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OCCURRENCE OF ANTIBIOTIC AND ANTIBIOTIC GENE RESISTANT CONTAMINANTS IN THE WATER ENVIRONMENT

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ABSTRACT

Antibiotics are an important part of health sector but also they have emerged as an effective pollutant of water environment. They are not only used in human medicines but also in other sectors like agriculture and aquaculture which eventually ends up in the environment. There increasing presence in water environment in last few decades has raised concern about the quality of water used for various purposes. The following review summarizes all recently published information about occurrence of antibiotics as well as antibiotic resistance genes in the water environment. An attempt has been made to put together all important aspects like importance, sources, effects, facts, methods of estimation and current status of various antibiotics and antibiotic resistance genes. Almost all the rivers and other water sources of the world are found to be contaminated with pharmaceutical pollutants. Various policies and methods for decreasing the contamination has been explained with more steps required to be taken in order to carry out proper usage of antibiotics at various levels and proper transfer to aquatic environment.

KEY WORDS : Antibiotics, Antibiotic Resistance Genes, Water Environment, Pharmaceutical Pollutants.

INTRODCUTION

Water is the major part for the social and economic wellbeing of humans and maintenance of a healthy and natural environment. The various water resources present in a country, marks the condition and development state of that particular country (Rakesh *et al.,* 2019). Water consumption increases with increased population and so is the increment in waste generation and environment pollution (Ranjusha *et al.,* 2020).

Previous decades have shown huge amount of disposal of micro pollutants into the environment due to various anthropogenic activities of humans. Out of all the pollutants, pharmaceuticals are of major concern due to their active persistence in the environment and long term effects on both the environment and humans (Hernandez *et al.,* 2018). With increasing industrialization and rapid urbanization, the enhanced used of pharmaceuticals has led to increment in use of chemical products in human and agricultural activities. It caused the introduction of new and strong organic and inorganic contaminants in water system (Smith and Rodriguez, 2015). They are present everywhere in natural environment including drinking water, soil, agriculture and surface water (Like Xu *et al.,* 2019; Fernando *et al.,* 2016). Also the environment acts as a reservoir for the Antibiotic resistant genes which marks the importance of studying the environmental reservoirs and develops a link between the health of animals, human and their environment (Thakur and Gray, 2019).

Many of the researches have shown the presence of antibiotics in coastal water for promoting selection of antibiotic resistance genes by getting adsorbed in the sediments (Bengtsson-Palme *et al.,* 2016). WHO has declared this as one of the most serious threat to human population in 21st century (Zhi Wang *et al.,* 2020). Also the genes present in marine environment can transfer to the biosphere. Toxicity of the marine environment can also be increased by some higher level of antibiotics which affects the marine organism. For example, the presence of Norflaxacin, Oxytetracycline and erythromycin-H_2O at Hailing Bay waters can cause excessive risk to marine species (Chen *et al.,* 2015). No antibiotics exist separately in the environment. In combination they show synergistic effects and cause joint ecotoxicity. In previous studies, a combination of amoxicillin, levofloxacin, tetracycline, norflaxacin and erythromycin showed synergistic effects when tested on green algae and cynobacterium (Gonzalez-Pleiter *et al.,* 2013; Huaijun Xie *et al.,* 2020). Some less hydrophobic antibiotics like fluoroquinolones and macrolides can undergo bioconcentration in marine organism and can also enter the food chain (Liu *et al.,* 2017)

In modern medicine, antibiotics have emerged as an important aspect in the treatment of bacterial infections. They have transformed the medical practices making possible the treatment of most lethal diseases, cancer chemotherapy and organ transplants (CDC). Use of antibiotics reduces the number, activity, structure and working of microorganisms (Bansal, 2019). Antibiotics are an important and widely used medical remedy for preventing infections in humans and animals and have largest global consumption (Perini *et al.,* 2018). Also antibiotics are used in animal breeding as food additive for the animal development (Xiaoyu Yang *et al.,* 2018). There consumption has also increased during the last decade. In 2010, 70 billion standard units of antibiotics were consumed by humans and around 63151 tons by animals which is expected to rise to 106000 tons by 2030 as stated by IMS (Bansal, 2019). Out of other countries, China is meant to consume highest amount of antibiotics in the world and the amount was estimates to be 160,000 tons in 2013 (Huaijun Xie *et al.,* 2020).

With each passing year, the production and intakes of antibiotics by humans is increasing. They do wonders for public health saving million people life from infections. With rapid industrialization, increasing energy demands and agriculture, their negative impacts can also be seen in the water system and environment (Ram and Kumar, 2020). Presence of antibiotics in water environment is one of major concern due to their capability of causing alteration in ecosystem. Although most of the antibiotics present in environment are in low concentration (around 110-610 ng/L) (Guo *et al.,* 2018), there impact andaccumulation in environment is increasing (Sun *et al.,* 2019). Presence of even low amount of antibiotics can cause the development of several antibiotic resistant genes/ bacteria in water system like drinking water, municipal waste and surface water. The antibiotic concentration varies according to season and bacterial community. Fish embryos present in antibiotic contaminated waste water gets damaged within 24 hours of development. The contaminated water has strong effects on decreasing the algal growth (Bielen *et al.,* 2017).

There are a lot of antibiotics that have been continuously detected in various water resources of the world like surface water and drinking water supply and also in the manure and agriculture (Zhang *et al.,* 2019). Fluoroquinolone, sulfonamide, tetracycline and macrolide are some important research antibiotics in previous time due to their large presence in wastewater and effect on human health (Hopanna *et al.,* 2020).

Most of antibiotics are not properly metabolized and ends up into natural water in huge amount (*Hao* Fu *et al.,* 2016). In general, about 30-40% of it is transferred to the environment (Huaijun Xie *et al.,* 2020). Antibiotic occurrence in environment (soil, water) depends on the properties like degradation, solubility, sorption of the compounds and other factors like pH and groundwater resilience time. Different antibiotics possess various properties for persisting in groundwater (Roura *et al.,* 2018).

Some important antibiotic that persist in environment have properties like tetracycline is the highest and frequently used antibiotic. They are having pH between 7 and 8 and are negatively charged in water. It allows its adsorption into the sediments. They are mostly present in the upper layer of soil due to their capability of forming stable complexes with organic matter. It makes the detection of tetracycline in the groundwater a bit difficult. Quinolones are polar with less water solubility at pH of 6-8. They do not show mobility in the soil which makes them to persist in the environment which makes it detectable through various studies. Some studies have shown the presence of quinilone in lower hundreds of ng/L in river water. Nitroimidazole is an antibiotic that was prohibited for animal husbandry in the European Union, but still they have been detected in several studies. An important antibiotic of this class is metronidazole which is lipophilic weak base. Next antibiotic are sulphonamides which are oldest and extensively studied antibiotics. Several studies have reported their occurrence in groundwater with sulfamethoxazole detected the most. They are highly mobile and prevalent in groundwater due to low biodegradability and low sorption by the soil. In a groundwater survey conducted by US geological survey. Sulfamethoxazole emerged as the most prevalent antimicrobial contaminants. Fluoroquinolones are highly accumulated in sediments. Their antibacterial activity depends on the pH. In normal pH range they exist in the form of zwitterions. Several studies suggested enrofloxacin as the most lipophilic compound. Antibiotics like ofloxacin, ciprofloxacin, norfloxacin and enrofloxacin move rapidly in water due to their high K_d values and lipophilicity. Ciprofloxacin is considered to be most widely prescribed antibiotic of this class and orbifloxacin as widely used veterinarian medicine (Boy-Roura *et al.,* 2017).

Antibiotic resistance can be seen as a condition where the microorganism is capable to reproduce and survive in presence of antibiotics. These resistance genes not only circulate within microorganism but also affect the animals and humans equally (Ram and Kumar, 2020). Antibiotic resistance present for different human pathogens can lead to the failure of the treatments, increase the treatment duration and contribute to high economic cost to mankind (Friedman *et al*., 2016). With the increasing use of antibiotics and antimicrobial agents in various human and vetenary medicine, horticulture, household use has lead to the enhancement of antibiotic resistance gene and antibiotic resistant bacteria in the environment (Bueno *et al.,* 2017).

Increasing resistance in the environment is generally due to the improper use of the antibiotics and their diffusal methods. Most of the unutilized antibiotic is excreted by an individual, health facility waste which is being disposed of inappropriately in soil, water bodies leading to formation of antibiotic resistance genes and contaminating the environment.

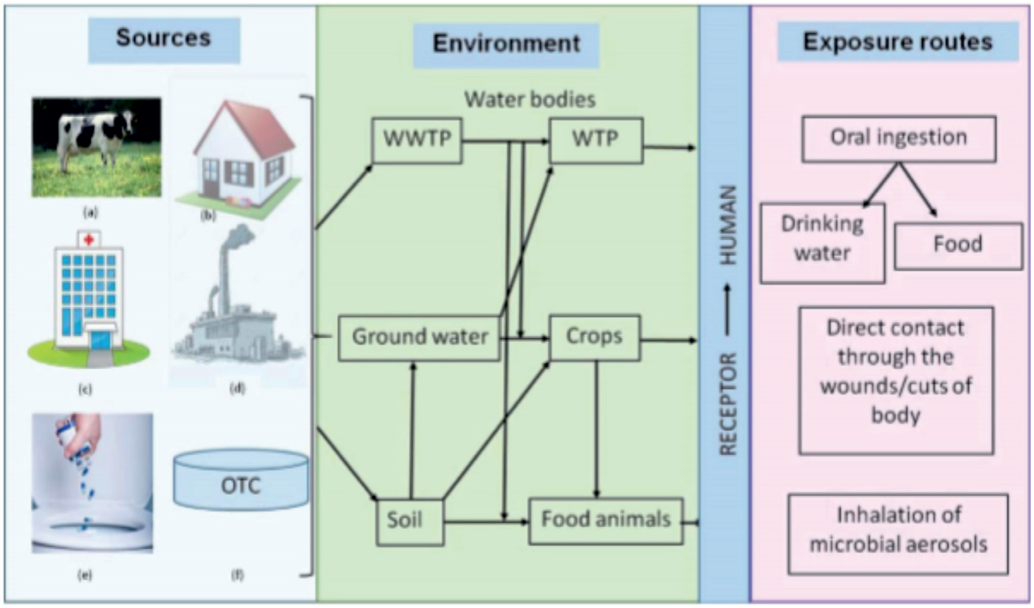
Source of antibiotics through which they enter the environment are the use of fertilizers in the fields causes the contamination of environment by directly increasing the antibiotic concentration. The anthropogenic sources include the human activities that cause the release of antibiotics in the environment. Most of which occur due to the improper handling and disposal of antibiotic. The urban wastewater contains the residue of antibiotics which were added through irrigation or as biosolid waste. Many conventional waste water treatment plants are not capable of removing low concentrations of contaminants like pharmaceutical products and personal use products which eventually ends up into surface water. The industrial process of making of the antibiotics and antimicrobials contribute largely to the environment pollution. During the transport, disposal of waste, the factories residue can enter the environment which contaminants the surface water as well as underground water. Recent studies have shown that a sheep administered with tylosin excretes about 11 percent of it residual antibiotic in urine and faecal matterupto 4 days. A different study reports India, Vietnam and Brazil as possible hotspots for development of antibiotic resistant mechanism.

These residues can travel to humans or animals and cause various infection. A study on fish from the farms of Baltic Sea shows the presence of antibiotic resistance genes in the fish intestine to be same as present in farm sediments which suggests the transfer of antibiotics to the fish during the time of hatching and rearing or from water borne ARGs. It concludes that major sources of ARB and ARG must be clinical and human activities alongwith the waste water treatment facilities and land waste..

Fate of antibiotics in environment (Wang *et al.,* 2018)

In agriculture, the pond water is used for animals and irrigation. Humans also use the stream and underground water for drinking and household purposes. In most areas all these sources are found to be contaminated by antibiotics and antibiotic resistance genes. Use of such water cause the transfer of antibiotics and resistance genes to crop during irrigation which eventually ends up in humans and animals (Danso *et al.,* 2019).

Some adverse effects of antibiotics can be seen on plants, humans and animals but they most likely affect the environmental microbiota. Antibiotics used for farming, effects human health mostly through foodborne pathogens like, *E. coli, Salmonella faecium* and *Campylobacter jejuni.* The strains of these pathogens can affect both animals as well as humans and can spread through closely related bacterial species. Antibiotics can persist in the animal tissues which makes it as food pollutant. It can trigger allergic reactions and development of antibiotic resistant bacteria in humans. Antibiotic pollutants entering into water and soil system cause the indefinite changes into the functions and composition of microbiota by selection of particular resistant species. Sulfadiaxine pollution in manure affects the nitrogen processes, microbial activities



and increase soil resistance. The resistance genes have an impact on population dynamics and physiology of microbial population (Kumar and Pal, 2017).

Hence today antibiotics resistance has become a global health problem. With time some strains are becoming more difficult to treat due to less available option without resistance. The resistant to a newly synthesis antibiotic develops very quickly which make it difficult for industries to develop new antibiotic. It is an estimates truth that more than 25000, 23000 and 38000 death occurs every year inEuropean Union, United States and Thailand. The conditions prevailing today can make the return of mankind to conditions before the invention of antibiotic. So recently more attentionhas been given to study the process responsible for development of antibiotic resistance in the environment and increasing antibiotic contamination (Berglund, 2015).

Development of awareness among local people for using the antibiotic free products can be the best way to control the spread of antibiotic pollution. Also people engaged with the municipal corporations and waste disposal should be educated about proper disposals of medicines and wastewater from various sources (Sivagami *et al.*, 2020).

Current Research Scenerio at National and International Level

Enormous amount of antibiotic discharged into the water body causes rapid increment in development of antibiotic resistance genes. Past research has suggested that most of the clinically developed ARGs and ARBs comes from the selection pressure caused by the antibiotic pollution but present and most latest research shows that most resistant genes exists due to faecal contamination and not from selective pressure (Karkman *et al.*, 2019). The pharmaceutical industries from late 1960s to early 1980s continue to deal with the problem of ARG by development of new antibiotics which has only contributed to more antibiotic abuse worldwide. In current scenario, ARGs are of major concern as contamination and their transport is an important aspect. There are many ways of transmission like when fertilizers are used and then ARGS are transferred to humans, animals and crops. Moreover, water is considered to be the major source of transportation.

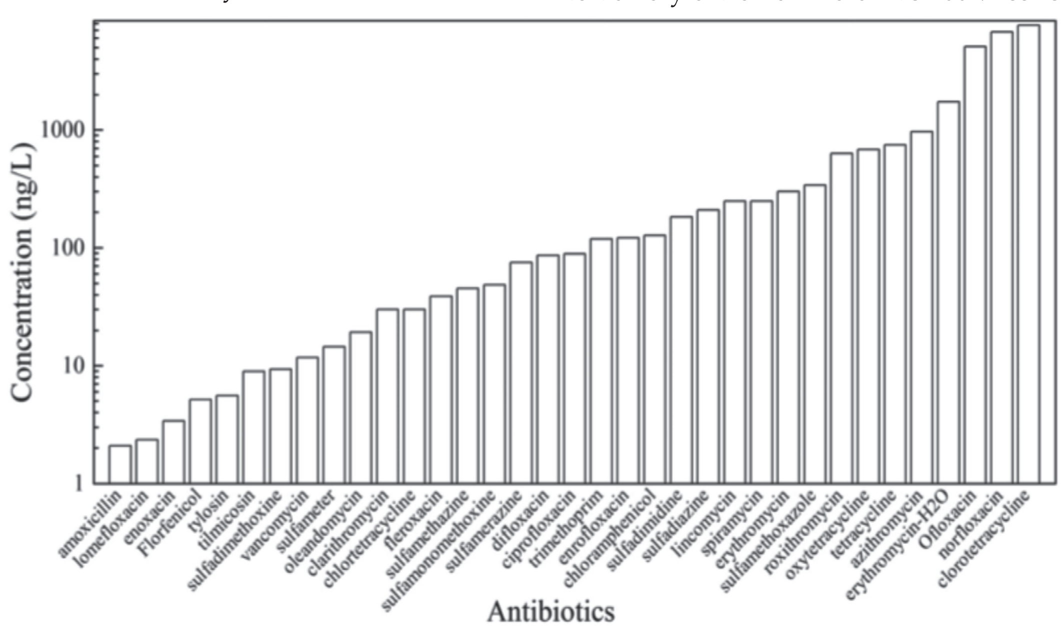
Based on many studies, the water bodies came out to be one of the major reservoirs of antibiotics and ARGs. It marks the importance of investigation of distribution of the enormous amount of contaminants on natural water and expresses the need of water quality assessment and pollution control. Due to this a lot of studies have been bone in recent times to examine presence of residues of antibiotic in surface water, drinking water, tap water and groundwater (Du *et al.*, 2019; Su *et al.*, 2018). But the studies must be conducted in same seasons for all samples as season has direct impact on the water flow, human activities, bird migration which affects the concentration of contaminants. Many studies are centered to the distribution of ARGs in various regions, difference in ARGs at different places in same season and the effect of time and location on the concentration.

It is well known worldwide that fluoroquinolones like levofloxacin, ciprofloxacin and B-lactam amoxicillin are the antibiotics which are effective for various diseases. Ciprofloxacin can also inhibit development of various multi drug resistant organisms (Sultan, 2014). In the surface water, groundwater and sewage water of Berlin drugs like ciplofibric acid and diclofenac were found. There is confirmed contamination in drinking water of United States and Canada. There are many studies shows that antibiotic resistant bacteria are present in stream of drinking water in various areas of USA and Europe. Different assessment has been done for detection fro concentration of antibiotics in drinking water in UK, Australia and US (WHO). The concentration was found to be more than 1000 fold less than the minimum therapeutic dose. Many literatures show solid phase extraction technique as a relevant method for extracting the antibiotic from samples where the identification can be done by Thin Layer chromatography, HPLC and capillary electrophoresis. By the evaluation in antibiotic in samples, information about development of resistant bacteria can be obtained (Mahmood *et al.*, 2019).

Antibiotic concentration in the surface water of different geographical regions all around the world (Wang *et al.*, 2019)

One of world's largest consumers and producer of antibiotics is china. They have excessive use of antibiotics for different purposes. According to the research, out of total antibiotics consumed by china in 2013, 48% was taken by humans. 46% of residues of these were released into the rivers and land through various sources. Research shows the presence of tetracycline, quinolone and sulphonamides in Beijing treatment plants and its effluents and same were detected in secondary effluents with levels as high as 195, 3866 and 2001 ng/L, respectively. Cephalexin, tetracycline, ofloxacin, norfloxacin and amoxicillin were highest used antibiotics in china in the same year. This intake of antibiotic was increased to 84,240 tons in 2015. This made it important the improvement of living conditions with upgraded sanitation so to reduce the consumption of antibiotics by animals and humans. Many studies in china in the surface waters of different rivers like yellow river, Bohai Bay were done that provided the development of antibiotic pollution. It leads to development of resistance genes causing infections and affecting not only china but every part of the world. WHO has found high rate of impacts due to these resistant bacteria in all countries along with China (Hanna *et al.*, 2018).

The variability of ARGs in different water bodies were studied by researchers in china to know about differences in ARGs in different water body, the impacts of different locations on them and current distribution of ARG. They sampled Poyang Lake, Bathing Beach seawater and Haihe River and studies 11 ARGS in 20 species. The results came out to be very extreme. There was 100 % concentration



of tetracycline resistance genes (tetQ, tetC and tetM) in seawater and Haihe River. Also 100% of sulfa ARGs were there in Poyang Lake and Haihe River. Among all three, Haihe River was found to be more seriously contaminated with 14 and 17 ARGs higher than in seawater and Poyang Lake respectively. Some highly abundantresistance genes were macrolide resistance genes (ermB) with concentration of 8.61 10^7 copies/L and anti-tuberculosis resistant genes with concentration of 1.32 10^6 copies/L in seawater and Haihe River respectively. It shows excessive increment in ARGS in natural waterbodies and close relations in production and life of humans and animals.

In almost every part of world, river plays an important part for each individual and country's economy. In certain countries like India they are also used for the religious gathering and seen as sacred(*WHO, 2018*).But also they have become disposal site of waste from each sector. A high antibiotic pollution is found in rivers like the Tamariparani River, Manjra River and the Musi River. Also the condition of Kshipra River is very bad causing health risks to rural communities and affecting the environmental conditions. This quality is further deteriorated due to various festivals like Simhastha Mahakumbh Mela organized at the bank of this river due to presence of millions of people all over the world. A study was also conducted in year 2018 to characterize the antibiotic residue levels. Resistance genes of *E. coli* and water quality variations from year to year which showed presence of various antibiotic residues of sulfamethoxazole and bacteria resistant to antibiotics like amikacin, tigecycline, gentamicin and ampicillin (Diwan *et al.,* 2018).

Sunderbans said to be the world largest coastal estuary which is located in delta of Meghna, Brahmaputra and Ganga River. In previous years, several studies have shown the presence of contaminants like heavy metals, antibiotics, PAHs etc, which affects the ecosystem. A study performed for the presence of ARBs and ARGs in the estuaries. Eight sediments samples were collected from Sunderban and they were analyzed using quantative real-time PCR for the presence of bla_{TEM} gene. In the analysis, about 18 multidrug resistant bacterial strains for the antibiotics kanamycin, vancomycin, ampicillin and tetracycline were isolated from the water system (Bhattacharyya *et al.,* 2018).

Theseantibodies stay in the environment for a longer time than in human body. A study in May, 2019 was done including survey of 91 rivers around the world and researchers found that around two-third sites of sampling from Thames to the Mekong to the Tigris were contaminated with antibiotics affecting the ecology of rivers to greater extent. Researches shows that most of the world's best known rivers are contaminated to a very unsafe level with the antibiotics used for serious infections. Danube River in Austria is the second largest river of Europe and was found to be the most polluted one. It contained seven antibiotics at four times higher than the safe concentration. It even includes clarithromycin used for the treatment of respiratory infections. Around 8 percent of sites were above safe limit. Even Thames which is seen as cleanest river of Europe was polluted with five antibiotics. Ciproflaxin was found at more than three times of its safe levels. Many low income countries had higher concentrations of antibiotic present in Tehri River with worst scenarios in Asia and Africa. Most residues were present near wastewater treatment plants which lack good technologies for removal of pollutants. The biggest problem for scientist is that no one is having a clear idea of cause and source of addition of antibiotics in the natural world. Many countries are having no data related to the concentrations of antibiotic in their rivers (*National Geographic*).

In most studies done on antibiotics in surface water of china, no particular attempt has been made to assess the risks at national level. So in 2020, a study was conducted at Northwest University of China. It proposed a holistic approach for the characterization of various risks of total 26 major antibiotic used in china. Some antibiotics included were sulfonamides, beta-lactam, tetracycline, macrolides and fluoroquinolones. At initial levels, exposure and hazard assessment was performed by available ecotoxicity data. Joint probability curve (JPC) was constructed for the assessment of 22 antibiotics which had sufficient ecotoxicity data and expected ecological risk came out to be less than 1%. For 26 antibiotics, assessment factor approach was applied and potential risk was detected for penicillin, amoxicillin and ciprofloxacin having risk quotient of 5.83, 1.04 and 1.54, respectively. This indicated that in china the risk of mot used major antibiotics seems to be low. But alsothere can be many uncertainties in the process due to data gaps. But the study has provided an importance of risk assessments for antibiotics in aquatic environment

(Qi Li *et al.,* 2020).

A study done by UCL at central London's freshwater source has shown the presence of high level of resistant genes. Many water sources like the Serpentine, the Regent's Canal, Regent's Park Pond and Thames, all contained genes resistant to erythromycin, tetracycline and penicillin and with maximum amount present in the Thames because it receives the discharge of large number of industries. These genes are found to come through human and animal waste. The team has developed a method to obtain information about number of resistant genes per litre of water. The method is DNA based. Then the experiments were done to remove the antibiotics using slow sand filteration with different proportions of sand, activated carbon and flow rates.

In 2020, the analysis for the presence of antibiotics in Douro and Leca rivers were done in Portugal. For the analysis, solid phase extraction was performed for obtaining the samples and they were analyzed using liquid chromatography along with tandem mass spectrometry. Leca river and its sediments was found to be more contaminated with pharmaceuticals with highest concentration of azithromycin detected as 2,819 ng/L in river water and 43.2 ng/g in sediments. Douro River has the presence of sulfamethoxypyridazine, but is lower in concentration as compared to Leca River. Some other antibiotics detected in lesser concentration in Leca River were ciprofloxacin, ofloxacin, trimethooprim, moxifloxacin and clarithromycin (Fernandes *et al.,* 2020).

A study conducted across 72 countries detected presence of antibiotics at 65% of the river sites considered. The respective project was carried out by the University of York providing around 92 kits across the world for sampling from respective sites. All the samples were then carried to University of York and were further analyzed there. Danube, Mekong, Thames, Tigris, Tiber and Chao Phraya were most important rivers among the sample sites. The data was compared with the safe limits given by AMR Industry Alliance ranging between 20 – 32,000 ng/L according to particular antibiotic. Asia, Europe, North and south America and Africa had most number of sites with exceeding the safe levels with highest levels at Ghana, Kenya, Nigeria, Pakistan and Bangladesh And a site in Austria. Trimethoprim which is used for the treatment of urinary tract infection came out to be present at 307 sites of total of 711. Metronidazole exceeded the safe levels by largest margin in Bangladesh having concentration 300 times more than the safe limit. Maximum antibiotic concentration in Bangladesh was 170 times higher than in river Thames. The total concentration detected in river Thames was 233 ng/L. Ciproflaxin is a drug used for treatment of large number of bacterial infections exceeded safe levels at 51 places, the results were eye opening telling about wide contamination of antibiotic all round the world (*Science Daily)*

As per UN reports, different antibiotics like trimethoprim, ofloxacin, norfloxacin, ciprofaxacin and sulfamethoxacole are prevalent in around 29, 15, 15, 20 and 47 countries, respectively. A study reveals that in Rio Granade, antibiotic concentration in hospital effluent came out to be 35500ng/L and in residential effluents it was 700 – 6600 ng/L. About 58% of samples taken from wastewater is contaminated with atleast one antibiotic and about 25% with more than three. The hospital effluents contained more than four antibiotics with ciprofloxacin, lincomycin, ofloxacin, trimethoprim, penicillin G and sulfamethoxazole as main contaminants. A study in Vietnam on hospital effluents shows that amount of contaminants depends on the usage by hospitals. 34 µg/L and 32.4 µg/L of antibiotic were reported in rural and urban hospital effluent, respectively. It came out to be total release of 61g antibiotics in environment per month. The concentration of antibiotic globally present in water body sulfamethoxazole 0.278 µg/L (maximum of 17.7µg/L), trimethoprim 0.037 µg/L (maximum of 13.6µg/L), ciprofloxacin 18.99 µg/L (maximum of 6500 µg/L) and norfloxacin 3.457 µg/L (maximum of 520 µg/L). Hong Kong river waters contained total antibiotic concentration of 580.4 ng/L including mostly sulfapyridine, doxycyline, ofloxacin, sulfadimidine, sulfadiazine and sulfamethoxazole. A study conducted in 2019 in 16 hospitals of Kenya reveled that antibiotics in hospitals were in range of 497-322735 ng/L in winter and 21.2-4886 ng/L in summer. Also in waste water there were present around 13-28% of the total antibiotics used (Bansal, 2019).

Most current research aims at finding the health risks of antibiotics in environment reservoirs. The policies prevent to exposure of more antibiotics to the environment from drug manufacturing facilities. Canada has implemented it domestic plan along with the global action plan of WHO to fight antibiotic pollution. The domestic plan of Canada, which is "Tackling Antimicrobial Resistance and

Antimicrobial Use: A Pan-Canadian Framework for Action" mainly focuses on Stewardship, Surveillance and Innovation (PHA Canada, 2017).

Europe always has a front foot in solving the problems related to antibiotic pollution and realized the need of more investments in the policies for this crisis. In June 2017, Europe implemented the "EU One Health Action Plan against AMR". The main objectives of the plan are boosting the research and innovation, making it the best practicing region and global development (Kraemer *et al.,* 2019).

India has taken new initiatives to prevent the contamination of environment due to antibiotic manufacture by controlling the effluents. A report by Antimicrobial Resistance Benchmark, said that many quality issues arises when proper systems are not used for manufacture leading to release of residues in the environment. The report emphasis on fact that this risk of contaminating the environment can be reduced by implementing proper environment Risk Management strategies.

India is considered as a biggest producer of ARB. It is occurring due to improper use and misuse of antibiotics for humans as well as livestock in poor sanitary and unhygienic conditions and over prescriptions. XDR and TDR superbugs have been identified in India. Recently a new variant of *Klebsiella pneumonia*has also been identified. The country has highest amount of multidrug resistant tuberculosis with 130000 or 27% tuberculosis cases out of the world cases in India.

With the growing concern for the presence of antibiotic and antibiotic resistant bacteria contaminants worldwide, an Antibiotic Manufacturing Framework was developed in 2018 by the AMR Industry Alliance (global group of private pharma manufacture, it ensures the control of emissions of effluents from various manufacturing units. The measures provided by this suggested calculation of the discharged amount of Active Pharmaceutical Ingredients. It is the antibiotic conc3ntration at which the development of resistant bacteria is minimum in environment. . The Environment (Protection) Amendment Rules, 2019 also proposed to set the limit on discharge of 121 antibiotic residues by drug production units.

WHO has declared the antibiotic resistance as a global threat for whole world. Due to this, 700000 people died in year 2014 and the consequence can become severe to deaths of ten millions by 2050 if conditions are not taken into control. In Hyderabad, around 56000 new born die each year due to presence of antibiotic resistance. According to WHO report of 2017, around 600000 patients of TB have become resistant to rifampicin which is one of the most effective drugs used. According to a study done in India, there has been an increment from 2 to 52% in number of carbapenems resistant bacteria (Bansal, 2019).

Many initiatives are also led by WHO to address the antimicrobial resistance. It includes activities like World Antibiotic Awareness Week which is held every November since 2015 with a theme of "antibiotics: Handle with care". Another is Global antibiotic Research and Development Partnership (GARDP) which is a joint initiative by WHO and Drugs for Neglected Diseases Initiative. It is supporting the researches through partnership of public and private sectors. Then the Global Antimicrobial Resistance Surveillance System (GLASS) is a system supported by WHO. It provides a standard approach of collecting, analyzing and sharing the worldwide data about the antimicrobial resistance at global level to make proper decisions and take action (WHO).

Future Perspectives of the Research

Ministry of environment, forest and climate change, on 23 January, 2020 has given some strict standards of the presence of different antibiotic concentration in the effluents of pharmaceutical industries into the environment. And with this it has become first state regulator of the world to take such measures for reducing drug-resistant bacteria. In current time, no such law is given by any other country for limiting the antibiotic resistance even by Europe or the USA. The Indian Government has adopted a National Action Plan on AMR with six priorities that include improving awareness about AMR, getting evidences through surveillance, controlling the contamination, optimizing use of antimicrobial agents, more investments and innovation in AMR and stretching India's leadership on AMR (MOEF).

The studies must be concentrated on developing techniques for better degradation of antibiotics. Some techniques are still present but come with disadvantages like high maintenance, unstable and slow degradation rates. Further efforts can be made to make the available technique more efficient and develop new more novel methods. Also new protocols are needed to be developed for the determination and quantification of antibiotics residues. They act as great tool for the detection of antibiotic contamination even at low levels, identifying biomarkers for environmental monitoring, development of new models for degradation pathway and tell the effectiveness of various processes for remediation.

Also the risks developing for human health and ecology due to presence of antibiotic and antibiotic resistance bacteria must be evaluated from time to time. The identification and quantification of the type of antibiotic is also an important part to be focused on. With the use of meta-omics technology and low cost techniques for sequencing, it is easy to find about the diversity of antibiotic resting bacteria and genes and also to compare their presence at different regional and global levels. Additionally, the presence of modern bioinformatics tool can help to better understand the evolution and transfer of antibiotic in the environment.

CONCLUSION

With increase in antibiotic usage over the past decades, their amount in water environment as pollutant has also been increased. About 30-40% of used antibiotics are excreted unchanged into the water bodies and at present almost every water supply of the world is polluted by antibiotics like fluoroquinilone, tetracycline, etc. Presence of antibiotic leads to development of antibiotic resistance genes in the environment which are further transferred to animals, humans and ecosystem causing allergic reactions, changes in function and structure of ecosystem and alters nitrogen processes.

Many studies done by the researchers showed presence of antibiotics and antibiotic resistance genes in the environment with chlortetracycline in maximum and amoxicillin in minimum amount present in water body all over the world. Surveys done over more than 72 countries confirm pollution in 66% of places. Different techniques like HPLC, TLC, solid phase Extraction and DNA sequencing proved to be helpful for correct determination of antibiotic in antibiotic resistance genes.

Countries have developed different plans to fight against antibiotic pollution and to balance the antibiotic discharge in the water environment. Also Antibiotic Manufacturing Framework developed by AMR industry alliance helped is the control of the emissions from pharmaceuticals. Environment Amendment Law gave by India in 2019 limits the discharge of some importance antibiotics from production units. Initiatives like World Antibiotic Awareness week taken by WHO has developed awareness at global level. A proper method and collaboration among various levels in system and setting of various regulatory standards by the authority can help to control dissemination of these pollutants in the environment.

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