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Antibiotics abuse in aquaculture and Phage therapy - as its potential alternatives

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ARTICLE ID: 033

INTRODUCTION:

Antibiotics are secondary metabolites produced by microorganisms that inhibit or kill a wide spectrum of other microorganisms. It is also called as 'societal drug' and effective only against bacteria. The use of antibiotics in aquaculture and other food production system has found to be drastic impacts on human health and lifestyle. The important aspect before insisting aquaculturists refrain from using antibiotics for different purposes is to show them alternatives for antibiotics. This article imparts documentation on possible alternatives and current research work for the implementation of the use of antibiotics in the arena of aquaculture.

The published information is based on the antimicrobial use (AMU) and AMR in aquaculture. Over the previous decade, aquaculture is expanding rapidly for many reasons of the world, and aquaculture product aggregate as an important food supply with increasing economic importance. The global aquaculture production over the period of 1994 - 2004 is doubled and in countries like Asia which accounted for 80%-90% of total production. As the data says, that the global production of food fish in 2010 was 79million tons, of which 48.19 million tons were produced by China alone whereas countries like India, Vietnam, Thailand, Bangladesh, and Indonesia together counted for 16.59 million tons. According to UN Food and Agriculture Organisation, aquaculture is growing more rapidly than all other animal food production sector (www.fao.org). It's contribution to global supplies of several species of fish, crustaceans, and mollusks increase from 3.9 percent of total production by weight in 1970 to 33% in 2005. It has been calculated that fisheries and aquaculture provides the world with about 110 million metric tons of food fish per year (FAO, State of World Fisheries and

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Aquaculture 2010.), providing per capita supply of 16.7 kg (live weight equivalent) among which 47% is derived from aquaculture production.

Age of antibiotics in aquaculture:

Due to the advancement in the fisheries sector, the use of antibiotics is increasing day by day especially due to the introduction of intensive and super-intensive aquaculture practices in the arena of aquaculture industry. The use of an antimicrobial agent is common in animal production but in the case of aquaculture, it is becoming havoc because in aquaculture these chemically active substances are regularly added to the feed which is then placed in the water where the fishes are kept. Sometimes anti-microbials may be added directly to the water which inhabits the range of micro-ecosystem and micro-biota of microorganisms such as bacteria. The frequent introduction of these chemical substance resulting in the emergence of a reservoir of antimicrobial-resistant bacteria among the finfish as well as shellfish.

Another major concern to avoid the use of antibiotics in aquaculture is, it favours the growth of antibiotic-resistant bacteria in the mean course of the aquatic environment. These bacteria are responsible for a disease outbreak in the case of the human population. Due to intensive use of the antimicrobial agent in feed or aquatic medium, the phenomenon of gene exchange for resistance to antibiotics between bacteria in the aquaculture environment and those bacteria in the terrestrial environment, such as bacteria or pathogens of animals and human pathogens. The procedures of antibiotics application also result in selective pressure in the exposed environments (usually water) which may involve a broad environmental application that affects a wide variety of bacteria. Several bacterial species may survive or adapt to unfavorable conditions or environmental changes after selecting mutations that improve their fitness and survival in the new conditions. Apart from these, bacteria take advantage of mobile genetic elements, such as transposons and plasmids. With these elements, bacteria could access a big arena itinerant gene pool that moves from one bacterial cell to another which could be further spread to the bacterial population.

Mechanism of antibiotic resistance:

The transferable resistance genes from the reservoir of drug-resistant bacteria which is formed due to selective pressure by an antimicrobial agent may disseminate by the

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phenomenon known as horizontal gene transfer and reach human pathogens, or drug-resistant pathogens from the aquatic environment or in adverse conditions it could reach humans directly. The phenomenon horizontal gene transfer may occur in the food chain, in aquaculture environment or in the human alimentary tract. In a general perspective, there are several mechanisms of antibiotics resistance that are readily spread to a variety of different bacterial genera. The microorganism may acquire genes in coding enzymes, while other antibiotic inactivating enzymatic reaction include adenylation, acetylation, and phosphorylation. Thus an important aspect of the huge amount of antibiotics used for fish in aquaculture and farm animals is the selection of pathogenic bacteria that is resistant to a variety of drugs which is also called multidrug resistance in bacteria. Many of the common antibiotics used in aquaculture such as tetracycline, chloramphenicol, etc. are labelled as critically important for use in human by World Health Organisation (WHO). So the horizontal gene transfer puts negative feedback on the therapeutics options on human infections.

Alternatives to antibiotics in aquaculture :

To abate the use of antibiotics and antimicrobials in aquaculture, many traditional and newly introduced alternatives are available which are quite effective on the bacterial pathogen of aquatic medium. These are:

Enzymes and probiotics – Probiotic are defined as "the cultured product or live microbial feed supplement, which beneficially affect the host by improving the intestinal (microbial) balance" or in other words, probiotic treatment is the implementation of beneficial bacteria to replace pathogenic bacteria in the aquaculture systems. It is frequently used in shrimp ponds in India to control the pathogenic bacteria. However, it is important to validate the efficacies of probiotics circulating in the market. Some Gram-positive bacteria used as probiotics such as *Bacillus subtilis*, *Lactobacillus sps.*, *Microbacteriumsps.*, *Streptomyces sps.* etc. while some Gram-negative bacteria used as probiotics are *Agarivoranssps.*, *Enterobacter sps.*, *Pseudomonas sps.* etc.

To facilitate the growth of these beneficial bacteria, prebiotics are needed which act as a medium for the growth of probiotic bacteria. It provides a favourable environment for

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probiotic to proliferate rapidly and change the gut microflora of the intestine such as dietary fibers

Genetic switch– It is the latest trend in which RNA molecule is used to control antibiotic-resistant bacteria by the application of tailor made RNA into the resistant bacteria should down-regulate the resistant mechanism responsible for the bacteria. It is called as riboswitches or riboregulators. In this method principles of RNA gene silencing are used.

Antimicrobial peptides (AMP) – It is responsible for innate defense mechanism produced from the host cell to destroy the invading bacteria/viruses that's why called as host defense peptides. It is less available in the market so research development is concentrated to develop more number of AMP.

Bacteriocins – These have biological protein moiety which shows the bacteriocidal property. The marine environment is the best source for these, which is not explored much. They are non-toxic and non-antigenic to animals moreover it is easily degraded by proteolytic enzymes of GI tract. e.g- Nisin(bacteriocin) used in food industries by US-FDA.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats):-It is used in a novel defense mechanism against bacteriophage by bacteria. CRISPR's and its associated proteins ..i.e.. Cas proteins collectively called CRISPR- Cas system provides a way to alter the the functioning of invading viral genes by binding with the phage gene which matches with CRISPR sequences of the flank section of RNA . Then the Cas enzyme bind with it and the target gene into various fragments.

While it is evolved in bacteria but this system of enzymes could be used in aquaculture too after certain modification by using the custom-tailored genome-editing tool, against disease-causing bacterial pathogen in place of antibiotics. The advantages of CRISPR over antibiotics are :-

- It is specific and target a single bacterial species.

- The anti-CRISPR gene is identified in phage, so it is nearly impossible for bacteria to develop the resistant against it because it could damage its own immune system , a suicidal move.

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Phage therapy – an effective remedy for an antibiotic alternative :

Bacteriophages are defined as viruses that could infect, multiply in and kill susceptible bacteria. They are both cosmopolitan and abundant in the environment, particularly in seawater, in which the total numbers of viruses frequently exceeds the bacterial concentration by a factor of 10. After their discovery in 1915, phages have been studied for their therapeutic properties and ability to control infectious bacteria; however these studies were later rejected due to the introduction of cheap, broad-spectrum antibiotics. Recently, after the introduction of the serious problem in aquatic biota .i.e. bacterial antibiotic resistance, phage therapy has come back as an effective alternative to the use of antibiotics. Till now there are many studies which prove the efficacy of therapeutic and prophylactic effects of phage therapy in animals and humans. There are several benefits of bacteriophages over other therapeutic agents such as :

1) They have specific narrow host ranges, which meansphages do not harm the normal intestinal fauna and microbiota;

2) Phages can self-replicate in the a exclusive bacterial target, which eliminates the need for multiple application and administrations

3) Till now, there is no side effects of bacteriophages is reported.

Phage therapy gives promising result in the field of aquaculture because phages can easily enter the fish body through the skin and gills and its administration through feed will be advantageous for infections in which the oral route is the the primary route for pathogen transmission, as is the case for *L. garvieae* infection of yellowtail and *P. plecoglossicida* infection of ayuphages. It can be detected in the kidney after dipping fish in phage solution. Bath administration of phages will be effective for those fish in which infection is initiated by bacterial colonization on the skin and gills. While administration of phages through is preferred when the oral route is the main path for transmission of infection. Characteristics of phages over antibiotics that supports its therapeutic application is elaborated below via table:

Factors	Limitations of antibiotics	Advantages of bacteriophages
Fate of drug	Metabolic destruction of the functioning molecule in the process	The exponential growth is seen as the therapy moves forward

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Concentration required of the drug	High concentration is required for ideal result	Very low because it is self replicating
Resistance by disease causing bacteria	Antibiotics become outdated over time through mutation.	While phages co-evolve to overcome the bacterial mutation and restricts their proliferation.
Ease of spread of bacterial resistance	It is broad spectrum	While it is host specific and narrow spectrum. It doesn't cross species boundaries

The phage therapy program consists of various steps such as primarily phage isolation from the sample collected from the cattle, poultry and farmed fish which is further deposited in a specialized Phage bank. Here the principles of genetic engineering are applied which is followed by efficacy, characterization, and safety of formed Phages. After all these procedures manufacturing and commercialization is followed. Many studies and reports are upbringing the use of this therapy over antibiotics. One of these is shown below:

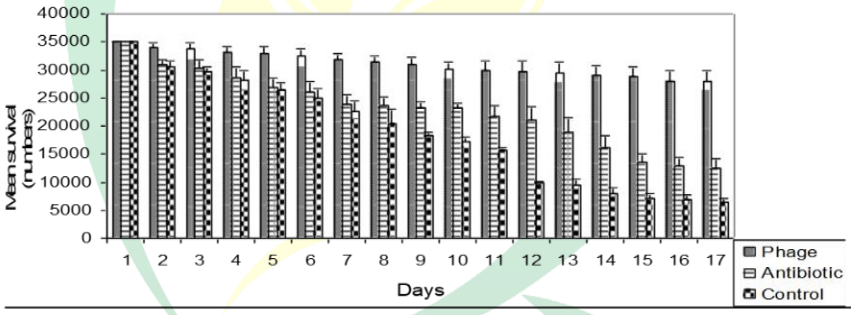

Fig. Mean survival of *Penaeus monodon larvae and standard error for* 3 replicate tanks of 35000 naupli larvae 17 reared for days (from zoea to post larvae) with 2 different treatments (Bacteriophage and antibiotic) and a control.

- **Conclusion :**

There are various reasons regarding the promising result of the phage therapy such as in this process the phages and bacteria are in suspension which is similar to lab conditions. Apart from this, therapeutic phages could have intimate contact with the pathogens of fish, mollusks, and crustaceans, and also as we know that natural phages are evolved to be successful in a liquid medium. These advantages of phages evolve them as a biocontrol agent because it is host specific and once the host population disappears, bacteriophages

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also disappear. Thus, it is harmless to other normal flora, does not affect useful bacteria associated with larvae, animals, or pond. Therefore, the use of phage therapy as an alternative to antibiotics is an eco-friendly management measure and an effective remedy for antibiotic resistance. While in broad perspective we could say that antibiotics need to be replaced with other solutions as antibiotic resistance is a major threat to human health. There are various options mentioned in the article that is being explored for dealing with the issue of finding alternatives for antibiotics. However, while the iron-clad solutions are being worked on, it is imperative that the farmers and the stakeholders employ BMP's in the meantime to control any problems in the pond without restoring the antibiotic usage.