



Occurrence of Antibiotic Resistance in Lotic Ecosystems

Sohini Bhattacharyya*, Ajay Kumar Harit, Manoj Singh, Urvashi Sharma and
Chaitramayee Pradhan

*Department of Zoology, Kalinga University, Naya Raipur, Chhattisgarh- 492101, India
Corresponding author: sohi.bhatta@gmail.com, sohini.bhattacharyya@kalingauniversity.ac.in*

ABSTRACT:

Antibiotic resistance is a matter of global public health concern. Antibiotic resistance is disseminated by antibiotic resistance genes (ARGs) which are carried by antibiotic resistance bacteria (ARBs). Lotic ecosystems like rivers which has flowing water can spread ARGs from one location to another. Such dispersion of ARGs can also move through food chains and food webs making the spread of antibiotic resistance more complex and widespread. Detection of these ARGs is important for understanding the origin and pathways of antibiotic resistance in our waterways. Both culturing and non-culturing methods can be used for detection of these ARGs. Overall, understanding the sources, assessing the presence, and determining the dissemination of antibiotic resistance is important for us to understand the level of antibiotic pollution in our waterbodies.

Key words: Antibiotic resistance, Antibiotic resistance genes (ARGs), Antibiotic resistance bacteria (ARBs), Lotic ecosystems.

LOTIC ECOSYSTEMS AND ITS IMPORTANCE:

Lotic ecosystems comprise of aquatic ecosystems with flowing water like streams and rivers (Ward 1989; Leff et al. 1992). Such lotic ecosystems are usually freshwater in nature and oftentimes vary structurally; but all of them are hotspots of various ecosystem processes (Leff et al. 1992; Rubin and Leff 2007; Mulholland et al. 2000). These aquatic ecosystems can help immensely in human survival. For example, freshwater streams can provide potable water, help in agriculture and cater for recreational purposes. Flowing waterways are also responsible for primary and secondary production, decomposition, and nutrient (nitrogen, phosphorus, carbon, etc.) cycling (Findlay 2010; Wymore et al. 2016; Baxter et al. 2013). Streams are also habitats for various aquatic plants and animals and provide refuge to small aquatic invertebrates which all are significant parts of the food chains and food webs of the respective environment. However, despite such importance of streams, anthropogenic activities

are consistently polluting these ecologically crucial ecosystems (Mulholland et al. 2000). Pollutants can be of different forms like pharmaceutical and personal care products, microplastics, nutrients, etc. Overuse and misuse of antibiotics over a long period of time has resulted in widespread antibiotic resistance which is a major concern of public health worldwide (Prestinaci et al. 2015).

ANTIBIOTIC RESISTANCE IN LOTIC ECOSYSTEMS:

Although streams can vary structurally and functionally, they all harbor different bacterial taxa which can perform vital ecosystem functions (Baxter et al. 2013, Roberto et al. 2019, Leff et al. 1992). Streams mostly follow the river continuum concept (Vannote et al. 1980) but the role of the microorganisms, including bacteria as per the continuum concept has been hardly elaborated in literature. Bacteria are capable of possessing genes that can facilitate element (nitrogen, phosphorus, etc.) cycling processes, developing metal and antibiotic resistance (Baxter et al. 2012; Roberto et al. 2019). Anthropogenic activities have enhanced global

pollution rates which have adversely impacted terrestrial and aquatic ecosystems alike. It is known that antibiotic resistance can occur naturally (McArthur and Tuckfield 2000); however, continuous, and constant uncontrolled use of antibiotics have resulted in increased antibiotic resistance in our waterways (Zhang et al. 2009; Pruden et al. 2013; Kumar et al. 2020). Antibiotic resistance is spread by antibiotic resistance genes (ARGs) which are carried by antibiotic resistance bacteria (ARBs). Increased antibiotic concentrations in the freshwater lotic ecosystems trigger prevalence of ARGs (Roberto et al. 2019). These ARBs along with their ARGs can be present in biofilms formed on benthic substrates, sediment, water column, and aquatic macroinvertebrates (Roberto et al. 2019; Bhattacharyya 2022; Winkworth 2013). Since lotic ecosystems are flowing waterbodies moving from upstream to downstream locations, chances of dissemination of ARGs from one spot to another is rapid and high. Additionally, smaller aquatic invertebrates are a part of the bigger food chain which means that their consumption might help in passing along of ARGs via the food chain and food web (Kumar et al. 2020). Consumption of aquatic organisms like fish that are resident of such streams and drinking water from such water sources can facilitate the flow of ARGs to humans too. This can be a matter of environmental and health concern.

SOURCES OF ARBs AND ARGs AND FACTORS AFFECTING ANTIBIOTIC RESISTANCE:

Human activities are major contributors of ARBs and ARGs in aquatic ecosystems including streams (Pruden et al. 2013). Major sources of antibiotic pollution are effluents from wastewater treatment plants, run-offs from agricultural fields and animal farms, aquaculture industries, and hospital wastes (Roberto et al. 2018; Winkworth 2013; Pruden et al. 2013). Samples of water taken from locations situated downstream or in close proximity to these sources have been shown to have significantly elevated abundance and diversity of ARGs in comparison to sites located farther from these origins of antibiotic pollution (Roberto et al. 2019; Manaia et al. 2018). The process of wastewater treatment does not fully remove the ARBs and ARGs which results in further downstream pollution of nearby streams. It is very common practice to treat animals in animal farms with excess antibiotics to prevent diseases outbreaks. Such animal husbandry industries severely pollute nearby streams with antibiotics and ARGs and the antibiotics are not completely broken in the guts of the animals and move out into the environment during defecation. Similarly, run-offs from agricultural land elevate the prevalence of ARGs in streams.

Various factors can control the abundance, dissemination, and diversity of ARBs and ARGs in the environment. One factor is close proximity to the sources of antibiotic pollution as mentioned above (Roberto et al. 2019). Secondly, elevated antibiotic concentrations in streams can trigger ARGs in the ecosystem. Thirdly, co-occurrence of additional factors like existence of metal resistant genes can positively affect occurrence of ARGs in the respective streams. Fourthly, it has been observed in some studies that the presence of certain bacterial taxa is correlated with the prevalence of specific ARGs (Roberto et al. 2019; Zhou et al. 2017). This is suggestive that some bacterial taxa can serve as hubs for certain ARGs. Therefore, the bacterial community composition of the respective ecosystem can influence the pattern of antibiotic pollution. Moreover, other factors like flow of water, discharge from nearby ARG sources (like wastewater treatment plants), substrate for biofilm formation, etc. can all be important predictors for antibiotic pollution in lotic ecosystems.

WAYS OF QUANTIFYING ANTIBIOTIC RESISTANCE IN STREAMS:

Over time multiple researchers have attempted to assess the level of antibiotic resistance in aquatic ecosystems. Detecting the abundance and diversity of ARBs and ARGs are the commonly used methods for this purpose. Both culture dependent and culture independent methods have been frequently used for detecting ARGs and ARGs in flowing waterbodies. In culturing methods bacterial colony counts in response to antibiotic concentrations used to understand the abundance of bacteria possessing the resistance genes for that respective antibiotic. Culture independent methods commonly use polymerase chain reaction (PCR) for detection of ARGs and quantitative polymerase chain reaction (qPCR) for detecting the abundance (using gene copy numbers) of ARGs (Leff et al. 1994; Roberto et al. 2019). However, it must be noted that these techniques have their own limitations. For example, colony counting methods do not capture the range of bacteria that are actually present in the ecosystem as specific media only support the growth of limited number of bacterial taxa (Hugenholtz 2002). For PCR and qPCR designing appropriate primers, assessing gene copies below the detection limits of the standards, precision of the reaction may be challenges capable of limiting the results. Hence, using bacterial community composition data generated from sequencing and metagenomics might be better options for detecting ARGs in aquatic ecosystems as they can overcome most of the limitations mentioned above (Schmieder and Edwards 2012). However, these advanced techniques are expensive and running

maximum replicates may not be feasible at certain times. Additionally, these parameters must be correlated with physicochemical attributes and antibiotic concentrations of the ecosystem studied to obtain more confirmative results. Moreover, multiple sites and sufficient replicates should be considered before drawing generalizations regarding the prevalence and dissemination of ARGs in lotic ecosystems.

CONCLUSION:

Overall, antibiotic resistance is an emerging threat to mankind which needs immediate attention. Lotic aquatic ecosystems may serve as hotspots for ARBs and ARGs as they provide habitat to an array of bacteria. Anthropogenic activities have increased aquatic pollution from several pollutants including antibiotics, however, the prevalence and dissemination of ARGs in streams and rivers is still not well understood. Several culture dependent and independent methods are used to study antibiotic resistance, but they all come with their own limitations. Therefore, future projects must work on understanding the pathways of spreading antibiotic resistance in our aquatic ecosystems.

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