Environment as Sink for Antimicrobial Resistance and Genes

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Abstract

Environment plays a crucial role in the antimicrobial resistance (AMR). Antimicrobials used across the sectors are directly or indirectly released into the environment. However, minimal focus was given on the "One Health" basis. The assessment of the influence of the environment on advancement and transmission of insusceptibility to drugs is of paramount importance. The status of AMR across the segments of ecosystems, viz., terrestrial and aquatic (rivers, lake), etc., is extremely important. In this chapter, consolidated evidence is provided to understand the AMR across the ecosystems. Studies that estimated the burden of AMR by metagenomics were also given a special criterion for understanding the impact on other sectors.

Keywords

Antimicrobial resistance · Lakes · Wastewaters · Metagenomics

1 Introduction

Antibiotics are low-molecular-weight secondary compounds produced from microorganisms (bacteria or fungi) that are involved either in the inhibition or the elimination of other microbes (Lancini et al., 1995; Stanton et al., 2020). In treating bacterial infections, antibiotics were used as the first resort and were once considered indispensable in modern medicine (Davies & Davies, 2010). Till 2005, several antibiotics were discovered or manufactured since the first antibiotic was discovered in 1928 (Zinner, 2007). Following the release of the antibiotics, they have been used in a variety of sectors (human health, veterinary, agriculture, and aquaculture). These drugs are mass-produced in millions of tons (Wang et al., 2010). Antibiotics that were overproduced or overused were then excreted or discharged into the environment as a result of which low concentration of sublethal antibiotics in the environment puts pressure on the bacterial population in the ecosystem. Because of this, antimicrobial resistance (AMR) has emerged in the bacterial population in the environment. More than ten million people are believed to be at risk as a result of the huge rise in drug-insensitive bacteria (O'Neill, 2016). Antibiotics enter the environment via three main routes: environmental release of antibiotics from production sites, antibiotics used in hospitals, veterinary, and aquaculture systems, and feed additives (Fig. 1). Antimicrobials are expected to be excreted in metabolized or active unmetabolized form from human and animal healthcare and are eventually released into urban wastewater, manure, or biosolids (10–90%). The percentage variance in excretion is caused by the organism's species, chemical structure, or dosage (Zhao et al., 2010). Unused and expired medications are frequently dumped into aquatic sources. Antibiotics used in irrigation and fertilization processes in the water and agroecosystems enter the environment via sludge, biosolids, and manure (Göbel et al., 2004; Yang et al., 2010). Antibiotic residue activity in the environment is impacted by an assortment of ecological dynamics, and water or soil quality

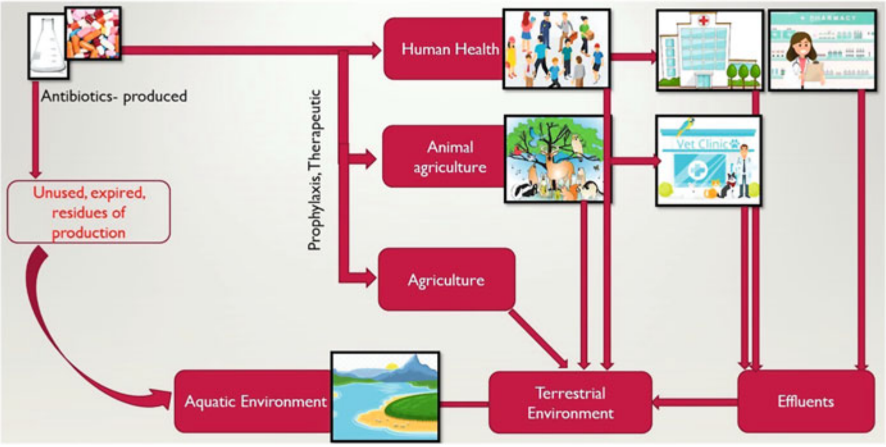


Fig. 1 Pathways of antibiotics used across the sectors entering the environment

parameters (pH, water content, organic carbon content, microbiota), including concentration, and its impact on terrestrial and aquatic ecosystems is not fully understood (Kümmerer, 2009). Similarly, antibiotic residue persistence is influenced by the types of biotic stress (microbes) present in the niche, as well as abiotic stress (pH, temperature, hydrolysis, oxidation, reduction, or photolysis) present in the environment.

The impact of antimicrobial resistance can be a major impediment to reaching most of the SDGs by 2030. They include SD1 and 2 – No poverty and Zero hunger; SDG 3 and 6 – Decent condition and well-being and Unpolluted water and hygiene; SDG 7 and 8 – Inexpensive and clean energy and Decent work and economic growth; SDG 11 and 12 – Viable metropolises and societies & Sustainable intake and making; SDG 14 and 15 – Submarine life and Life on land; and SDG 17: Partnerships for the goals.

2 Major Concerns of AMR in the Environment

The ecological conditions play a crucial role in the development of AMR. The resultant human activity, namely, antibiotics and antimicrobials released into the environments, is an important reason for drug insusceptibility in bacteria, and they, in turn, spread to the microbes in soil, rivers, and seawater. Human consumption of antibiotics increased by 36% in the 2000s. In 2000, the human consumption of antibiotics ranged from 9.2 to 10.5, with an average of 9.8 defined daily doses (DDD). By 2018, it has increased from 37.2 to 43.7, with an average of 40.2 billion DDD. The rate of intensification from 2000 to 2018 was 46%, with an annual mean consumption of 14.3% in 2018 varying from 13.2 to 15.6, and the study reported data at a 95% uncertainty level (Browne et al., 2021). When the antibiotics are taken, 20% are absorbed and the remaining 80% are expelled through exudates. Human consumption of drugs amounts to 30% of the antibiotics. Compost fertilizers are also a source of antibiotic pollution in surface runoff, groundwater, and drainage networks. It is important to observe that the plants and crops absorb the antibiotics. Of the total antibiotics produced, nearly 70% are used for animal purposes only. The intensification of farming and the pressure to enhance animal growth result in antibiotic use, particularly in developing countries. By 2030, there will be a significant increase in antimicrobial use in livestock to 67%. Major waste flows, including drainage, manures, and agricultural runoff, contain residues of antibiotics and AMR bacteria. It is known that the concentrations of antimicrobials in most sewages are too less to an extent of causing lethality to bacteria, but it is possible that the low concentrations are enough to develop AMR. The occurrence of ARB in fresh source water and water subjected to treatment cannot be ruled out. An enormous collection of pollutants in public and industrial wastewater puts a huge burden on microbes to develop resistance. The landfills and open dumps contain but more than 50% solid wastes from civic sources. Unused drugs and expired drugs are also a part of municipal waste. Reports indicate the occurrence of MDR bacteria in marine waters and sediments, which are in the vicinities of aquaculture, industrial, and civic discharges.

The contaminants of aquatic sources include wildlife: exudes from wild animals; landfills: discharges from landfills; dredging: dredging of the sediments; aquaculture: resultant aquaculture activities; human healthcare: originating from clinical sources in the form of surplus solids and fluid seepages; livestock and farms: application of droppings and stowage; releases: sewage and discharges from effluent treatment plants (ETP); runoffs from agriculture fields; and antibiotics: effluents from the manufacture of antibiotics are also considered the source for antibiotics in the environment.

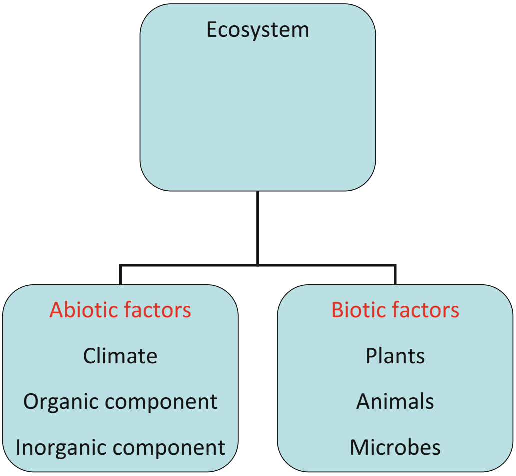
Similarly, air pollution occurs due to emissions from livestock, clinical environments, and human airways. Soil pollution occurs due to excretions from wildlife sources, wastewater treatment plants, sewage: sludge/discharges, livestock, and farms; manure application; and discharges.

Due to various other reasons, humans are impacted by potable and recreational water; domestic animals: direct contact with animals; wildlife: all types of wild animals; livestock: ingestion of livestock products; and polluted air: including fellow human sources. Wildlife is impacted in the same way by ingestion of contaminated foods; direct contact: anthropozoonosis; clinical sources in the form of surplus solids and fluid seepages; and livestock and farms: application of droppings and stowage, releases, and runoffs from agriculture fields.

The thermal stability of antibiotics is also important in their breakdown (Turiel et al., 2005; Dantas et al., 2008). The persistence of drugs in the environs induces an adaptive response to alleviate stress, resulting in the advent of a new-fangled phenotype obdurate to drugs either by mutation in the genome of bacteria or by obtaining genes of insusceptibility by means of horizontal or vertical gene transfer mechanisms (Tenover, 2006). These residues, when present in concentrations lower than the minimal inhibitory concentration, change the signaling cascade, affecting virulence, biofilm, and quorum-sensing mechanisms, as well as non-target bacteria in aquatic and terrestrial ecosystems (Chee-Sanford et al., 2009). This chapter offers researchers sufficient information about what an ecosystem is, how it is classified, and how AMR is reported in ecosystems.

3 Ecosystems and Their Classification

A place with a specific and recognizable landscape (forest, grassland, desert, wetland, or coastal area) and an abiotic component (sunlight, temperature, and rainfall) that supports the circumstances of the biotic components (living organisms) is referred to as an "ecosystem," according to Tansley's definition (Blew, 1996). Various biotic and abiotic factors influence the different ecosystems, according to Odum and Smalley (1959). They include abiotic factors differing substantially between habitats, such as terrestrial (temperature, sunlight, and water) and marine (temperature, sunlight, and water) (salinity and ocean currents). Odum and Smalley categorized these abiotic elements into three categories: climatic, inorganic, and organic components (1959). These biotic components include living species such as plants, animals, and microorganisms that can be divided into three categories: producers, consumers, and decomposers. The majority of bacteria and fungi are classified as decomposers or reducers. Natural ecosystems, which are built based on the interactions between biotic and abiotic components and occur naturally in a cyclic manner, and artificial ecosystems, which are sustained with human intervention, such as farms, are two types of ecosystems. Freshwater and marine environments make up aquatic ecosystems. Flowing water/lotic environment (streams, rivers, etc.) and standing waterbodies/lentic environment (ponds, lakes, swamps, etc.) are two types of freshwater ecosystems that are completely distinct from one another. Natural disasters such as floods and droughts alter the species that live in lotic environments.



4 AMR in Different Ecosystems

4.1 Aquatic System

The aquatic environment not only acts as a maintenance medium for the AMR but also acts as a mixing and mobilizing medium for intra- and inter-species levels because water in the biosphere has a vast cell biomass of $>10^{30}$, which contributes more toward the maintenance, mixing, and mobilization of ARGs. These bacterial or microbial cell biomass act as a pathogen in various living plants and animals, but are also involved in various biogeochemical cycles. There are extreme environments that also harbor extremophiles, and a lot can be understood from these extremophiles. Therefore, these antimicrobials at minimal residuals also derange the bacterial transcription mechanism. These result in co-selection of other population with developing resistance to antibiotics prevalent as residual concentrations. It also facilitates the horizontal or vertical gene transfer of resistance genes through various mechanisms. The microbes with ARGs can be transported via water as a medium to any place easily, and, therefore, it forms a niche for the spread of ARGs to the next population (Taylor et al., 2011).

4.2 River and Lake System

Rivers are any natural flow of water that flows within a definite bank and are well nourished by the precipitation of organic content in water as well as by the runoff from nearby terrestrial areas. Due to human activities, the runoff majorly contains heavy metals and other pollutants, which can be either organic or inorganic. Contamination of river ecosystem can occur mainly from the release of sewage, which contains organic pollutants and pharmaceutical waste, including fecal and pathogenic microorganisms that can coexist and cause transformation with autochthonous microorganism.

Liu and others (2018) studied the genes unresponsive to drugs "*ARGs Antibiotic Resistance Genes*" from all lacustrine sources across China in which the ARGs of surface waters exhibited substantial distance–decay association. The study revealed efflux pump as a vital tool of resistance with predominance of MDR genes. The ARGs' normalized richness was lower in the northern region and higher in the southern and central regions of China. The topographical dissemination of ARGs is impacted by various environmental mechanisms, namely, aligned and nonaligned processes.

Systems such as river–lake are securely linked to land-dwelling bionetworks and appear to be sinks of ARGs and distribution pathways for resistant bacteria for the reason of predisposal by anthropological events sans difficulty. There is currently a scarcity of methodical information on the incidence, transmission hazard, and cause of ARGs in the conformations of river–lakes. Chen et al.'s (2020) study centered on high-throughput summarizing and dynamics of source sink in unraveling drug-unresponsive genes in the deposits of interrelated Fuhe river–lake structure and the resultant unloading brook Baiyang located in the northern region of China. In the study, 40 outward deposits were acquired and subjected to metagenomic shotgun sequencing. The summary and synchronized incidence of genes unresponsive to drugs in dregs of the river–lake environs, along with the mobile genetic elements (MGEs) that harbor ARGs and their impending dispersal hazard of resistome, were categorized systematically. A novel bacteriophage, namely, CrAssphage, was used to trail the effects of human fluid contamination on ARGs. Fast Expectation-Maximization Microbial Source Tracking (FEAST), an innovative method, combined with Linear Discriminant Analysis Effect Size (LEfSe) scheme was used to assess the impact of river residues on the incidence of ARGs in the receiving lake (Chen et al., 2020). Furthermore, the discovery of nascent ARGs, namely, *mcr*-1, *tetX*, and carbapenemases in the deposits of the river–lake system, is one of the important findings of the study. The ARGs' incidence in the dregs of environs of river–lake is attributed to contamination from human exudes as evidenced by source tracking, and the distribution assessment showed that >80% of ARGs in Lake Baiyang originate from Fuhe River. Grid assessment proposed nonrandom coincidence forms of ARGs intra- and between categories. One more significant observation is that numerous MGE-carrying contigs were recognized with an equal holding of ARGs of one or more, resulting in possible advanced resistome hazard in Lake Baiyang. It is not the same case with most of the lacustrine sources of the world.

In Bangladesh, the research on various components of the environment (wastewater, rivers, ponds, and household waste) revealed the abundance of *Plasmid Mediated Quinolone Resistance* (PMQR) genes, mostly *qnrS* in *E. coli* isolates. This was followed by genes aac (6′)-lb-cr, *oqxAB*, *qnrB*, and *qepA,* which were detected in abundance. Penicillin, cephalosporins, fluoroquinolones, sulfonamides, aminoglycosides, and carbapenems were also detected (Amin et al., 2021). In the Republic of Czechoslovakia, when the Morava River waste and surface water was screened for the presence of 10 antibiotics at predicted no-effect environmental concentrations (PNECs), the wastewater samples exceeded the PNEC and vancomycin-resistant enterococci were identified in both water samples of polluted and surface (Hricová et al., 2021).

The copious and tenacious presence of microbes that are unresponsive to drugs in the environs poses a significant risk to the human well-being. Antimicrobial resistance (AMR)-associated mortalities occur more often than malignancies and traffic accidents combined per annum (O'Neill, 2016). Multiresistant bacteria could spread from humans to aquatic habitats via water purification plants that dump treated contaminated water into other aquatic sources after treatment. Antibiotic resistance genes (ARGs) discovered in gene-transfer units, as well as the growth of MDR, may have ramifications for the well-being of humans and the development in environs (Martinez, 2009). AMR can be caused by chromosomal DNA mutations or mobile element horizontal gene transfer. Integrons are a type of mobile element that are associated with the internment, mobilization, and transmission of genes that are unresponsive to drugs in Gram-negative microbes. Gene cassettes are genomic platforms that allow resistance determinants to be integrated and rearranged (Mazel, 2006).

While the emergence of novel microbes that are insusceptible to different antimicrobials poses new investigative and healing tasks, India continues to battle dreaded diseases, viz., tuberculosis and malaria organisms, which always remain unaffected by treatment (Chaudhry & Tomar, 2017). Poverty, illiteracy, overcrowding, and hunger are the factors aggravating the problem (Swaminathan et al., 2017). Public's lack of knowledge of infectious diseases, as well as lack of access to healthcare, frequently prevents them from seeking medical advice, which usually leads them to seek antimicrobial drug self-prescription without expert knowledge of the prescription and length of treatment (Laxminarayan et al., 2016).

The growth of the pharmaceutical industry has coincided with an increase in the quantity of trash created by these industries. This garbage reaches waterbodies due to a lack of strong supervisory and legislative procedures, and serves as a perennial cradle of AMR in the environs (Lundborg & Tamhankar, 2017).

ARBs and ARGs have been discovered in a variety of Indian water sources. The discharges of drugs and hospitals into aquatic sources are the two major sources, especially to the adjoining areas of water resources sans proper treatment. The rate of incidence of third-generation cephalosporin-insusceptible *E. coli* was 25% in household water, 70% in domestic and hospital waste, and 95% hospital effluent, respectively (Akiba et al., 2015). The Ganges and Yamuna, India's two major rivers, traverse enormous areas of land and receive several creeks with varied concentrations of bacteria that are immune to drugs. In north Indian rivers, 17.4% of Gram-negative bacteria were ESBL producers (Azam et al., 2016), with the detection of resistance genes such as *blaNDM-1* and *blaOXA48* (Ahammad et al., 2014). The occurrence of third-generation cephalosporin insusceptibility among *E. coli* was 100% in the Cauvery waters of Karnataka (Skariyachan et al., 2015). Water intended for potable and leisure purposes sourced from surface and underground has been shown to harbor *E. coli* insusceptible to third-generation cephalosporin at 17% (central India; Kumar et al., 2013), 7% in Kashmir (northern India; Rather et al., 2013), 50% in Sikkim (eastern India; Poonia et al., 2014), and 100% in Hyderabad (southern India; Lübbert et al., 2017).

The water samples for these studies were procured from various sources, viz., waterways, meres, lochs, springs, manually operated water, and borewells. ESBL, CRE, and quinolone-unresponsive *E. coli* and *Klebsiella* sp. estimation in the Msimbazi River in Tanzania revealed a burden of these resistant isolates of >50% (Kimera et al., 2021). QNL/PEN/SUL, QNL/PEN/TET, and CEP/QNL/PEN were the most common combinations, which might be related to their widespread usage in animals and people, as well as their discharge into the environment populated, and also the disposal of waste is not up to the rate of urbanization (Said et al., 2015). A significant gradation of environments is infected with microbes obdurate to one or more than antimicrobials prevailing in Africa, owing to a high concentration of AMU in fauna and people, undertakings associated with agriculture, and deficiencies in antimicrobial control and disposal (Kimera et al., 2021). The water samples procured from the St. Clair and Detroit river area contained 48% *E. coli* of specified pathotypes and were unresponsive to drugs (Hamelin et al., 2007). The transboundary waters in these locales cater to the needs of millions across Canada and the United States representing a critical shared resource. Many towns depend on these aquatic sources for potability and leisure, and quality is a major concern. Beach closures are now listed as a major water usage limitation in numerous *Areas of*

Concern between the countries, and pathogen contamination and the growth of ARB in the Great Lakes basin environment have also been identified as concerns. The screening revealed *tet(A), tet(B), blaTEM,* and *sulII* genes of drug obduracy are abundant in the study area (Hamelin et al., 2007).

In Germany and Australia, the water samples procured from external areas harbored 24 ARGs active against eight classes of antimicrobials that were assessed using culture-independent techniques (Stoll et al., 2012). The most common ARGs were *sulI*, *sulII* (77–100%), and *dfrA1* (43–55%), which code for sulfonamide and trimethoprim obduracy, respectively. The presence of the gene *ermB* that is responsible for insusceptibility to macrolides was observed in external waters of Germany and was high (68%); however, in Australia, its presence was relatively low (18%). The chloramphenicol resistance gene *catII*, in contrast, was found more commonly in Australia at 64% than in Germany at 9% level. Similarly, the β-lactams resistance gene *ampC* was found to be high in Australian samples (36%) than in German samples (19%). Stoll et al. (2012) emphasized the wide-ranging resistant genes of antibiotics to sulfonamide, trimethoprim, macroline, etc.

4.3 Effluvium and Sewage Sludge

Effluvium is produced in various areas such as industries, hospitals, agriculture, and domestic areas. Effluvium is usually collected in effluvium treatment plants or sometimes directly channeled to some waterbodies. Due to overuse of antimicrobial agents, the effluents from these areas become a hub for antimicrobial residues, antimicrobial-insensitive genes, and microorganisms. This will eventually contaminate the surface parts of groundwater body (Walters et al., 2010; Rizzo et al., 2013). Effluvium produced from hospitals contains antimicrobial obdurate pathogens. Hence, discarding these effluents directly into the waterbody can lead to harboring of AMR pathogen strains in the aquatic environs (Kümmerer, 2001; Carraro et al., 2016).

Sludge removed from sewage and effluvium are used as landfills and manure in the agriculture sector. Since sludge settles most of the antimicrobial residues, AMR genes, and ARB in wastewater and sewage, it contaminates surface water and groundwater. Contamination occurs mainly by leaching process and is also carried along with rainwater as “runoff” (Urbaniak et al., 2017). Application of contaminated sludge as manure can reshape the movements of metabolism and environmental multiplicity of soil (Knapp et al., 2010). Some antibiotics tend to adhere to the soil particles and form stable nonbiodegradable molecules, including fluoroquinolones, sulfonamides, and tetracyclines. The biodegradation process of these antibiotics is very slow than the other antibiotics. Since they are stable in soil, they can leach into groundwater as well as reach surface water (Czekalski et al., 2014).

Likewise, urban effluvium entering the treatment plants is also of major concern in the context of the environment as it is a rich source of cellular biomass and contaminants received from various effluents, including pharmaceutical effluents, hospitals, and domestic. Hence, any lacunae in the treatment process in the sewage sludge result in the accumulation of pathogens, ARBs, ARGs, and antimicrobial residues, which act as a sink for the pathogens to interact with the antibiotic pressure and thereby facilitate the evolution of newer or novel drug resistance in bacteria (Ferro et al., 2016; Bondarczuk et al., 2016; Waseem et al., 2017).

A study has identified that Tn25 MGE enriches the integron class I in the effluents. In addition, many other transposases and ARGs were also detected and were abundant in the inlets of the treatment (Caucci et al., 2016). There are reports on the correlation of ARGs with the transposable elements in the effluvium environment, along with the prediction of the possible role of heavy metal genes in the spread of resistance (Di Cesare et al., 2016) *sulI* and *int1* were correlated, so were the genes insusceptible to heavy metals (*czcA* and *arsB*) and *tetA*, *ermB*, and *qnrS* genes.

5 Cyanobacterial Blooms and Their Effect on ARGs of Free-Living (FL) and Particulate-Attached (PA) Bacteria

Antibiotic-unresponsive genes and the blooms of cyanobacteria in freshwater structures drew worldwide attention as a public health hazard. The effects of the blooms of cyanobacteria on the taxonomic groups of bacteria are substantial. In contrast, the influence of these blooms on the function of drug obdurate groups was not clearly identified. Based on this, Guo et al. (2018) carried out contemporaneous studies in bloom and bloom-free settings in a subtropical reservoir on ARGs of free-living (FL) and particulate-attached (PA) bacteria using high-throughput methods. The study detected ARGs amounting to 145 and MGEs 9, of which 68.93% were MDR and efflux pump tools being varied and predominant. The bloom-free conditions have shown profusion of ARGs both in FL and PA bacteria than in the bloom condition. The study also revealed that free-living bacteria harbored lesser ARGs in blooming conditions compared with conditions that are free from blooms. Whereas the quantity of ARGs in PA bacteria was consistent. Despite more than 96% ARGs being common in bacteria of free-living and particulate-attached kind or in periods free from blooms or otherwise, the purposeful clusters in particulate-attached bacteria were further intensely affected by blooms of cyanobacteria than the free-living bacteria. The study also assessed the association of the structural conformations between taxonomy and function, and observed that the AR groups were highly adaptable and showed lesser relationship among bloom and non-bloom conditions than that observed in the taxonomic structure, excluding FL bacteria. Guo and others (2018) showed that the blooms of cyanobacteria seem to have a robust suppressive influence on ARG abundance in FL bacteria, and have a significant impact on the structure of AR groups in PA bacteria. The study also proposed that both nonaligned and discerning developments interactively impacted the ARG structural crescendos of the FL and PA bacteria. At the same time, the AR group of FL bacteria demonstrated an advanced progressive sequential conjecturable pattern post bloom period than PA bacteria. Guo et al. (2018) also stressed on the bacterial way of life as a pivotal tool, leading to varied reactions of AR groups to the blooms of cyanobacteria.

The ARG dynamics in potable river reservoirs by high-throughput sum up indicates that complete profusion of ARGs and MGEs was stimulated potentially by periodic precipitation. The dissemination configuration of ARGs and MGEs is dependent mainly on the ecological gradient. In the river as well as reservoir structures in the ARGs' sketches, the MGEs play a dominant role (Chen et al., 2019).

5.1 Agriculture

Exponential growth in population has increased the need for agricultural products such as plant crops and animal products. Since the availability of area is getting reduced due to an increase in the population, animals are reared in congested places because of which there is a high risk of infections and deaths, which leads to less productivity. To enhance the productivity, use of antimicrobials has become a quotidian practice to thwart contagions and associated diseases. Antibiotics are also used as growth stimulators in subtherapeutic dosages (EMA and EFSA, 2017). Commonly used antimicrobial classes such as penicillins, tetracyclines, aminoglycosides, macrolides, quinolones, and sulfonamides are critically important for the treatment of humans. Since there is an uncontrolled usage in the agriculture sector, advancement of antimicrobial resistance is also high. Studies suggest that AMR and ARB can transmit through the food consumed. Hence, the major source of AMR in humans is agriculture (Kümmerer, 2004).

5.2 Airplane Wastewater

International flights can facilitate the dissemination of AMR across the world. The screening of human exudes from flights revealed the incidence of AMR genes of the most commonly used antibiotics. The most commonly identified AMR genes were of tetracycline, macrolide, and beta-lactams (Nordahl Petersen et al., 2015). A study conducted on frequent South Asian travelers revealed that *blaCTX-M* carrying *E. coli* was found with polyclonal acquisition, suggesting that ARGs are acquired during the international travels (Bevan et al., 2018). The detection of resistance genes in these long-distance flights has signaled to tap the genomics to source the track of transmission (Hendriksen et al., 2019, b).

5.3 Travel by Bus, Truck, Waterway, and Air, and Contribution to AMRs

The studies revealed that among the global population India and China contribute to >35% of AMR. In India, 23 million people travel in trains daily in 12,619 trains. In addition, 8395 million people travel per year across the country. By adding the mobility across the world and within the country in trains, buses, trucks, waterways, and airways, the anthropogenic pressure on the global spread of antimicrobial pressure could be predicted. Similarly, within 24 hours a passenger from one part of the world reaches the other part of the world. This situation asks for the exact role of travel by different means within and between nations in the spread of AMR.

6 Metagenomics for ARGs' Determination in the Environment

Resistance of bacteria in the environment due to the overdosage of antimicrobial reagents is considered a major issue in global health (Giedraitienė et al., 2011). The phenomenon of the emergence of drug obdurate microbes in the environment started in the 1940s with the advent of large-scale production of penicillin. The indiscriminate use of antimicrobials caused a foremost challenge in healthcare settings, agriculture, livestock farming, and aquaculture. The emergence of AMR and ARGs from these fields can sink into the environment.

Resistance is a natural mechanism used by bacteria, which can be induced or noninduced. The widespread use of antibiotics, in contrast, has accelerated the occurrence of extremely antimicrobial-unresponsive microbes. The drug obduracy spreads predominantly among microbes of the identical genus and, to a lesser extent, amidst phyla, resulting in the creation of potentially dangerous bacteria (von Wintersdorff et al., 2016).

Various scientific approaches were developed to identify bacterial virulence, resistance mechanism, and other metabolic activity of bacteria. Schmieder and Edwards (2012) observed that most of the bacteria in the environment are in the VBNC state. In gauging the entire mechanism of resistance in both culturable and nonculturable bacteria, methods such as high-throughput DNA sequencing made the task much easier. Metagenomics facilitated the purpose of identifying the antimicrobial resistance in any samples (Bello-López et al., 2019).

In 1998, Hendelsman coined the term "metagenomics." This method was derived from traditional microbial genomics and reflects the fact that sequencing does not require pristine cultures (Handelsman et al., 2002). Meta-genomic research aids in the discovery of new species and the reconstruction of genomes of creatures that cannot be cultivated *in vitro* (Alves et al., 2018).

The development of metagenomics has aided in the understanding of the microbiome's involvement in a complex environment (Venter et al., 2004). The culture-independent technique gives useful information on the vast range of microbial populations that cannot be propagated in laboratory conditions. The detection of ARGs by collecting gut microflora, sediment, or water, and processing the directly extracted meta-DNA from these samples for the presence of ARGs is a commonly employed culture-independent approach. PCR, PCR-cloning, qPCR, meta-genomic technique using next-generation sequencing, and microarrays can all be used to determine ARGs using a culture-independent method from this meta-DNA.

Next-generation sequencers with better resistome analysis employing meta-genomic data are a recent breakthrough in sequencing methodologies. The multiplicity of ARGs, together with the microbiome/diversity/bacterial community structure, can be determined by sequencing the meta-genome taken from sediment or animal gut microbiota. In contrast to culture-dependent approaches for microbial community analysis, metagenome analysis employing high-throughput sequencing-based analysis (HTS) provides a thorough community structure that includes both dominating and diverse flora, and the data can be statistically compared between samples.

The key constraint of this strategy is the involvement of millions of dollars (USD) in expenditures for the creation of the infrastructure. This method offers a deeper understanding of the resistome targeted as well as developing unique ARGs. Furthermore, comparison analysis and data storage necessitate both skill and space. However, in recent decades, there has been a significant reduction in the price of analysis, and the creation of multiple commercial laboratories for outsourcing analysis helps tide over the limitations. In aquaculture, a number of investigations have been carried out.

The sequencing-based metagenomics were primarily used to establish resistomes in sediment, animal gut/tissues, or water; determine the intricate processes at work in the host–environment relationship; identify the beneficial flora bearing these transmissible ARGs; and locate resistance materials in elements such as MGEs for mobility assessing the transmission ability. The influence of drugs is specified by feed makeup and metabolic capacity of gut microbiome (Yang et al., 2013; Vaz-Moreira et al., 2014; Kokou et al., 2020).

The research of AMR bacteria and ARGs in the targeted and nontargeted, namely, the culture-based and quantitative PCR and metagenomics methods, is employed (Venter et al., 2004; Sommer et al., 2009; Zhang et al., 2011). Conversely, only 1–10% of bacteria can be cultured depending on the environmental sources (Vaz-Moreira et al., 2014). From this point of view, employing amalgamation of culture-based and culture-free procedures in scrutinizing the environmental resistome is possibly the most effective tool (Hashmi, 2020).

A combination of metagenomics and meta-transcriptomic analysis could reveal the active population in the environment harboring these resistance genes, proving the idea that the existence of antimicrobial drugs at sub-MIC concentrations favors the spread of resistance. There are more studies on efflux pump-mediated resistance and enzymatic breakdown of antibiotics in specific bacteria that are important to human health (Wright, 2005). The total relative abundance of both portable colistin-insusceptible mcr gene variants and tigecycline-insusceptible *tetX* gene variations was more in combined farms than in monoculture farms (Xu et al., 2020). The possibility of ARG transfer from *Acinetobacter baumannii* to *Klebsiella* and *Pseudomonas* was discovered, as well as the need for a paradigm for global epidemiology based on resistome.

Functional metagenomics is a relatively new approach for identifying unknown genes unaffected by antibiotics in the environs, with the ability to disentangle hundreds of resistant genes with a similarity of more than 65% to the existing database. The sensitivity and specificity of resistome analysis are reduced if the rate of ARGs increases due to allelic changes. Researchers are exploring new tools to tackle the complexity of sequence-based analysis. One of these is "target capture platforms," which selectively enrich antimicrobial, heavy metal, and other resistance-related sequences. ResCap is a platform that searches for resistance in >8600 genes (Lanza et al., 2018). Antibiotic Resistance Genes Database (ARDB) is one of many platforms now accessible for evaluating antibiotic resistance genes utilizing a sequencing-based technique. sraX, ResistoXplorer, MEGARes, SARG, DeepARG, and PRAP: Pan Resistome Analysis Pipeline are some of the other tools and software available for determining AMR from sequencing analysis data (Dhariwal et al., 2017). These tools can analyze the resistome diversity of a range of sequencing data outputs.

The bacterial resistome of untreated sewage that belonged to 79 locations in 60 nations was subjected to metagenomics analysis for categorization (Hendriksen et al., 2019, b). The study also observed systematic variations in the quantity and diversity of AMR genes among Europe/North America/Oceania and Africa/Asia/South America. Furthermore, the study reported a significant correlation of socio-economic conditions, well-being, and dynamics of the environment in the forecast of AMR gene profusions in all nations. The outcome of the study indicated that genetic variations of AMR and its profusion varied with area worldwide and that increasing sanitation and health could help reduce the all-inclusive AMR burden.

7 Conclusion

In the transmission and spread of AMR, the environment plays a critical role in both direct and indirect ways. Among the numerous ecosystems, the aquatic environment plays a very pivotal role in the AMR diffusion as it gets connected to different other ecosystems. However, much emphasis has not been given to the global action on the control of AMR. It is indeed a very much important component of one health and much complex in nature for the control. Even though the antimicrobial residue in the environment is crucial in development, this is not the only factor that drives the AMR in the environment. The transfer of resistance rate has also to be determined holistically in the environment and the way it is carried out with clinical strains. Moreover, the naturally occurring antibiotics may also facilitate the development in the environment that has to be delineated from the evolution that occurs through the application of chemicals. Technologies, viz., biological- and chemical-based available for treating the ARBs containing various ARGs for reducing the risk associated with the environmental persistence of AMR or ARGs, are to be in place for bringing down the burden of AMR in the environmental context. Regarding climate change, there is a huge scope for a shift in the pathogenic flora from the environment to the several hosts and the emergence of newer pathogens with varied patterns of antimicrobial resistance.

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