

ANTIMICROBIAL RESISTANCE (AMR): DIFFERENTIAL DRIVERS ACROSS THE ECOSYSTEM[†]

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Antibiotics and antibacterials are used in both terrestrial and aquatic agricultural system for better health management. Indiscriminate use of these chemotherapeutic and chemoprophylactic agents lead to the development of antimicrobial resistance with subsequent adverse impact on the global economy. Resistance against the drugs develops due to biochemical and genetic changes within the bacteria or social changes in the clinical settings. Although the most of the bacteria of aquatic origin are not characterized, aquatic ecological systems seem to serve as a reservoir of antibiotic resistance and platform to spread the clinical resistance amongst medical, veterinary and aquaculture system. Recent advancement of molecular techniques will be of great use to explore the resistome across the different ecosystem. Antimicrobial-resistant bacteria and responsible genes have been reported from both groundwater and surface water in India. Wide range of bacteria species pathogenic to human, animal and piscine population are reported to be resistant against various antimicrobials. This communication dealt with present status, possible mitigation and necessity of multidisciplinary tactic involving researchers from medical, veterinary, aquaculture, environmental and extension science to address this burning worldwide dispute.

Key words: Animal, Antibiotics, Antimicrobial resistance, Aquatic system, Fish

The history of antibacterial goes back to the first synthetic agents –sulphonamides which were introduced to the early twentieth century for controlling the bacterial infections in the field of clinical science. Later on, antibiotics from microbial origin entered into medical science with one of the most important discoveries- penicillin, which could able to treat various complicated infections. Till date, antibiotics are playing a great role in human and animal health. In agricultural animals including both terrestrial and aquatic origin, antibiotics and other antibacterials are applied now -a- days with the modus operandi of health management and enhancement of food production. Thus, indiscriminate use of antibacterial has become one of the great

problems which create further complication through the development of antimicrobial resistance (AMR). Fall of 3-8% livestock population with decrease in 2-3.5% gross domestic product (GDP) due to AMR is expected by 2050 (O'Neill, 2014).

Resistance to any drug or AMR develops when pathogenic microorganisms will not respond to that particular drug against which it was sensitive earlier and effective to treat them.

AMR can lead to the following issues:

- some infections being harder to control and staying longer inside the body
- longer hospital stays, increasing the economic and social costs of infection

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- a higher risk of disease spreading
- an increased rate of fatality

The major concern of AMR is presently effective antibiotics will no longer be effective in future. Therefore, the easily treatable infections could be more dangerous and even difficult to tackle.

Distinguishing between antibiotic and antimicrobial resistance is important. Antibiotic resistance means microorganisms resist any antibiotics. AMR refers that the drugs developed to kill the microorganisms are ineffective.

Resistance against drugs develops due to various biological or social changes. After a certain time of introducing a new drug, there is a chance of developing antimicrobial resistance against the drug due to genetic or biochemical changes within the microbes. These changes come about in different ways:

Mutation: During the process of reproducing, at any point of time, mutation may occur resulting in gaining the ability to survive against antimicrobials.

Selective pressure: Development of dominant antimicrobial-resistant microbes carrying resistance genes.

Gene transfer: This is the process of sharing resistance genes from other microorganisms. Thus, a microbe becomes resistant after picking up resistance gene from a resistant microbe.

Phenotypic change: Microbes can change some of their characteristics to become resistant to common antimicrobial agents.

Over the years, increasing numbers of resistant pathogens emerged in every niche of the environment. Along with developing new drugs, it is also very important task to properly recognize AMR and understand the ecology and pathway of antibiotic resistance to prevent and increase the lifespan of antibiotics (Suzuki *et al.*, 2017). The incidence of antibiotic-resistant infections rose along with the number of multidrug-resistant pathogens. There are well documented prove on

development of 'resistome' across the clinical settings, terrestrial and aquatic environment (Watts *et al.*, 2017). Aquatic ecological systems serve as a reservoir of antibiotic resistance and platform to spread the clinical resistance amongst medical, veterinary and aquaculture system. Various aquatic bodies such as rivers, reservoirs, dams, wetlands, wastewater effluents, and overspill of agricultural land, serve to hoist antibiotic resistance genes (ARGs) and their transfer to other pathogenic organisms (Michael *et al.*, 2013). Latest microbial genomic approach revealed that these genes of resistant determinants can move vertically within the microbial population and horizontally across the species and genera (Port *et al.*, 2014). This movement is allowed by the 'mobilome', -the genetic elements that facilitate and contribute to horizontal gene transfer (HGT). Till date, it is not clearly understood about the role of nonpathogenic bacteria as a reservoir or in transferring the ARGs to other potential pathogens and pollutants in strengthening this prospective.

Reports on antimicrobial resistance in India

Antimicrobial-resistant bacteria and responsible genes have been reported from different water sources of India. The leftover water from pharmaceuticals and hospital discharges released to the adjacent wetlands without treatment are key factors. Very recently, Taneja and Sharma (2019) reported that 25-95% of *E. coli* from domestic waste and hospital discharges were resistant to cephalosporin. Different levels of drug resistance were recorded in the large area of land across the river Ganges and Yamuna in India. This may be due to the several inlets into the adjacent agricultural land. It is also reported that extended spectrum beta-lactamase (ESBL) were 17.4% of bacteria isolates with *bla*_{NDM-1} and *bla*_{OXA48} genes from these rivers. From the river Cauvery, Karnataka, 100% resistance against cephalosporin were also noted from another study (Skariyachan *et al.*, 2015). Number of reports suggested that both the surface water (rivers, lakes, ponds and springs) and groundwater (tube well and hand

pumps) in diverse agro-climatic zones of India are contaminated with cephalosporin-resistant *E. coli* (Kumar *et al.*, 2013; Akiba *et al.*, 2015; Lübbert *et al.*, 2017).

Most of the bacteria from the aquatic origin are un-cultivable or difficult to culture. This character makes these bacteria distinct from the pathogens of medical and veterinary importance which can easily be isolated and cultured using standard bacteriological techniques. With the help of standard methods, only 0.1% or less of the aquatic bacteria could be monitored for ARB and ARGs due to complicated culture characteristics which are yet to be standardized. With the advancement of the next-generation sequencing (NGS) and metagenomics technologies, the behaviour of ARBs and ARGs are going to be open for the researcher. High thorough-put genetic assays altogether are required to match ARGs for detail appreciation of various factors involved in ARGs in aquatic ecosystems.

Aquaculture and AMR

Aquaculture is one of the fastest-growing sectors, however, there is an argument on its sustainable growth and intensification. High-density dependent intensification is a major risk for disease outbreaks in aquaculture (Krkosek, 2010). Disease outbreaks include trans-boundary aquatic animal diseases, infections due to viruses, bacteria, fungi, parasites and other pathogens. Disease outbreaks in aquaculture, thus, are impeding both economic and social development. It is difficult to determine economic loss due to disease, however, it was reported as 10–15% of the total value of fish production world-wide (Klesius *et al.*, 2008). In India, Andhra Pradesh contributes a major share of 40,000 tonnes of major carps, worth of 600 million rupees per year, but loss due to disease outbreak is supposed to be Rs.40 million. Shankar *et al.* (2000) reported that a bacterial pathogen, *Aeromonas hydrophila* alone responsible for causing mass mortalities (30–70%) in Indian

major carps rearing. Antibiotics administered in fish are excreted with urine or faeces and reach the sewage treatment. Antibiotic and antibacterial use in aquaculture system has serious consequences due to the diffusion of antibiotic resistance, which is a major global concern at the present scenario. To control different bacterial diseases antibacterials are applied in aquaculture which causes further consequences through leaching and due to less palatability in the whole aquatic system.

Status on antibiotic resistance pattern in pathogenic bacteria of fish

Our study on the bacterial isolates of fish origin from Andhra Pradesh and Odhisa revealed that *Pseudomonas* spp, *A. hydrophila* and myxobacteria - are resistant to various antibiotics which are generally used in clinical settings for human, terrestrial and aquatic animal chemotherapy. Few results are given below:

***Pseudomonas* sp.:** Out of 32 antibiotics screened, *P. putida* was resistant to cephotaxime, co-trimaxime, ampicillin, cephalothin, ceftazidime, tricarcillin and cloxacillin; *P. aeruginosa* was sensitive to gentamycin, tobramycin, netillin and intermediate sensitive to chloramphenicol, oxytetracycline, tetracycline; while resistant to other antibiotics. *P. fluorescens* was sensitive to tobramycin, intermediate sensitive to gentamycin; resistant to rest other antibiotics. Tiwari *et al.* (2017) reported that injudicious use of drugs is one of the main contributors to the emerging resistance of *P. aeruginosa* to antimicrobial therapy. It also contributes to the appearance of multidrug-resistant strains of bacteria.

Aeromonas hydrophila: One of our studies suggested that most of the strains of *A. hydrophila* are resistant to cephotaxime, cephalixin, co-trimaxazole, ampicillin and cefuroxime. In another set study, we found that *A. hydrophila* isolated from fish at eastern India are mostly partially resistant to tetracycline (38.5%) ciprofloxacin, gentamycin and flumequine (30.8%) (Samal, 2015). It is reported

earlier that *A. hydrophila* are resistant to tetracycline, streptomycin, erythromycin, cephalosporins (cephalothin, cefotaxime and cefoxitin), nalidixic acid, sulfadiazine/trimethoprim, penicillin-G, colistin, ampicillin, novobiocin, amoxicillin, oxytetracycline, amikacin, gentamicin, oxacillin, nitrofurantoin, augmentin, piperacillin, ampicillin-sublactum, cefotaxime, levofloxacin, ciprofloxacin, lincomycin, rifampicin, chloramphenicol, amoxicillin, metronidazole, neomycin, ticarcillin, mezlocillin, sulfamethoxazole, trimethoprim, vancomycin, fluoroquinolones, clindamycin, erythromycin, polymixin-B, co-trimoxazole, sulphafurazole, carbenicillin, chlortetracycline, cloxacillin, doxycycline, cefaclor, norfloxacin, bacitracin, kanamycin and cefodoxime (Abbott *et al.*, 1992).

All isolates of Myxobacteria were resistant to cefuroxime, cloxacillin, cephalothin, ampicillin, penicillin G and trimethoprim. Resistance to co-trimoxazole, bacitracin, neomycin, nitrofurazone and norfloxacin was also noted in most of the isolates.

Vibrio sp.: All the strains were found to be resistant to cefuroxime, cloxacillin, cephalothin, ampicillin, bacitracin and neomycin. Behura (2015) studied the resistance pattern of antibiotics against 21 different antibiotics. He reported that most of the *Vibrio* isolates from fish were resistant to cefuroxime, cephalothin, ampicillin, bacitracin, neomycin, penicillin and cloxacillin.

Staphylococcus aureus: All strains were found to be resistant to cefuroxime, cephalothin, ampicillin, penicillin and co-trimoxazole. Most of the strains were sensitive to ciprofloxacin, nalidixic acid, chloramphenicol, tetracycline and erythromycin.

Edwardsiella tarda: The strains of *Edwardsiella tarda* were sensitive to trimethoprim, tetracycline, neomycin, gentamicin, cefuroxime, cephalothin and resistant to ampicillin, erythromycin, nalidixic acid, oxytetracycline and amikacin, respectively.

Mitigating AMR through alternative approach in aquaculture

One of another possible solution to chemotherapies in aquaculture is related to the use of herbal extracts. Plant extracts and different algae contain pharmaceutically important natural compounds, like phenolic compounds, polysaccharides, proteoglycans and flavonoids, those are capable of stimulating piscine immunity and, thus, effective to play important role in preventing infections (Das *et al.*, 1999; Sahu *et al.*, 2007). These are recognized as eco-friendly alternatives for therapeutic and prophylactic purposes in health management of aquatic animals, which combine sustainable production systems with high quality of seafood products. Nevertheless, further studies are needed to assess any potential impact of these substances on the host microbiota and on the environment.

The control of antimicrobial resistance is a challenge for all the developed and developing countries but depending upon regional policies, practices, technological development and constraints the response may differ. Therefore, it is the urgent demand to work together in a multidisciplinary approach involving researchers from medical, veterinary, aquaculture, environmental and extension science. Finally, policymakers and administrative personnels are required to be involved for the promulgation of strict rules and regulations on the use of antibiotics in each and every health sectors.

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