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AMR prevalence in Indian Rivers and the hitherto health risk assessments -An overview.

Prevalencia de RAM en Indian Rivers y las evaluaciones de riesgos para la salud hasta ahora: una descripción general.

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ABSTRACT

The advancement in antibiotic chemotherapy and related drugs led to the development of antimicrobial resistant microbes (AMR) in the environment. Recent studies show that AMR is one of the major challenging threats to the modern world. India has been referred to as 'the AMR capital of the world. India ranks among the highest antibiotics consumers for human use and faces immense water pollution, highlighting the relevance of AMR studies. The present paper summarises the major AMR studies in Indian rivers. The surveillance and health risk assessment are essential in the Indian scenario. Lack of proper sewage-water treatment plants, less extensive monitoring facilities, and lack of public awareness are major factors that trigger the adverse effects of AMR concerns in India. Advance technologies (in monitoring and treatments) and adequate studies such as health risk assessment alleviate AMR concerns.

Keywords; AMR; antibiotics; sewage; water treatment plants; health risk assessment

RESUMEN

El avance en la quimioterapia antibiótica y medicamentos relacionados condujo al desarrollo de microbios resistentes a los antimicrobianos (AMR) en el medio ambiente. Estudios recientes muestran que la AMR es una de las principales amenazas desafiantes para el mundo moderno. Se ha hecho referencia a India como "la capital mundial de AMR". India se encuentra entre los mayores consumidores de antibióticos para uso humano y enfrenta una inmensa contaminación del agua, lo que destaca la relevancia de los estudios de AMR. El presente documento resume los principales estudios de resistencia a los antimicrobianos en los ríos de la India. La vigilancia y la evaluación de riesgos para la salud son fundamentales en el escenario indio. La falta de plantas de tratamiento de aguas residuales adecuadas, instalaciones de monitoreo menos extensas y la falta de conciencia pública son factores importantes que desencadenan los efectos adversos de las preocupaciones sobre la resistencia a los antimicrobianos en la India. Las tecnologías avanzadas (en el seguimiento y los tratamientos) y los estudios adecuados, como la evaluación de riesgos para la salud, alivian las preocupaciones.

Palabras clave; Resistencia antimicrobiana ; antibióticos; aguas residuales; plantas de tratamiento de agua; evaluación de riesgos para la salud

INTRODUCTION

Emerging contaminants are the pollutants monitored commonly in the environment but can potentially cause adverse effects on the ecosystem and human health. These include chemicals of natural and synthetic origin and microorganisms (Rosenfeld *et al.*,2011).

Pharmaceuticals, including analgesics, antibiotics, and anti-inflammatory and antiepileptic drugs, are also recognized as emerging contaminants (Barber, 2014).

Antibiotics are the chemical agents used to treat various infectious diseases in humans and animals. Their application extended to the agriculture and bee-keeping sectors, where they were used as growth promoters (Moreno-Bondi *et al.*,2009). The commonly used classes of antibiotics were of natural and artificial origin, such as sulphonamides and macrolides (Hernandez *et al.*,2007).

Antibiotics are considered as one of the categories of emerging pollutants of concern. Due to the indiscriminate usage, continuous input and persistent environmental behaviour, even at lower concentrations. India's most commonly used antibiotic classes include cephalosporins, broad-spectrum penicillins and fluoroquinolones. Broad spectrum application as an antibiotic class increased the clinical consumption and the production output of cephalosporins (Das *et al.*,2019). Antibiotic residues in the environment were subjected to a different transformation through Physico-chemical changes and microbial actions. They can exist in the environment in original parent forms and other metabolite residues. Due to the complex antibiotic application and pathway, they also exist in multicomponent chemical mixtures. (Moreno-Bondi *et al.*,2009). This increase the challenge of monitoring antibiotic concentration in different ecosystems. The observed concentrations in various matrices also showed indifferently, such as in hospital effluents, municipal wastewater and surface waters.

Chapter 1 Antimicrobial Resistnace

Selman Waksman first described the word "antibiotic", who was the discoverer of streptomycin. It implies a chemical compound's use, a laboratory effect, or an activity. Formerly there was no mention of the class of these compounds (Hawkey 1998).

A resistant microbe describes a microbe that is not killed by the antibiotic with a standard treatment time (WHO,1998). Further studies proved that exposure to antibiotics over a period of time, even at low concentrations, forces microbes to adapt or die, termed selective pressure). The survived microbes are antimicrobial-resistant, carrying antibiotic-resistant genes (ARGs). ARGs and other genetical elements that impart resistance are transferrable to other strains of the same or different species or genus(Wise *et al.*,1998). The risk factor of AMR uncontrolled spread through different matrices could be triggered. This also points towards the flow of ARGs through environment microbes to pathogenic agents and the emergence of disease outbreaks.

Bacteria possess naturally (intrinsic) resistance and can be acquired (Wise *et al*.,1998). Natural resistance is restricted to chromosomal and not transferable from strain to strain (Hawkey 1998). Acquired resistance is specific traits in a certain bacteria, such as penicillin G resistance in *Staphylococcus aureus*. The bacteria which showed susceptibility previously towards antibiotics are eventually developing resistance against termed acquired resistance (Tomasz *et al*.,1995). Acquired resistance can be developed in bacteria via two distinct mechanisms: (i) mutations and (ii) horizontal (lateral) gene transfer.

Chapter 2 AMR in Pathogens

The antibiotic-resistant bacteria with resistance to multiple antibiotics are called multi antibiotic Resistant bacteria. These are now well-known as superbugs and are of great concern in the modern medical field. Pathogenic superbugs are the most challenging problem of current science due to the possibility of associated disease outbreaks and treatment failure. Two important classes in superbugs were commensal bacteria and opportunistic pathogens. The human commensal flora gained multi-resistance over time and became virulent such as Meticillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug-resistant *Escherichia coli*. The opportunistic pathogens are of environmental in origin and have intrinsic resistance to multiple antibiotics. *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *A. baumannii* and *Burkholderia cepacian* are opportunistic pathogens.

The most prevalent Gram-negative pathogens, such as *Escherichia coli, Salmonella enterica*, and *Klebsiella pneumoniae*, cause various diseases in humans and animals. Over the past half-century, a strong correlation has existed between antibiotic use in treating these diseases and antibiotic resistance development. Several studies identified a correlation between introducing the lactam class of antibiotics and new inactivating enzymes. In studies, up to 1,000 resistance-related -lactamases were identified (Davies and Davies, 2010).

The mutation is the most typical mechanism of resistance development in bacteria, regardless of whether the antibiotic is exposed to bacteria. *Mycobacterium tuberculosis* ('o) is an example of this which gained resistance through several mutations. The resistance genes resulting from mutation are transferred to other strains through horizontal gene transfer, viz., plasmids and transposons. The emergence of multi-drug-resistant tuberculosis has now been identified in over 100 countries (Tomasz *et al.,*1995).

Thus, the human and animal gut harbour variety of flora and works as a bioreactor for breeding ARBs. These ARBs transfer resistance genes among native flora through horizontal gene transfer events, vectors and quorum sensing. The well-documented zoonotic diseases are anthrax caused by *Bacillus anthracis*, bovine tuberculosis by *Mycobacterium tuberculosis*, brucellosis by *Brucella abortus*, and hemorrhagic colitis by *Escherichia coli.* The unmetabolized or partially metabolized fate of antibiotics leads to the food chain and bioaccumulation. The persistence period of antibiotics ranges from 1 to 3466 days in the environment (Dafale *et al*.,2020). The bacteria have a shorter generation time and are liable to strong selection pressure from host immunity and antimicrobial drugs (Dafale *et al*.,2020).

Antimicrobial resistance (AMR)microbes, the emerging biological contaminant, pose a major threat to public health (Moellering 2007). The commensal bacteria harbour in the gut and upper respiratory tract are significant in disseminating resistant genes. Food-born AMR is also a primary spreading source through food products (Chugh,2008).

The information on antimicrobial resistance in India is incomplete, and most studies have occurred only in recent years. Bala *et al.*,2007 reviewed comprehensive studies conducted on AMR of *N. gonorrhoea* in India. *Meningococci* are well studied AMR due to the pan India outbreak. Ciprofloxacin-resistant strains are critical from an AMR point of view; such strains of *Meningococci* were reported in Delhi (Singhal *et al*.,2007). Similarly, Manchanda and Bhalla,2006 reviewed the present status of AMR resistance in *N. meningitidis* in India.

Higher rates of prevalence were observed for ß-lactamase production in microbes. Microbes showed resistance to the ß-lactam group of antibiotics such as penicillin due to altered plasmid-mediated ß-lactamase. In contrast, a study in Punjab reported susceptible isolates to penicillin and ampicillin. Isolates caused the outbreak in Delhi in 2005 and showed sensitivity towards penicillin, ampicillin, rifampicin and ceftriaxone.

Another relevant study is on Shigella isolates by Rahman *et al.* 2007. Antibiotics such as azithromycin, ceftriaxone and ciprofloxacin were observed as ineffective to them. Among AMR microbes, the prevalence of extended-spectrum β–lactamases(ESBL) producers is highly relevant due to their broad nature of resistance.WHO suggested ESBL *E .coli* as an AMR indicator recently. Similiarly Amp C β–lactamase and carbapenemases are also highly relevant in community health (Rahman *et al.* 2007).

The development of antimicrobial resistance gives rise to new diseases and the re-emergence of old diseases. Chugh,2008 reviewed the Indian status of emerging and re-emerging diseases. It has been a threat in India at public health and socio-economic levels.

According to the report by the government of India As per the '11, under the government of India, more than 70 per cent isolates of *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* and nearly half of all *Pseudomonas aeruginosa* were resistant to fluoroquinolones and third-generation cephalosporins (scoping report on antimicrobial resistance in India,2017).

The current scenario in infectious diseases has generated a new era that identifies the "One Health" approach. This approach integrates the compartmentalized studies of sharing and managing etiological agents under one umbrella. Under this context, the relevance of zoonotic diseases generates primary concern (Dafale *et al.,*2020).

Chapter 3 AMR studies in rivers

In the Indian scenario, comprehensive AMR studies are still incomplete due to the lack of sufficient information on the extent of AMR in the environment (Neelam and Sharma,2019). A total of 90 studies on AMR in the environment in comprehensive Indian institutions published 2152 studies on AMR.

A few studies were conducted that dealt with AMR prevalence in Indian rivers. Most studies were associated with the two largest rivers in India -the Ganges and Yamuna (Reddy and Dubey, 2018; Mittal *et al*.,2019). The vast river bed and multiple inlets from urban sources lead these rivers to carry massive concentrations of drug-resistant bacteria (Reddy and Dubey 2018). Most studies were conducted to investigate the effects of urbanized environments on the rivers.

Near all the rivers in India are highly exposed to sewage and hospital effluents without proper treatment (Reddy and Dubey, 2018, Marathe *et al*.,2017, Karkman *et al*.,2019; Yadav *et al*.,2020). The vast works are related to detecting the abundance of AMR *Escherichia coli* in surface water. Maloo *et al*.,2017 detected the presence of multiple antibiotic-resistant pathogenic *Escherichia coli* on recreational beaches of Mumbai.

The different investigations involve detecting both antimicrobial-resistant bacteria and antibiotic-resistant genes (ARGs) in various water sources in India.

In the Yamuna river, genes in the MtrC-MtrD-MtrE efflux system were identified and found to be the dominant mechanism in resistance (Mittal *et al*.,2019). A total of 164 genes encoding β-lactamases were identified.

Similarly, resistance genes were identified in third-generation cephalosporin-resistant *E. coli* isolates from the south Indian river Cauvery which showed 100 per cent resistance to third-generation cephalosporin. The genes detected in the study were blaNDM-1 and blaOXA48, which led to the production of beta-lactamase.

Metagenomic study in the river Ganges showed 23 different ARG types in the water and sediment. Beta-lactam, multidrug and elfamycin were identified as major ARG types. (Reddy and Dubey,2018).

A specific study for the AMR *Vibrio sp* from the Narmada river. The study showed ineffective resistance to five antibiotics Viz., ampicillin, ceftadizime, erythromycin, chloramphenicol, and cefuroxime. Class one integron dominated the river's multidrug resistance determinant (Sharama *et al*.,2009).

Karkman *et al*.,2019 analyzed bacteriophage crAssphage abundance and suggested it as the indicator for environmental faecal pollution. For this, detected resistant genes were crAssphage sequences.

The study in Mutha river Pune city by Marathe *et al*.,2017 identified a high abundance of antibiotic resistance genes (ARGs) in river sediments in the city compared to the similar sample from upstream. ARGs detected in the sediment include carbapenemases.

Chapter 4 Potential for spread of resistance genes

In intrinsic resistance, genes that impart resistance are of chromosomal origin and responsible for host insensitivity. The chromosomal-resistant genes are non-transferable and cannot be transferred to other bacteria. Dissemination of a resistance determinant is lower if it is stabilized in the chromosome.

In transferable traits, dissemination occurs through horizontal gene transfer. Genetic elements such as transposons, integrons, Insertion Sequence (IS) details and the 'new' Insertion Sequence Common Region (ISCR) elements have also contributed to the increase in antibiotic resistance (Lekunberri *et al*.,2017).

A new type of genetic element, the Common Region (CR or ISCR), has been closely associated with the spread of many antibiotic resistance genes. Generally, they can be divided into two groups: those that form complex class 1 integrons (ISCR1) and those associated with non-class 1 integrons (ISCR2–12 at the time of writing this article) (Lekunberri *et al*.,2017; Walsh,2006).

Studies showed that environmental bacteria are more intrinsically drug-resistant than the commensal organisms causing infectious disease. The environmental resistome is the origin of the resistance mechanisms to many antibiotics. Antibiotics trigger the mobilization of the highly efficient resistance genes and circulate in the environmental resistome into pathogenic bacteria. Therefore, the synthesis and application of antibiotics should concern the potential of environmental resistome.

Genes that contribute antibiotic resistance in bacteria include not only the antibiotic-resistant genes but also the genes which have the potential to act as resistant genes. Thus the antibiotic resistome term consists of all type of genes that impart resistance in microorganisms.

Resistance genes from pathogens form only a small fraction of the resistome. Resistance genes from non-pathogenic bacteria include antibiotic producers and cryptic resistance genes. Multiple genes encode proteins with modest resistance, or antibiotic binding functions are also included in the resistome. These resistance precursor genes are the ultimate external source of antibiotic resistance.

The spreading of ARGs is facilitated by the horizontal gene transfer and the selective pressure provided by antibiotics. The genes for β-lactamase enzymes are such widely spread and most studied genes. The role of selective pressure is well studied for methicillin resistance.

Clinical evidence supporting the idea that reducing antibiotic use results in a reduced frequency of resistance is weak. Only two large-scale epidemiological studies of penicillin resistance in *S. pneumoniae* and erythromycin resistance in *S. pyogenes,* have been interpreted as supporting reversibility of resistance in community settings (Andersson 2003).

Chapter 5 METAGENOMICS

99% of the microorganisms cannot be cultured in an environment by standard techniques. Culture-independent methods are essential to understanding most microorganisms' genetic diversity, population structure, and ecological roles. Metagenomics is one of the efficient culture-independent methods. It can be used to compare the river resistome (collection of all ARGs) and mobilome which includes integrases, transposases and insertion sequence common region "ISCR" elements (Lekunberri *et al.,*2018). Studies showed that untreated or partially treated urban waste enriches the receiving river microbiota with ARGs, MGEs and potential bacterial pathogens. Such environments pose risks for the transmission of ARGs and pathogens to humans.

The disadvantage of culture-independent methods is significantly underestimating the actual variety of microorganisms (Krsek and Wellington,1997). For assessing the diversity of microorganisms in the environment, efficient methods are required to extract nucleic acids from microbial cells or directly from the environment (Milling *et al.,* 2005).

Morgan and coworkers (2010) suggested multiple DNA extraction procedures for a single environmental sample to increase efficiency.

Sampling is a crucial step when using metagenomic approaches, as the sample should be of representative size (Thomas *et al*., 2012). It should represent the whole population for the biodiversity studies (Wooley *et al*., 2010), and in the survey that details a habitat, samples should represent the habitat (Handelsman *et al*., 2007).

Isolation of bacterial DNA directly from environmental samples leads to the discovery of new genes and ways of resistance to antibiotics or describes microbial biodiversity in a specific environment (Felczykowska *et al*.,2015).

Metagenomic tool-based project has been launched recently in Ganges River. The high-throughput metagenomic sequencing of the microbial community in the river Ganges revealed a broad spectrum of resistance genes in sediments. The untreated sewage and pollution exposure develop combine resistance to antibiotic and metal resistance genes in microbial communities (Reddy and Dubey,2019).

Chapter 6 HEALTH RISK ASSESSMENT

Human health risk assessment (HHRA) of antimicrobial resistance is of significant concern in the current scientific world. HHRA is the systematic method to estimate the probability of health risks concerning hazardous contaminants in a particular exposure dose. The essential steps in the risk assessments involve identifying risks and managing goals. [U.S. Environmental Protection Agency (EPA) 2012].

The major health risk assessment application from a microbiological point of view was in the food Industry. Different models were established for specific pathogens that cause infectious diseases. Health risk assessment for AMR was in an emerging state, and a few studies were conducted in the area. In most cases, established models for pathogens were reapplied. The Codex Alimentarius Commission (2011) described eight principles specific to risk analysis for foodborne antimicrobial resistance applicable to risk analysis of environmental ARB. A theoretical risk assessment involves three major factors; surveillance data on the occurrence and types of antibiotics used, scientific data on ARG and ARB in the various environmental compartments, and c) related disease information. Data varies depending on the regional to national level. The study outcome depends on antibiotic use, resistance patterns, and human exposure pathways, which were different at regional and national levels.

Ben *et al.*,2019 conducted a health risk analysis of antibiotic resistance focusing on the antibiotic residues in the environment. The essential four elements of health risk assessment were applicable in the study also; hazard identification, exposure assessment, dose-response assessment, and risk characterization (Figure a). The study suggests three core steps for the analysis ; monitoring antibiotic residues and antibiotic resistance in different ecosystem set up, The dependency of pathogenic antibiotic-resistance development on antibiotics, and dose-response model for pathogenic antibiotic-resistant bacteria.

The coherent conceptualized model assesses the risks of infection and death from human exposure to antibiotic resistance. Quantitative microbial risk assessment (QMRA) is a successful tool to assess the human health risk associated with hazardous exposure to microbial agents (Ashbolt and Schoen, 2010; Soller *et al.*, 2015). Most studies are restricted to foodborne hazards, but investigations were also conducted to assess the risk of microbial contaminants in waterbodies. Chereau *et al.*,2017 evaluated the risk of the spread of antibiotic resistance in South East Asia and suggested it is the highest of the World Health Organization regions.

George *et al.*, 2013 reviewed the application of quantitative microbial risk assessments and their implication in managing the risk posed by *Escherichia coli* O157: H7 in drinking water. The model is appropriate to provide proper input for the water safety plans and can be used to efficiently and effectively manage drinking water safety.

CONCLUSION

Extensive study of antibiotic resistome concepts yet to be studied in India. Taxonomic analysis of antibiotic resistant bacteria in aquatic systems is also a gap area that should be extended. Analysis of samples from wastewater treatment plants for the presence of antibiotic-resistant bacteria or genes is essential to understand the removal efficiency. The sources and pathways of antibiotic resistance bacteria in aquatic systems may different from case to case.Thus fate of AMR and antibiotics should be done specifically for particular ecosystems. Even though the health risk assessment of antibiotic resistance is of serious concern, it is less studied due to the lack of established dose response models for AMR. Metagenomics tool is yet to be explored in AMR studies and it can be utilized in taxonomic analysis and ARGs detection.

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