International Journal of Fisheries and Aquatic Research
ISSN: 2456-7248
Impact Factor: RJIF 5.44
www.fishjournals.com
Volume 3; Issue 3; July 2018; Page No. 01-04

Antibiotic resistance in aquaculture

NR Chattopadhyay[1], PP Ghorai[2], N Bagra[3]
[1] Department of Biotechnology, Govt. of India, India
[2] Department of Zoology, Vidyasagar University, West Bengal, India
[3] Department of Zoology, Rajiv Gandhi University, Itanagar, Arunachal Pradesh, India

Abstract
Aquaculture is a rapidly growing industry that currently accounts for almost half of the fish used for human consumption worldwide. Intensive and semi-intensive practices are used to produce large stocks of fish, but frequent disease outbreaks occur, and the use of antimicrobials has become a customary practice to control them. The selective pressure exerted by these drugs, which are usually present at sub-therapeutic levels for prolonged periods in the water and the sediments, provides ideal conditions for the emergence and selection of resistant bacterial strains and stimulates horizontal gene transfer. It is now widely recognized that the passage of antimicrobial resistance genes and resistant bacteria from aquatic to terrestrial animal husbandry and to the human environment and vice versa can have detrimental effects on both human and animal health and on aquatic ecosystems. A global effort must be made to cease antimicrobial overuse in aquaculture and encourage stakeholders to adopt other disease prevention measures. Shaping a new path is crucial to contain the increasing threat of antimicrobial resistance.

Keywords: aquaculture, antimicrobials, antimicrobial resistance, horizontal gene transfer, one health

Introduction

Antibiotic resistance became an issue with the advent of bactericidal agents such as antiseptics and disinfectants, which were in use long before the actual antibiotics were discovered. The major problems associated with antibiotic resistance though have come about recently, the rise of antibiotics started in the 1930's. With the discovery of sulphonamides and penicillin, which were relatively easy to produce and effective, antibiotics became common as cures for previously dangerous diseases such as Pneumonia. The more antibiotics were used, the more bacteria became resistant to the antibiotics, and over the decades the practice of using antibiotics subsequently made more bacteria becoming resistant to many drugs. Many factors that contribute to the rise of resistance include repeated and over use of antiseptics and disinfectants and overall misappropriation of the medicine, improper use or prescription of antibiotics. Fortunately, many institutes, namely CDC, the FDA, the USDA and NIH, are working towards finding a solution. Again, a draft "Public Health Action Plan to Combat Antimicrobial Resistance" has been framed by some leading Institutes which clearly uphold the role of government to fight antibiotic resistance. Public Health Action Plan to Combat Antimicrobial Resistance (Action Plan) was developed by an interagency Task Force on Antimicrobial Resistance that was created in 1999.The Action Plan provides a blueprint for more specific and coordinated federal actions to address the emerging threat of antimicrobial resistance. The primary goal was to promote the development and use of new and existing AR products that reduce the risk of the development and transfer of antimicrobial resistance to humans, as well as new approaches to reducing agricultural and veterinary use of antimicrobial drugs.

One of the most important factors of an antibiotic or disinfectant is that it targets both the bacterial and host cells. There are two main types of antibiotics:

i) Bactericidal, which kills the bacteria and ii) bacteriostatic which keeps the bacteria from multiplying so that the body's immune system can destroy those remaining. Antibiotics may be either a broad spectrum that kills many different kinds of bacteria or they target certain types more specifically. Broad spectrum antibiotics are used when cause of an infection is not known, so the option is to use an antibiotic with broad spectrum effect means that is potent with killing a large variety of bacteria. This carries with it an increased risk developing resistance because as it is not target specific so the non-target one by being resistant will multiply rapidly. In general, saprophytic bacteria are effective than vegetative bacteria in transmitting infection. Again, as the bacteria, gram positive and negative for example, differ in regard to the constitution of cell wall and membranes so differential treatment is required specific type of bacteria. This is because primary interactions with antibiotics occur with outer membranes.

Mechanism of Resistance

Antibiotic resistance develops with the initiation of a mutation, either in the chromosomal genes of bacteria or in its plasmids. Resistance develop out of chromosomal genes is called intrinsic resistance and that develop due to plasmids is called acquired resistance. When a non-specific antibiotic is used to kill whatever bacteria are available i.e. both

pathogenic and non-pathogenic, eventually some will develop resistance to it. This is why broad-spectrum antibiotics are more dangerous because it increases the chances of natural selection to occur. If the rest of the bacteria are killed off than these bacteria will be the one to reproduce and so it's genes or plasmids will be spread (If it a plasmid that has this mutation it is called an R- plasmid). This might not be a problem the first time a person or animal has the disease because the bacteria might be killed off, or the mutated bacteria might not be a pathogen, but eventually the resistant bacteria will survive and multiply.
Microbes resist antibiotics in many ways through their adaptations. Some alter the ability of the antibiotic to be absorbed in to the bacteria by decreasing the permeability of the plasma membrane, altering the receptor sites, or developing pumps that are able to move the antibiotics out of the cell. Others create enzymes that break down the antibiotics or change the metabolic pathway that was affected.
With greater understanding of the mechanisms by which bacteria acquire resistance to antibacterial agents and more information on the high-risk procedures with in aquaculture practices, fish health promotion strategies can be developed to prevent and control disease with the minimum application of drugs, use to maximum effect.
Resistance arises in bacteria either by chromosomal mutation or by the acquisition of plasmids. Chromosomal mutations are alterations in the bacterial chromosome which cause resistance that can be passed on to daughter cells on cell division. Plasmids, being molecules of extra chromosomal DNA, can replicate independently of the chromosomal DNA and may be passed to the daughter cells but also to other bacteria of the same or even different species. Both this mechanisms are important in aquaculture- affecting different groups of widely used antibacterial agents.
P=Plasmid
C=Chromosome

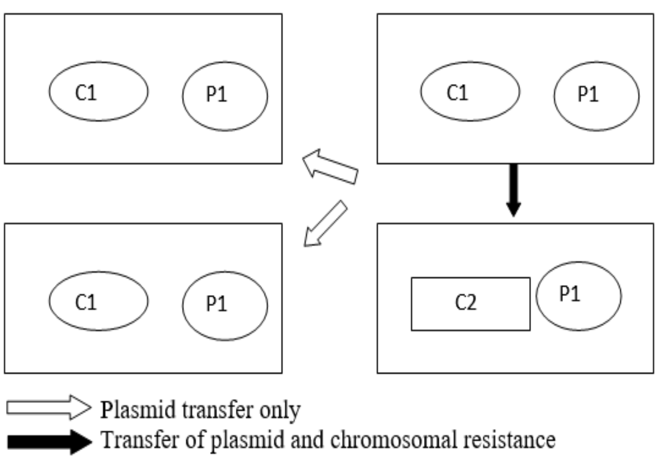

Fig 1: Plasmid mediated antibiotic resistance and through chromosomal mutation

Chromosomal mutation to resistance can be passed subsequently only to daughter cells, whereas plasmid encoded resistance can be passed to daughter cells, transferred to other bacteria within the genus and to species in other genera.

Plasmid Mediated Resistance
In addition to the nucleoid, many bacteria often contain small non-chromosomal DNA molecules called plasmids. Plasmids usually contain between 5-100 genes. Plasmids are not essential for normal bacterial growth and bacteria may lose or gain them without harm. They can, however, provide and advantage under certain environmental conditions. For example, under normal environmental growth conditions, bacteria are not usually exposed to antibiotics and having a plasmid coding for an enzyme capable of denaturing a particular antibiotic is of no value. However, when the bacterium is exposed to particular antibiotic then the plasmid coded enzyme help the bacteria to survive and grow by overpowering antibiotic.
Plasmids are small molecules of double stranded, helical and non chromosomal DNA. Plasmids code for synthesis of a few proteins not coded for by the nucleoid. For example, R-plasmid, found in some gram-negative bacteria, often have genes code for both productions of conjugation pilus as well as multiple antibiotic resistance. Through a process called conjugation, the conjugation pilus enables the bacterium to transfer a copy of the R-plasmid to other bacteria, making them also multiple antibiotic resistance and able to produce a conjugation pilus. In addition, some exotoxins, such as the tetanus exotoxin and *Escherichia coli* enterotoxin are also coded by plasmids.
At present, therefore, it is not possible to evaluate the significance of the presence of plasmids in fish pathogens in the emergence of resistance. Nevertheless, there is little direct evidence that plasmid-mediated transfer of resistance is driven by use of drugs in aquaculture. Study indicates that 11 out of 40 isolates of *Aeromonas Salmonidae* carry plasmids encoding resistance to oxytetracycline which could be transferred.

Chromosomal Resistance
Chromosomal resistance is particularly important among the quinolone group of drugs and here, there have been no reports of plasmid involvement. Oxolinic acid (Anti-bacterial to gram negative bacteria) is generally used when fish die for no apparent reason and when other medications are not helpful, works well for Koi affected by Aeromonas (hole in the side) when applied by mixing with feed. It has widely been used in the U.K. along with flumequine, possess fluorine in position 6 and is a so-called second-generation quinolone. The fluorination increases antibiotic potency and activity against gram-positive bacteria.
In general, the bioavailability in fish is higher for flumequine than for oxolinic acid. In Atlantic salmon the bioavailability ranges from 38 to 47% (Martinsen, 1993) with corresponding values of 31% in halibut (Samuelsen and Ervik, 1997), 65% in cod (Hansen and Horsberg, 2000) and 59% in turbot (Hansen and Horsberg, 1999).
in France and resistance is now frequently encountered. Mutations to resistance occur readily in *Aeromonas salminicida* cultured in the laboratory in the presence of oxolinic acid. This may also be associated with resistance to both oxytetracycline and oxolinic acid where it is postulated as being due to chromosomally induced changes in the permeability of bacterial outer membranes.

Aquaculture and Anti Biotic Resistant Bacteria

Antibiotic is used as feed additives in animal husbandry generate populations of resistant bacteria in the animal gut. These are introduced via slurry in to the ecosystem soil. The influence on the ecosystem soil does not seem significant, due to inactivation and dilution slurry and soil, and the probability that this pathway contributes to the problems of antibiotic resistance in human medicine is viewed as low. No evidence could be found that residuals of antibiotics in slurry have a negative influence on the ecosystem soil, due to preliminary results obtained by similar field trails with disinfectant. The application of antibiotics in aquaculture should be regarded more critically.

Strains of *Aeromonas hydrophila* isolated from skin lesions of the common fresh water fish, *Tilapia mossambica*, were screened for the presence of plasmid DNA and tested for susceptibility to 10 antimicrobial agents. Of the 21 fish isolates examined, all were resistant to ampicillin and sensitive to gentamycin. Most isolates were resistant to streptomycin (57%) tetracycline (48%) and erythromycin (43%).

Antibiotic Resistance in Bacteria Isolated From Hatchery-Reared Larvae and Post-Larvae of *Macro Brachium Rosenbergii*

Recently, intensive interest has been generated to culture giant fresh water prawn, *Macrobrachium rosenbergii*, because of minimum risk and assured market demand. In the hatchery, the antimicrobials such as ciprofloxacin (2-5mg/l), oxytetracycline (5-10mg/l), nitrofurantoin (5-10 mg/l) or formalin (5-25mg/l) were being administered by immersion method to control the bacterial load. The potential consequences of antibiotic use in the treatments are the development of antibiotic resistant microorganism, multiple antibiotic resistance, resistance transfer to pathogenic bacteria and reduced efficacy of antibiotic treatment for diseases caused by resistant pathogens.

The objectives of the present study are to determine the antibiotic resistance in bacteria isolated from larvae and post larvae of *M. Rosenbergii* and to find out the alternatives for the antibiotics. The bacteria associated with larvae and post larvae of *M. Rosenbergii* showed fluctuations in number on different larval stages. Vibrio sp. have been found to be a serious problem in the hatcheries and farms of marine shrimp and fresh water prawn. It is, therefore, necessary to control the bacterial population of larvae and post larvae in the hatcheries to minimize the danger of bacterial infections. Use of antibiotics, a hypochlorite solution, an idophore and formaldehyde have all been found to be effective in controlling the bacterial infection of crustaceans, more than 90% of the bacterial isolates isolated from the larvae and post larvae of fresh water prawn showed resistance to erythromycin, oxytetracycline, and furazolidone. The present investigation disagrees with the prophylactic use of antibiotics in the aquaculture systems because of the development of the resistant bacteria.

Based on MIC (Minimum Inhibitory Concentration) data, formalin was found to be effective to inhibit bacteria isolated from larvae and post larvae of *M. Rosenbergii* at the concentration of 50 mg/l. Hence, the present study strongly recommends the prophylactic use of formalin to control bacteria and also fungus.

Antibiotic Resistance in Bacteria Isolated From Artemia Nauplii and Efficacy of Formaldeyde to Control Bacterial Load

Artemia nauplii form an important live feed for a variety of fin fishes and shell fishes and are given to over 85% of aquaculture species around the world. Careless use of this live food organism may be responsible for the development of disease and mass mortalities in larvae of fishes and shellfishes. Artemia nauplii vary a heavy bacterial load and some bacterial species have been reported to be the source of infections and high mortalities in fish larvae and live food organisms are thought to be responsible. In the present study, the total number of aerobic heterotrophic bacterial flora ranged from 3.8 X 10^3 to 8.1 x 10^3 CFC/ nauplius on sea water nutrient agar and 9.4 x 10 ^{2} to4.3 x 10^3 CFC/ nauplius on TCBS agar. It is therefore necessary to control the bacterial population of *artemia nauplii* to minimize the danger of bacterial infection before their use in aquaculture. The effects of chemotherapeutants, ultraviolet irradiation treatments and freezing have been investigated to minimize the danger of bacterial infections associated with feeding live food. Use of antibiotics, a hypochlorite solution, an iodphorand formaldehyde have all been found to be effective in suppressing the bacterial flora of artemia nauplii. A wide range of antimicrobial compounds such as chloramphenicol, oxytetracycline, kenamycine, nifurprizine, oxolinic acid, sodium nifurstyrinae and flumequin is now being used in aquaculture. In the present study, the common antibiotic used in the aquaculture systems were tested against the bacteria isolated from *artemia nauplii* for their resistance and the results showed that more than 60% bacteria were resistant to erythromycin, nitrofurazoneand oxytetracycline, and 43% to chloramphenicol and 21% to tetracycline. The prophylactic and use of antibiotics drying larval rearing, including disinfecting livefeed, results in increasing the frequencies of antibiotic resistant bacteria in a aquaculture system. Formaldehyde and sodium hypochlorite were tested for their efficacies to reduce the bacteria associated with *artemia nauplii.* It is evident that chloramphenicol (150 mg/l) erythromycin (200 mg/l), oxytetracycline (200 gm/l) or tetracycline (100mg/l) could inhibit the growth of bacteria associated with artemia nauplii; whereas formaldehyde could eliminate the bacteria at the concentration of 50mg/l). This concentration of formaldehyde was found to be less toxic to *artemia nauplii.* Lower concentrations of formalin are effective for prophylactic control of fungus. Based upon these observations, the present study strongly recommends the use of formaldehyde to disinfect live feed before introducing in to rearing system.

Problems Arising From Antibiotic Resistance

Resistance affects all of us and is particularly a problem when it comes to hospitals where people are already vulnerable because of whatever illness put them there, and also to live stock and plants because many of the antibiotics used on human are used on them too, which increases the changes of bacteria developing resistance. This antibiotic resistance may cause economic losses as the outbreak of different diseases may not be treated efficiently. Control of bacterial growth is known to vary with the antibiotic, and no one antibiotic would be expected to inhibit all bacteria under most culture conditions. Chloramphenicol, another widely used antibiotic in aquaculture system, is extremely toxic and it has been shown to breakdown in light to form cytotoxic compounds that affect symbiotic algae and their hosts.

Antibiotic resistance is reported widely in the scientific literature for all the fish pathogens with comment that the incidence and the extent of this is increasing. To solve these problems, fish vaccines can significantly reduce specific disease related losses resulting in a reduction of antibiotic use. The final result is the decrease of overall unit costs and more predictable production. Fish vaccines have advantages over use of antibiotics because vaccines are natural biological materials that leave no residue in the product or environment and will not result in increases in antibiotic resistant strains of disease organisms. Even though commercial vaccines for aquaculture work really well in terms of protecting the fish against certain diseases, they should be used only as part of the overall health management programme.

Recent studies indicate that Streptococcus, Leuconostoc, Lactobacillus, and Carnobacterium are part of the normal microbiota of the gastrointestinal tract in healthy fish. Pathogenic strains of such lactic acid bacteria as Streptococcus, Enterocossus, Lactobacillus, Carnobacterium and Lactococcus are also found in various organs. Disease caused by these organisms seems to spread with the development of fish culture. Antibiotic treatments and vaccinations have been proposed to cure or prevent these diseases. It has also been reported that some lactic acid bacteria isolated from the gastrointestinal tract of fish can act as probiotic. These candidates are able to colonise the gut, and act antagonistically against gram negative fish pathogens. These harmless bactriocin producing strains may reduce the need to use antibiotics in future aquaculture.

Conclusion

Even with a greater understanding of the husbandry practices necessary to control outbreaks of bacterial disease, and the use of vaccines where they are available, antibacterial agents are still essential in aquaculture. Treatment failures are reported but less well documented. In addition, there is growing public concern about risks to consumers ingesting drug residues and about effects on the environment. At present, there is little hard evidence of human health problems arising from eating aquaculture products with residues of antibacterial. Although allergic responses have followed the consumption of if milk and some meats containing antibiotics, such reports as do exist have implicated residues in fish.

Against this background, the present review sets out to evaluate problems associated with the use of antibiotics in aquaculture to ascertain their extent and origin to consider how disease control practices might be modified to ensure maximum prolonged benefit of the antibacterial drugs available to aquaculture.

Acknowledgement

Author is thankful to the Dept. of Biotechnology, Govt. of India, for providing fellowship and necessary funds to complete the work

References

1. Aquaculture and Biotechnology- Iddya Karunasagar, Indrani Karunasagar, Alan Reilly
2. Aquaculture Journals
3. www.Google.com.
4. Lunestad BT, Samuelsen O. in Improving Farmed Fish Quality and Safety, 2000.Veterinary drug use in aquaculture