Fluoroquinolone antibiotics: an emerging threat to the environment and their remedial measures

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In our soil and water ecosystems different pharmaceuticals are present as chemo-pollutants. Considerable focus has to be given to antibiotics as the use of this group of pharmaceuticals is increasing day by day. Consequently, numerous multi-drug-resistant bacteria are developing which are becoming hazardous to human health. Additionally, once antibiotics enter into the ecosystem, they may disturb microbial community structure and functioning. This may cause bacteriocidal and bacteriostatic effects leading to alteration of some microbial populations. The indirect impact includes resistance to antibiotics by some bacterial strains and the development of antimicrobial-resistant bacteria[1]. The prescribed antibiotics after entering the living body either partially metabolized or completely unaltered may be excreted through mammalian urine and faeces and reach the wastewater treatment plants (WWTP)[2]. Conventional treatment plants do not remove antibiotics and therefore these molecules directly enter the ecosystem. Clinical establishments and pharmaceutical industry effluents, hospital waste disposal, and aquaculture are significant sources of aquatic contamination by antibiotics. Manure application to the soil and irrigation with recovered water contribute to the dispersal of antibiotics and antibiotic resistance genes in the soil and living systems therein. Though there is no information available regarding any adverse impact(s) of antibiotics intake by plants, it is speculated that they might cause allergic or toxic reactions and/or could enhance antibiotic resistance in humans.

Quinolones are derivatives of quinine which have emerged as the most potent antimicrobials today after their discovery in the early 1960s. Fluoroquinolones (FQs) group of antibiotics, discovered in the 1970s and 1980s, are the most important antibacterial agents till today. Their antibacterial activity is due to the inhibition of the bacterial nucleic acid synthesis by disruption of enzymes topoisomerase IV and DNA gyrase which consequently leads to the breakage of bacterial chromosomes. FQs block the final stage of the topoisomerase reaction and the ligation of both ends of DNA strands[3].

After their discovery, quinolones had very confined use and just in two decades they became one of the most prescribed antibiotics with global sales of US$ 3.04 billion in 1997, and this figure is continuously increasing since then[4]. In 2015 alone, doctors in the US recommended this group of antibiotics (32 million prescriptions recommended these drugs) in such quantity that the FQs have emerged as the country's fourth-most popular class of antibiotic. In Europe, especially in Zurich and Zagreb, higher amount of FQs (190 to 326 g d^{-1}) were released from households in the WWTP[5]. In India, over 46 kg d^{-1} of quinolone load consisting of ciprofloxacin, ofloxacin, norfloxacin and lomefloxacin was observed in the effluent from approximately 90 industries[6]. The FQs are also present in the wastewaters from livestock farms. Manure from livestock consists of 5.3 μg kg^{-1} to 1.4 g kg^{-1} of FQs and exceptionally high concentrations of enrofloxacin and norfloxacin were detected in chicken manure samples collected from China in 2007 (ref. 7). FQs volatilize in very negligible amount and have high tendency to get adsorbed on solid surfaces including soil.

Ciprofloxacin (CIP), one of the second generation FQs, is the most widely prescribed FQ in the world and the global value of its sale by November 1999 had exceeded US$ 1.3. Data on prescribed quinolones to outpatients in 25 countries of Europe showed that more than 50% of quinolone used are second-generation quinolones (mainly CIP). In Switzerland, the major FQs consumed by human are CIP and norfloxacin (NOR)[4]. The World Health Organization (WHO) presents a model list of minimum essential medicines required for basic human healthcare system, which are efficient, safe, and cost-effective and CIP is among the one in the list[8,9]. In 2016, CIP accounts for more than seven million prescriptions and it was the 102nd most prescribed medication in the US[10]. It was earlier reported that in India the levels of CIP in the environment were around 31 mg l^{-1}. This is approximately one million times greater than that being regularly detected in treated municipal sewage effluents and is toxic to a range of organisms.

Ofloxacin (OFL) is the second most widely prescribed second generation FQ in the world, followed by levofloxacin, lomefloxacin, norfloxacin, sparfloxacin, clinafloxacin, gatifloxacin, moxifloxacin, sparfloxacin and trovafloxacin. OFL is used to administer orally and it is active against most Gram-negative and Gram-positive bacteria, in addition to being active against some anaerobes. The global value of OFL by November 1999 was approximately US$ 900 millions[4]. OFL is a semisynthetic antibiotic and its L-enantiomer is available as levofloxacin. In 1990, OFL was approved for the use in the US, but in 2009 its initial sponsor had discontinued it partially because of its adverse side effects[11]. More than 3 million prescriptions of levofloxacin were reported in 2016 and at that time it was the 161st most prescribed medication in the US[10]. Another FQ, viz. NOR is one of the first generation FQs and is currently discontinued in the US since 2014 but is still very common in Europe[12].

Since the last few decades, extensive use of FQs has led them to enter the environment through manufacturing and application sites. In many third world and developing countries, WWTPs are not well equipped with appropriate and modern techniques. Therefore, filtration and removal of harmful chemo-pollutants from industrial and household effluents are not successfully achieved and consequently, antibiotics are released to near-by water channels. Rivers and other water bodies get contaminated with these antibiotics.

This expanded and aimless utilization of anti-infection agents for treatments in aquaculture, animal husbandry and human medication has generated the new family of antibiotic-resistant bacteria (ARB) during the last two decades. Antibiotics leftover in the wastewater exert selective pressure which leads to the increase in ARB in the environment. In Indian rivers, the escalating rate of ARB seems to be a major challenge because of their negative human health effect. Such bacteria have been isolated from major Indian rivers including Yamuna, Ganges and Cauvery[13]. In the long run, multi-drug-resistant bacteria in water sources will be a major health hazard to society. A study in Kolkata demonstrated the risk of co-transmission of FQ and β-lactam resistance amongst uropathogenic *E. coli*[14]. According to GLASS Report, 8–65% of *E. coli* linked with urinary tract infections showed resistance to CIP – an important FQ frequently used to treat this condition[15]. This is one of the most concerning public health issues that restrict proper treatment options. Goswami and Rao[16] showed changes at genome-level expression in bacteria with respect to glutathione exposure which act as a trigger for FQ antibiotic resistance. Due to the emergence of multidrug resistance bacteria, these antibiotics have been gradually losing their importance and this situation forced the scientific community to discover new antibiotics.

In bacterial evolution, horizontal gene transfer (HGT) plays a key role. Antimicrobial resistance has also resulted from HGT[17]. HGT is promoted by microbial community as stress response which is induced by antibiotics present in the environment, and this further leads to dissemination of resistance genes[18]. If these mutated bacteria are pathogenic, the situation becomes even worse. Environmental stress due to rising global temperature further leads to more number of enzymes in antibiotic resistant and pathogenic bacteria to become active. Also, there is a possibility that such bacteria will further transfer their resistance genes to other microbes. A recent study[19] of microbes in water and sediments in the river Ganges showed that the river harbours a wide range of resistance genes, mostly in sediments.

During floods, numerous water channels and rivers flow off and thus get contaminated with pathogenic ARB which may cause epidemic diseases and this kind of situation is hard to control sometimes. Urban WWTPs are hotspots of ARB as they receive residual antibiotics from different sources through wastewater which leads to spreading of ARB and antibiotic resistance genes (ARGs). Few studies also reported that ARB of farm animals could transmit to consumers through meat and milk products causing infections in humans and creating other unhealthy consequences for them[20].

Ongoing initiatives for antibiotic remediation

There are no specific (or detailed) data related to FQs discharged in the environment reported from India as well as from other developing countries. Lack of information in this regard is not only harming the local people residing nearby the pharmaceutical manufacturing industry but it also puts the entire environment and ecology at risk. Currently, many methods have been adopted to remove antibiotics from the environment which include conventional treatments (biological processes and filtration), oxidation processes (advanced oxidation processes, electrochemical oxidation and chlorination), adsorption processes, photodegradation, membrane processes and combined processes.

Biodegradation is one of the eco-friendly processes in this regard usually considered as the most promising way to remediate environmental pollutant; however, the probability of success of FQs remediation by microbes in the environment is still unsolved. Previous findings suggested that FQs are difficult to biodegrade while few mixed bacterial cultures were able to biotransform them in laboratory condition and/or in wastewater treatment bioreactor. Biodegradation of this class of antibiotics can be done by a group of microorganisms (bacteria and fungi) capable of degrading fluoroaromatic compounds. The common groups of microorganisms used for the degradation of fluoroquinolone antibiotics are *Microbacterium* sp., *Labrys portucalensis* F11 and *Gloeophyllum striatum*[21]. The biodegradation of FQs has rarely been accounted for and recently, just a couple of microscopic organisms and parasite species have demonstrated to have the option to biotransform/biodegrade FQs. In a recent study, the CIP-degrading network was distinguished in nitrate-diminishing conditions which involved *Comamonas*, *Arcobacter*, *Dysgonomonas*, *Macellibacteroides* and *Actinomyces* genera while in sulphate-decreasing conditions the network was chiefly formed by microorganism's subsidiary to *Desulfovibrio*, *Enterococcus* and *Peptostreeptococcus*[21]. In another experiment, 85% of CIP was degraded under sulphate-diminishing conditions and 62% was degraded under nitrate-lessening conditions[22]. Biodegradation of CIP by blended culture was additionally studied and it was discovered that CIP-degrading bacterial network was principally made out of the classes *Gamma-proteobacteria*, *Bacteroidia* and *Beta-proteobacteria.* Microorganisms from genera *Pseudoxanthomonas*, *Stenotrophomonas*, *Phenylobacterium* and *Leucobacter* also reported for CIP biodegradation[21]. Biodegradation of NOR by *Trametes versicolor* was also reported[21]. A plant growth-promoting bacterium, *Microbacterium azadirachtae*, isolated from municipal wastewater biotransformed NOR to metabolites with less antibacterial activity[23]. Another fungi, *Ganoderma lucidum* JAPC1 strain and *Pleurotus ostreatus* were also reported to degrade NOR[21].

Future directions

India was one of the highest consumers of antibiotics in 2014, followed by China and the US. Non-remedial utilization of anti-microbials is also common in the Indian subcontinent, as they have been reported in poultry, apiary, aquaculture and farming. Traces of erythromycin, enrofloxacin, ciprofloxacin and chloramphenicol were detected in honey samples of different brands collected from Indian market. In Tamil Nadu, India, anti-infection agents are additionally added to crustacean seafoods and fishes before delivery to other provincial states. Most of the sewage generated in India remains untreated and is directly discharged into nearby water bodies, which subsequently contaminates the rivers and other water channels leading to dissemination of ARB in this environmental segment. The research community in India has to find out ways to overcome the limitations of antibiotic removal from the environment. It is also important to monitor and quantify the prevalence of FQ antibiotics and their transformed products in effluents originating from pharmaceutical industries. A user friendly and efficient analytical technique should be adopted/implemented to detect the traces of FQ antibiotics in the different environmental compartments. A few specific remedial solutions are mentioned below:

- Developing techniques in conventional wastewater treatment plants to filter out these antibiotics from the effluents of pharmaceutical industries can be helpful.

- Adsorption and/or biosorption (chemi- and/or physi-sorption) of FQs at discharge site is recommendable.
- Already proposed techniques for removal and bioremediation of FQs should be used in treatment of effluents of pharmaceutical industries.
- Different FQs degrading microbes which have been screened till date need to be brought up in the field. All the *in vitro* experiments performed which showed positive results have to be validated *ex-situ* and commercialized as soon as possible. This initiative which is employing the existing microbial bioresource will further minimize the FQs' pollution from our environment.
- The awareness of improper use of antibiotics and the related human health effect should be disseminated among the general public. Mass media should be engaged to raise such awareness and should sensitize people about antibiotic resistance and its public health linkage. General public should be advised not to use any antibiotics without prescription from doctors.
- Information about manufacturing sites and medicines produced from the firms should be made publically available so that research communities can target the sites to examine the extent of pharmaceutical pollution in those areas and can adopt proper remediation processes.

Overall, the Government of India which has already initiated a 5-year National Action Plan (2017–2021) to combat antibiotic resistance should implement the existing laws to overcome this issue.

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