment, determined species-specific transfer potentials of mobile genetic elements to 5 cyanobacteria from bacterial donors, as a function of temperature and population density. A survey of over 800 cyanobacterial isolates from habitats, including free-living strains from fresh- and marine waters, terrestrial sources, and symbioses, has found AMR genes to be widely encoded, including in members of major cyanobacterial orders (Nostocales and Oscillatoriales), which occur in cyanobacterial blooms and mats (Timms *et al.*, 2023).

Cyanobacteria have different inter-specific sensitivities to various classes of antibiotics, up to 5 orders of magnitude (Le Page *et al.*, 2017). The mode of action of antibiotics is assumed to be the same in bacteria and cyanobacteria, and the different susceptibility seems to be related, as in bacteria, to diversity in mechanisms of uptake (e.g., variability in porin channels for the passage of small molecules, e.g., beta lactams), or in efflux pumps systems to eject AB outside the cell or in the presence of degradative enzymes (Le Page *et al.*, 2019). Dias *et al.* (2019) found lower susceptibility in strains isolated from WWTPs than in those from natural aquatic environments. In laboratory experiments, we have also observed intra-specific different susceptibility between *Planktothrix* strains and between *Microcystis aeruginosa* strains (manuscript in preparation) within the framework of a small pilot project funded by the Istituto Superiore di Sanità (ISS) in Italy with the aim of investigating aspects of the relationship(s) between cyanobacteria and AMR. Our results indicate that antibiotics can influence the cyanobacterial community, selecting more resistant strains, whatever the mechanism they use.

The development of biofilms in drinking water treatment plants is a further important aspect that has been studied by Xu *et al.* (2020). They looked for cyanotoxins and ARGs in the complex cyanobacteria-containing biofilms along different steps of a treatment system in a Drinking Water Treatment Plant (DWTP). They found both ARGs and cyanotoxins, particularly after water treatment with activated carbon, with a weak correlation between the presence of ARGs and the concentration of Microcystins (MCs). Subsequently, they demonstrated in lab experiments that cyanotoxins enhance the rate of horizontal ARG transfer between bacteria and speculated that the interactions between ARG and MCs can be increased in DWTP biofilms, favoring the emergence of new antimicrobial-resistant cells (Xu *et al.* 2020). These authors found an up-regulation of genes involved in conjugative transfer and in genes related to anti-oxidant response and suggested that oxidative stress could increase cell membrane permeability, thus

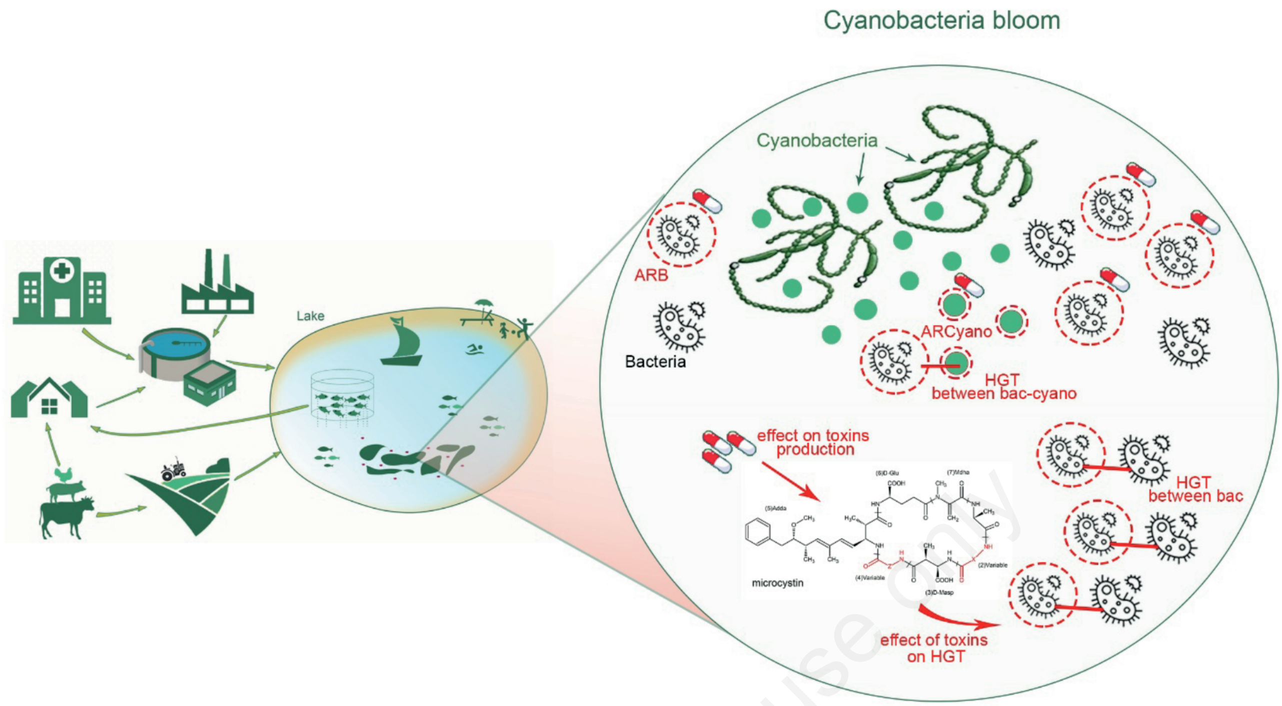

Figure 1. Diagram illustrating the relationships between cyanobacteria, antibiotics, and Antimicrobial Resistance (AMR) from a One Health perspective. Antibiotic-resistant bacteria and antibiotics flow into surface waters (like a lake in the example) from various terrestrial and aquatic sources. In the water, cyanobacteria, stimulated by increasing temperature and nutrient runoff, can interact with antibiotics and antibiotic-resistant bacteria and contribute to the spread of antibiotic resistance by: a) developing resistance or being selected for resistant strains; b) stimulating the passage of antibiotic-resistant genes to other bacteria through the release of cyanotoxins and; c) supporting the growth of antibiotic-resistant bacteria through the release of freshly produced organic matter during their blooms. Finally, cyanotoxin production seems to be stimulated by exposure to antibiotics. Humans can be exposed to AMR through drinking water and recreational activities.
ARB, Antibiotic-Resistant Bacteria; HGT, Horizontal Gene Transfer; ARCyano, Antibiotic-Resistant Cyanobacteria.

facilitating the passage of the resistant plasmid. To our knowledge, this is the only study on cyanobacteria, cyanotoxins, and ARGs in biofilms, and on the possibility that microcystins enhance the transfer rate between donor and recipient bacteria in horizontal gene transfer. Further studies are needed to substantiate these results, but if they are confirmed, we hypothesize that toxic benthic cyanobacterial mats, especially in proximity to outflows from WWTPs, could be another important hotspot for the transfer of mobile genetic elements, including ARGs, and recommend that these should be a focus of future studies.

A further but not less relevant aspect of the relationships between cyanobacteria and antibiotics, is the production of cyanotoxins, which can be stimulated after repeated exposures to sub-lethal concentrations of antibiotics (Wu *et al*. 2020). In our pilot study, we have also observed an increase in cell quota of cyanotoxins during a 14-day exposure to amoxicillin of a monoclonal strain of *Microcystis aeruginosa* CCAP 1450/6 in a closed system (Manganelli *et al*., 2023).

Concluding remarks

These findings indicate that cyanobacteria have an intricate role in influencing the evolution and environmental dissemination of AMR, potentially acting as conduits between environmental AMR and humans and animals via environmental aquatic exposure, water consumption, and food chains, given their extensive diversity. Additionally, our findings indicate that it is crucial to investigate whether cyanotoxins, a prominent environmental health concern in drinking water (Buratti *et al*., 2017; Chorus and Welker, 2021), may exacerbate horizontal gene transfer, or be stimulated by environmental antibiotics or acquired AMR, thereby heightening the risk of cyanotoxin exposure via waterborne routes. This need is intensified by the increasing evidence that the incidence of infectious microbial disease, and ARB, ARG, and cyanobacterial mass populations are all favored by climate change (Huisman *et al*., 2018; Rzymski *et al*., 2024).

In conclusion, the One Health approach to AMR should consider the environment in its complexity and should include cyanobacteria among the environmental drivers of AMR, in an effort to better understand the weight of the various drivers. Meanwhile, mitigation measures to reduce toxic cyanobacterial blooms should be implemented with the double aim of reducing both the risk of exposure to cyanotoxins and their impact on the spread of antibiotic resistance.

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