IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 9, Issue 4 Series I (July. – August. 2023), PP 42-53
www.iosrjournals.org

Therapeutic Application Of Bacteriophages Against *Aeromonas Spp*. Mediated Diseases In Aquaculture: A Critical Review

Mrunalini Sonne[1*], Nivedita Ghayal[2], Sangeeta Ahiwale[3]
[1*]*Annasaheb Kulkarni Department of Biodiversity, MES Abasaheb Garware college, Pune- 411004, Maharashtra India*
[2]*Department of Botany MES Abasaheb Garware College, Pune- 411004, Maharashtra India*
[3]*Department of Microbiology RSS Mahatma Phule College, Pimpri Pune 411017, Maharashtra India*

Abstract
Aquaculture uses a variety of broad spectrum antibiotics to manage and prevent a variety of diseases, without understanding their mechanisms of action. This has led to water pollution in the modern world. The necessity for alternate control measures against bacterial illnesses in the aquaculture sector is highlighted by issues brought on by antibiotic-resistant bacteria and the dearth of effective control strategies. Bacteriophages (phages) have shown promise as therapeutic agents for the efficient management of bacterial infections in aquaculture. In the current study, a variety of investigations were conducted to determine if utilizing lytic phages to reduce Aeromonas spp. infection in fish aquaculture was appropriate. Motile Aeromonas septicaemia is a fish disease that has caused financial harm to the aquaculture sector. The best way to avoid infectious illnesses is immunisation; however there are still relatively few vaccinations that are commercially accessible in the aquaculture industry. Currently, the production of aquaculture depends significantly on antibiotics, which adds to the worldwide problem of the rise of bacteria that are resistant to medicines and resistance genes. To decrease the usage of antibiotics in aquaculture systems, it is crucial to create efficient antibiotic substitutes. A potential strategy to manage harmful microorganisms in farmed fish is bacteriophage (or phage) treatment. In order to successfully use phage treatment to reduce infection in fish caused by Aeromonas, further phage therapy research in aquaculture is needed, according to this study. The importance of phage as biocontrol for aquaculture is encompassed in this review.
Key word*: Aquaculture, Aeromonas, Bacteriophage, Fish.*

Date of Submission: 08-08-2023 Date of Acceptance: 18-08-2023

I. Introduction

The aquatic genus Aeromonas, which is frequently isolated from environmental and clinical samples and is thought to be native to such habitats. Freshwater like lakes, rivers, reservoirs and groundwater, chlorine-treated and untreated wastewater, brackish water, seawater, swimming pool water, reclaimed and drinking water are typical habitats for these bacteria. Aquaculture's economics is impacted by a number of illnesses that *Aeromonas spp.* may occur in marine fish species, farmed freshwater and wild species. Numerous illnesses have links to different types of water exposure, leech therapy (since they have a symbiotic connection with certain germs), or consuming tainted water or food. Epidemiological links between strains found in drinking water and episodes of human diarrhoea have lately been made. The pollutants in most environmental contamination situations impact a number of fish organs. Since they are simple to spot, non-specific lesions on the gills, skin, and fins are frequently utilized as signs of polluted surroundings[1]. Particularly in agricultural settings, some farmer practises may unintentionally expose fish to toxins over short or extended periods of time, which may be extremely dangerous for both the fish and the fish consumer gave an intriguing example when he documented deaths brought on by the usage of net antifoulants that included dangerous copper compounds.[2] The severity of the corresponding disorders can be significantly influenced by certain biotic or abiotic causes. For instance, stressed fish often seem more vulnerable to environmental contamination[3]. Many authors stated that the study of those impacts is indicative of the overall quality of the environment since the effects of the majority of contaminants on fish and, generally, all aquatic creatures, appear to be non-specific. The majority of contaminants tend to havenon-specific impacts on fish[4].

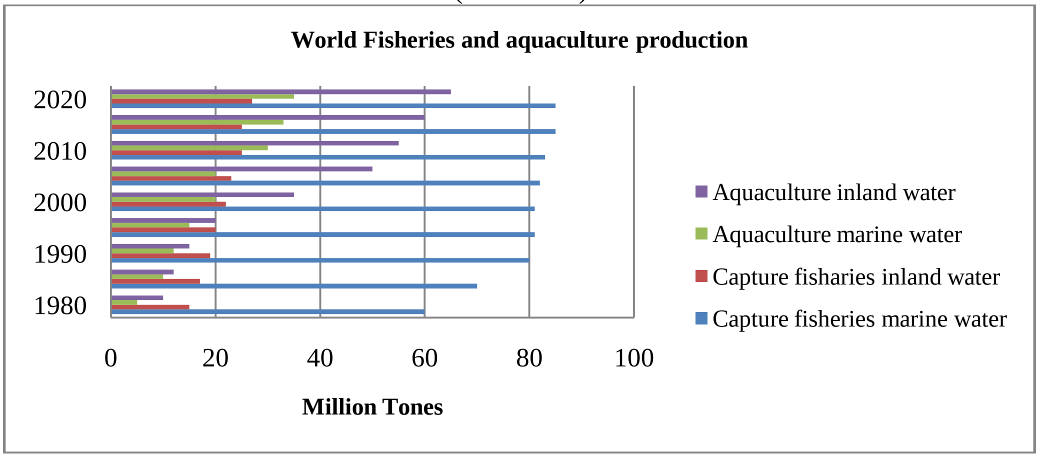
Disease presents a serious hurdle to aquaculture in India, as it does in many other countries throughout the world, and a constraint on societal and economic development. The future growth of the sector has also been seriously harmed by several illnesses, which have also seriously harmed the livelihood of fish farmers. Several illnesses affecting modern aquaculture have high stocking densities and also emerged and stressful conditions that promote the occurrence and spread of infectious disease.[5] This is because aquaculture methods have been intensified without a fundamental understanding of the complex interactions between environment, host, and pathogen. Fish and shellfish consumption has increased along with the country's growth into intensive and semi-intensive aquaculture production systems. Diseases in shrimp culture were believed to have cost the industry $1 billion in total[6]. Higher morbidity or mass mortality, as well as lower production, have frequently been caused by parasite, viral, and increasing bacterial disease incidence because of the vertical expansion of fish culture with different increased stocking density and species.[7] Farmers in rural areas with little resources and little to no expertise in managing fish health are the ones that suffer the most and suffer significant economic losses. It is well known that the intricate interactions between the environment the host, and the pathogen, lead to the development of illness.[8] Many stresses, a high microbial load, poor water quality, high stocking densities, and malnutrition may make it more likely for opportunistic pathogen infections to occur in aquatic habitats.[9]Antibiotic use has most frequently reduced losses; but, continued use of antibiotics has produced germs that are resistant to them, making these therapies less and less effective. Even though antibiotics are often used in many countries, there is a need to transition to more natural treatments instead of probiotic therapy and vaccination. One such treatment is the use of bacteriophages to reduce morbidity and mortality in various aquaculture environments. The term "phage" refers to viruses that attack certain strains or species of bacteria.[10]

Aquaculture industry Production

Aquaculture industry is one of the industries with the greatest growth rates globally. In Asia China, India, Bangladesh, Pakistan, Taiwan and many other countries provide enormous prospects for fishing in both marine and inland waterways due to its extensive coastline region. The nation has the second-largest proportion of the worldwide aquaculture business, mostly because of its 2.36 million hectares of ponds and tanks, 7,500 km of coastline, and 1.1 million acres of brackish water that provide a place for fish farming. There are 28 million individuals in India who make their living from the fishing industry. By 2020, Asia will produce 89% of all fish produced worldwide, according to the Food and Agriculture Organisation (FAO). The output of fish for human consumption worldwide increased to 96.4 million tonnes in 2018, the greatest amount ever. Inland fishing and marine catch were the main contributors. According to Ministry of agriculture Government of India China, Indonesia, Peru, India, Russia, the US, and Vietnam were the top seven main producers, contributing for around 50% of the total world capture output. 7.58 percent of the world's output comes from India. Between 2019 and 2020, the nation's fish output peaked at 14.16 million metric tonnes, an all-time record. The gross value added (GVA) from the fisheries sector is 1.24% while the GVA from agriculture is 7.28%.[11]

In India, fisheries and aquaculture have a big impact on food production by ensuring the nutritional security of the nation's food supply, boosting agricultural exports, and employing around 14 million people in various occupations. The nation has had consistent and sustained increases in fish output since gaining independence because of its various aquatic resources. The aquaculture sector, according to data from the Management of Fishery department of Animal Dairying and Fisheries, is responsible for 6.3% of the world's fish production, 5.15% of agricultural GDP, and 1.1% of GDP. The inland sector of the aquaculture industry in India contributes close to 65% of the total 10.07 million metric tonnes of fish produced, and cultural fisheries contribute similarly. However, the prevalence of disease has become a major barrier to the production and sale of sustainable aquaculture products, impacting the socioeconomic standing of fishermen in nations like India. Infection by opportunistic infections can be brought on by a variety of stress conditions, including insufficient microbiological quality of culture water, poor nutritional condition, and an excessive stocking density, among other physical and chemical factors. It also takes into account the possible difficulties in managing health concerns in aquaculture for sustainable development.

Fig1: The State of the World Series of the Food and Agriculture Organization (FAO) of the United Nations (2020-2021)



Labeo rohita (Rohu), *Catla catla* (Catla), and *Cirrhinus mrigala* (Mrigal) are the three principal species of Indian Major Carps (IMC), whereas three exotic species include *Ctenopharyngodon idella* (Grass carp), *Cyprinus carpio* (Common carp), *Hypophthalmichthys molitrix* (Silver carp). This is done by way of polyculture. Freshwater aquaculture also involves the production of pangasius (*Pangasiandonhypophthalamus*), freshwater prawn *Macrobrachium rosenbergii* (Scampi), air-breathing and non-breathing catfish, and tilapia (*Oreochromis niloticus*; *Oreochromis mossambicus*).(Katiha Pradeep K et,al., 2005). Between 70% and 75% of all freshwater fish are produced using IMC, with the remaining 25% to 30% being made up of common carp, grass carp, silver carp, and catfish[11].

These losses can happen at any point in the grow-out process, including at facilities for larval rearing and hatcheries. The water, contaminated surfaces, feed aerosols, or transmission from one animal to anotherare all potential sources of disease exposure for fish and shellfish. Many illnesses in aquaculture can go untreated until animal stress makes them susceptible to infection since they are opportunistic in nature. Unfavourable pH, temperature, or salinity conditions, or sudden changes in these parameters, insufficient oxygenation, ammonia buildup, overcrowding, excessive handling, overfeeding, and overall poor water quality are among the many stresses that are regularly experienced. According to FAO Fisheries and Aquaculture Dept.,fish and shellfish can get opportunistic illnesses from a variety of germs, and it is important to determine the cause of diarrhea and treat the specific organism. Aeromonas is always present in the environment and provides several chances for human interaction. It was widely known as a cause of soft tissue infections and septicemia in aquatic animals before it was discovered as an etiologic agent in human illnesses.[12]

Aeromonas causing Diseases

Aeromonas is the third most common bacteria in people with diarrhoea after Campylobacter and Salmonella.[1314] Aeromonas defeated Salmonella, According to a study done between 2005 and 2008 in Dhaka, Bangladesh, the most prevalent enteric bacteria were Aeromonas spp. (12.8%), Vibrio spp. (42.9%), Shigella spp. (20.3%), and Salmonella spp. (6.4%).[15] Children's diarrhoea cases in Egypt were examined, and it was discovered that Aeromonas was more common than Salmonella and had an isolation rate similar to Shigella. Aeromonas was, however, only second to Shigella in Iran.[16] However, it was also shown that in Cuba, Aeromonas isolated at a higher incidence than Salmonella.[17] Aeromonas was identified as the predominant pathogen in a prospective matched case control study on diarrheal disease in children that was carried out in Bangladesh and Pakistan. Diarrhoea mortality due to child infections with Aeromonas was expected to be 12.3 in 1990; however, in 2013 it was estimated to be 5.5 (per thousand).[7] Aeromonas instances are sporadic and difficult to follow, with a 10.6 occurrences per million incidence in India and other Southeast Asian nations. Bacterial fish illnesses are highly prevalent, often saprophytic and malnourished, and can lead to high mortality. The prevalence of ulcerative illness in India has also drawn attention to the danger of disease epidemics pose to fish farmers[18].

Bacterial skin disease, also known as red disease, is a common bacterial illness in fish culture. It causes reddish patches on the torso, an abdominal depression, and enlarged eyes. Motile Aeromonas Septicaemia is a significant bacterial illness that affects freshwater fish culture the most frequently and severely. Motile Aeromonas septicaemia can cause superficial to deep skin lesions, significant morbidity, and even rapid death with or without any clinical signs. Skin lesions with variously sized zones of bleeding and necrosis are typically seen at the base of the fins. The death rate often approaches 100% if prompt intervention is not taken[19].

Table 1: Lists the specifics of prevalent *Aeromonas spp*. illnesses in aquaculture that have been documented .[19]

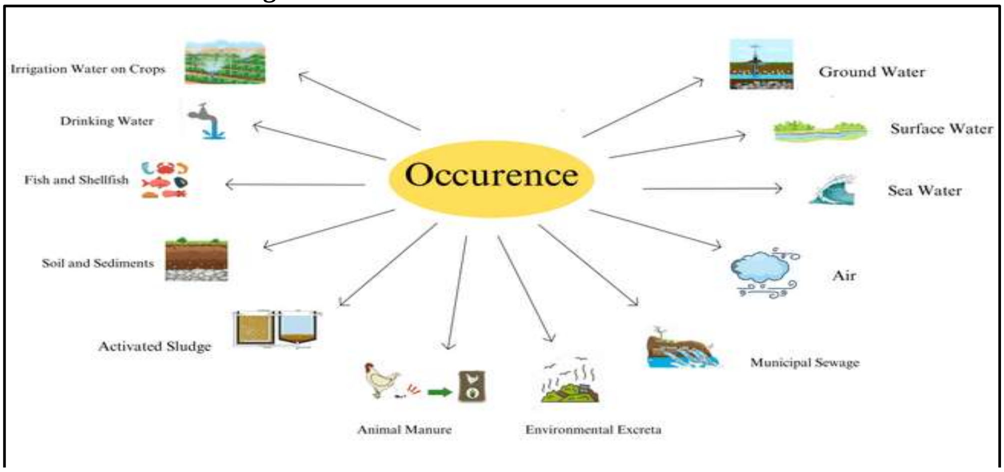
Involvement of Pathogens	Disease Condition	Symptoms
A.hydrophila,	Fin rot And Tail rot	Fin and tail disintegration discolouration, and erosions
Aeromonas hydrophila, A veronii bv. Sobria, A. sobria	Aeromonas septicaemia or Aeromoniasis Motile	Ulcerative lesions on skin fins, and, head, exophthalmia haemorrhagic
Aeromonas liquefaciens	Eye region infection	Cornea, Cataract of eyes, eyeball begins to rot.

In aquaculture three Indian major carps (IMC), which include Catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala)*, exotic carps like silver carp (*Hypophthalmicthys molitrix*), grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), along with catfishes (Clarius bat Exotic catfish, (*Pangasiandon hypophthalamus)*, and pacu, (*Piaractus brachypomus*), culture have both grown over the past few years. In pond culture systems, 3.0 tonnes of fish may now be produced in a cage that is 6 feet by 4 feet by 4 feet, as opposed to 10 tonnes per acre. The development of genetically modified tilapia for sale as a low cost source of protein has been the main emphasis. However, with a production of over 3.25 million tonnes, carp production accounts for the majority of fish production in Indian freshwater aquaculture[11]. Disease is thought to contribute 10-5% of the cost of production[20].

Brief history of *Aeromonas spp.*

Aeromonas spp. have been identified from a wide range of foods, including vegetables, meats, seafood, and dairy products. They are widely distributed in the aquatic environment.[21]Bergey's handbook describes *A. hydrophila, A. caviae, and A. sobria* as motile members of the genus Aeromnas in the family Vibrionaceae.[22] Motile aeromonads are opportunistic infections that cause diarrhoea in human and bacterial septicaemia illnesses in channel catfish and other fish.[23]

Fig 2: Aeromonas Occurrences in Environment[1]



Four Aeromonas species were first recognised: the pathogen *A. salmonicida, A. hydrophila, A. sobria*, and *A. caviae*.[22] Previous research on these four species, it was discovered that they could be divided into distinct biochemical groupings (designated phenospecies) and distinct DNA hybridization groupings which led to variation in species designation and classification, these facultative gram-negative bacteria are found in a lot of foods and water sources. Their contribution to foodborne disease is still up for debate.[23]

Aeromonas hydrophila

Fish pathogenic strains of *Aeromonas hydrophila* were discovered in sewage and surface water alongside sick and healthy fish, according to Heuschmann et al., 1978. According to Kuo et al., 1980 contact-based infection of virulent gliding bacterial strains was more successful than intraperitoneal injection. Additionally, they noted that stagnant water had a greater occurrence of pathogenic strains than did moving water. Injection, oral delivery, and immersion procedures were all shown to result in fatal infections in the experimental fish, according to Muroya et al., 1981 paper. Bang 1983 reported finding a strong inflammatory response followed by mortality after studying bacterial infection with the sickness of colour carp. In cases where sand gobies were injected with *Aeromonas hydrophila* and displayed illness symptoms, he found 50% mortality within 96 hours of inoculation; the majority of the dead fish were also sluggish. Previous research shown 80%

of fish who were artificially infected with *Aeromonas hydrophila* developed illness. In South East Asia, numerous fish species were found to have Epizootic Ulcerative Syndrome in connection with virulent strains of the *A. hydrophila* bacterium.[24]

Aeromonas Salmonicida

Furnunculosis, also known as typical furunculosis, is caused by the rod-shaped gram-negative, facultatively anaerobic, non-motile *Aeromonas salmonicida subsp. salmonicida* bacteria and results in acute or persistent hemorrhagic septicemic haemorrhage. This bacteria has a wide variety of host preferences and may infect both freshwater and saltwater fish in addition to salmonids, as the name suggests. Furunculosis manifests itself in several ways in fish. The most prevalent kind of furunculosis that affects aquaculture causes deaths within a few days [19].

Melanosis, lethargy, anorexia, and haemorrhages at the base of the fins are some signs of this systemic illness in fish. Internal bleeding frequently occurs along the abdominal walls, in the viscera, and in the heart. Older fish are more likely to develop sub-acute or chronic furunculosis, which poses less of a threat in aquaculture. Internally, it results in organ and muscle haemorrhaging. The sub-acute form may manifest externally as reddish fins, bloody navel and vent discharge, lethargy, and protruding eyeballs. Adult fish typically bounce back from sub-acute forms. Additional signs of furunculosis include unique boil-like skin lesions and on the kidneys show white nodules.[25]

Fish disease prevention and control measures in aquaculture

According to relative standards, it is fair to say that controlling infectious diseases in aquaculture is more difficult than controlling diseases in terrestrial animals because of the environment in which fish live and the characteristics of the fish themselves. Fish cannot be observed closely enough, as we can with terrestrial animals, and the environment can quickly facilitate the spread of diseases. The diagnosis of fish disease presents a different set of difficulties from those of diseases in terrestrial animals, where the focus is on the individual animal. Due to the characteristics of the water where fish reside, the situation differs in the analysis of diseases in aquaculture.[26] The development of *Aeromonas spp.* can be impacted by a number of conditions, including low pH, low temperature incubation, nitrite, and salt. It was discovered that tropical-zone suitable methods for fish preservation were successful, but that growth at 5% salt and below 5 C was not possible. At 25–37 ℃, 5% salt inhibited growth, as did a combination of 1000 ppm sorbate; at 37 ℃, liquid smoke only effectively suppressed growth when an initial inoculum of 102 CFU/ml was utilised. At any temperature, it was shown to diminish muscle tissue by 5.6' 0.2.[27]

Washing: Grill carcasses are washed and chilled with water. Significantly decreased *Aeromonas hydrophila*, but refrigeration at 1.1 C for 48 hours significantly increased it.[28]

Oxidizing- When *A. hydrophila* was exposed to oxidising raw ground fluids with high Fe2+ levels (460-1.070 lmol), it immediately dropped by 2-3 log unit.[29]

Smoking: Smoke concentration from a variety of various wood smokes might harm *A. hydrophila*.[30] Cold smoking is one traditional method of fish preservation. rainbow trout that was cold-smoked vacuum-packed, and preserved at 4 C for 21 days., Sunen et al., 2003 examined the antimicrobial activity of four wood smoke condensates.[31]

Probiotics: Carbon dioxide, Lactobacillus casei, and low storage temperature can all work together to reduce Aeromonas hydrophila survival in ready-to-use mixed salad greens. Two *Aeromonas hydrophila* strains by bacteriocin-producing lactic acid bacteria were recovered from retail slices of beef. Aeromonas hydrophila was shown to be inactivated by sodium tripolyphosphate, 2% of any polyphosphate and 3.5% NaCl in BHI broth; this inactivation was temperature-dependent. Palumbo et al.[32]

Heating: In order to inactivate Aeromonas hydrophila in liquid egg, Sheldon et al. (1996) discovered D-values (1.5, 0.10, and 0.03 min) at 51, 57, and 60 ℃. This suggests that such heating treatments can provide a considerable safety factor.[33] Maximum populations of *Aeromonas hydrophila* were found in rice pudding after 22 days at 4 ℃ and after 6–9 days at 12 ℃, according to Papageorgiou et al.'s 2003 study.[34] Tomatoes should be transported, kept, and stocked in stores at low temperatures (6℃), according to research by Velazquez et al. from 1998. *Aeromonas hydrophila* levels should also be reduced by treating tomatoes with chlorine at a 50 ppm concentration.[35]

Alcohol therapy: Therapy for alcohol *Aeromonas hydrophila* loads in or on oysters could not be reduced by 5 ml vodka for 10 minutes or chilling for 7 days at 5 ℃.[36]

Vaccination: In 1942, Cutthroat was the first fish to get a vaccine against *Aeromonas salmonicida* infection. Because there is currently no sophisticated knowledge of fish immunology, traditional vaccinations are being used. There are injectable, oil adjuvant vaccinations available. One of the major problems in the upcoming years will be the development of vaccines against viral and intracellular bacterial infections. In such circumstances, a DNA vaccination will be useful.[26]

Antibiotic: Antibiotics are made to stop the spread of harmful microorganisms and to destroy them. Typically, they disrupt cell membranes, interfere with protein or DNA production, or reduce enzyme function to exert their effects. Because of their specific toxicity to ribosome activity, cell membranes, or enzyme activity in prokaryotic cells, substances having antibiotic action are chosen for use in human and veterinary medicine. The typical procedures employed in the aquaculture, especially in underdeveloped nations, where a lot of antibiotics have been used to prevent illness.[37]

Challenges with fish disease control measures:
Antibiotic resistant pattern among Aeromonas strain

All Aeromonas isolates from the soil samples tested positive for resistance to beta-lactam antibiotics, according to the study's findings (ampicillin and penicilin). This resulted from the isolates' possession of -lactamase enzymes[38]. In their investigation of antibiotic resistance among Aeromonas isolates from water and stools, Ramalivhana et al. (2010)discovered that all tested isolates, 100 percent of them, were ampicillin-resistant.[39] However, all of the Aeromonas isolates used in this investigation were Gentamicin-susceptible, with the exception of 3 (5.7%) sea cucumber isolates. This was not the case with the Jalal et al., 2010 reported[38]. In certain research, it was discovered that isolates of *Aeromonas hydrophila* from water, food, clinical samples, and other sources did not often respond to antibiotics. The antibiotics neomycin, sulfamethoxazole, trimethoprim/sulfamethoxazole, chloramphenicol, and streptomycin were all effective against *Aeromonas hydrophila*. All isolates were resistant to tetracycline, streptomycin, ampicillin, bacitracin, and penicillin; sulfisoxazole, kanamycin, gentamycin, erythromycin, nalidixic acid, and neomycin, on the other hand, were sensitive.[40]Antibiotic resistance was tested in 80 *Aeromonas hydrophila* isolates from 238 channel catfish fillets.[41] Oxytetracycline, sulfamethoxazole, chlortetracycline, neomycin, chloramphenicol, and oxytetracycline were the most often resistant antibiotics for the majority of the isolates. Aquaculture has a major impact on motile aeromonads' antibiotic resistance, particularly *Aeromonas hydrophila*. Antibiotic resistance varied among the 319 strains of *A. hydrophila* found in fish and prawns. Chloramphenicol rendered all strains susceptible, whereas novobiocin, methicillin, rifampicin, and bacitracin rendered them resistant. The presence of *Aeromonas spp*. was checked in these samples. 11.5% *Aeromonas hydrophila* and 69% *Aeromonas spp*. were found in the samples that were examined.[42]

The Food and Drug Administration has recognised a number of "low regulatory priority aquaculture medications" as novel animal medicines with low regulatory priority. Aquaculture has been prohibited from using certain medications, chemicals, and antibiotics, as well as items with applications to human health. Marine Product Export Development Authority and the Coastal Aquaculture Authority have also prohibited the use of a variety of pharmaceuticals, chemicals, and antibiotics in aquaculture in accordance with a notification from the Government of India.

Bacteriophage therapy

The two main categories are phages that are lysogenic and lysogenic. Lytic phages replicate phage components like the nucleic acids and capsid proteins using the host to infect host bacteria by attaching to the bacterium, inserting the phage genome into the host cell, preventing the synthesis of host components, lysing the host, assembling new phage particles, and releasing progeny phage. Lysogenic phages are perfect for the creation of phage therapies for use in treating animal ailments and decreasing infections in various foods and the environment because they replicate fast and cause the host to die and lyse.[43] One potential substitute is bacteriophages, naturally occurring bacterial viruses that may destroy particular bacteria without leaving behind any chemical traces and without affecting other flora.

The efficacy of phage therapy is comparable to that of chemotherapy because it induces bacteriolysis through a mechanism that is completely different from that of antibiotics[44]; it has a high host-specificity toward bacterial pathogens, making it impossible to replace microbes; and it has an immediate counteraction like a mutation. From an economic standpoint, the cost of phage therapy is much, much lower than the dosage of an antibiotic; there have been no instances of phage and phage products, notably lysin, having negative effects on eukaryotic cells.

Since antibiotics may kill the desired bacteria, disrupt the host's normal flora, and upset the biological balance of the aquatic environment, they are crucial for both disease prevention and treatment. Due to the widespread use of antibacterial drugs in aquaculture and drug residues such vancomycin, ampicillin, cephalothin, and colistin, *A. hydrophila* strains are becoming multidrug resistant (MDR). To combat this, novel antimicrobial medicines are needed, such as phage multiplication by auto-"dosing". A modest dosage of phage can have a positive therapeutic impact, as shown by a prior study which showed that mice intraperitoneally injected with the phage KEP10 could quickly spread to all organs and decreased mortality rates of mice transurethrally injected with a pathogenic strain of *Escherichia coli* that is multidrug resistant. [45] Phage treatment has been shown to be effective in some cases of Aeromonas disease cases, with pAh1-C and pAh6-C being the most effective. However, not all phages are useful as medicines, and the effectiveness of therapy will be decreased due to phage supplementation. Pave stability and restricted host ranges of phages must be isolated for phage treatment.[46]

Challenges with using phages as bio-control agent in aquaculture
Multiplicity of Infection (MOI)

The efficiency of phage therapy is significantly influenced by the MOI value. It varies depending on the diseases infected animals have and the phages utilized in in vivo experiments. Initially identified as the initial target for phage treatment in aquaculture in 1981, *Aeromonas species* are now the third most frequently investigated aquatic microbial disease in phage application research. [47] *A. hydrophila* phages were obtained by researchers, with AH1 being chosen to study the biological control of illness among the loach *M. anguillicaudatus*. Three hours after giving *A. hydrophila* to an infected *M. anguillicaudatus* loach, researchers saw that the bacterium's ability to kill animals and spread infection had totally vanished. Even at a MOI of 0.001, *A. hydrophila* mortality and infectiousness were reduced to 40% .[47] Low titres are preferred for the production and widespread commercialization of phage products in order to lower the costs of purification, preparation, and application. *A. hydrophila* was treated with phage treatment in unfiltered fish pond water. Within 8 hours, 99% of the strain of *Aeromonas hydrophila* in the water had been eliminated when the number of cases of infection (MOI) was 0.23. Phage treatment provided a protective effect against *A. hydrophila*-induced mortalities in the loach, whether it was administered by injection or diet.[48] The outcomes showed that the phage treatments had no negative impact on the fish. Another factor that can increase efficiency is increasing the MOI of the phage inoculum.[49] The percentage of the number of phage to the number of bacterial hosts should not be larger than 1 in order to determine the MOI value, one-step growth curve, or ratio. If the multiplicity of infection is 1, it means that there are equally many phages and host bacteria. It is necessary to have a precise host cell density in order to calculate MOI value. More thane10 MOI might be a reduction of pathogen load. [50]

Phage stability

The most critical element to achieving effective phage-mediated control of harmful microbes is making the right viral selection. When making and choosing phage suspensions for phage therapy, It is crucial to take into account variables such as host range, adsorption rate, lytic activity, traits of growth (burst size and latent duration), bacterial inactivation efficiency, safety, and environmental stability.[49] There is scant evidence of negative phage immune responses, and phages are not toxic. Even so, it's crucial to maintain the purity and absence of bacterial elements in phage preparations. When selecting phages for therapeutic usage, primarily virulent strains should be isolated from environmental sources.[51]Temperate phages shouldn't be used in phage treatment due to the host's capacity to create immunity against similar or identical phages.[52] The genome may be searched for enzyme-coding genes to identify lysogenic conversion. It is also necessary to assess and take into account a phage's potential to spread harmful phage components, such as pathogenic genes, between bacteria. If phages are to be used as therapeutic drugs, those with genes linked to lysogenic conversion or other possibly detrimental genetic traits should be ruled out from further study.[52]

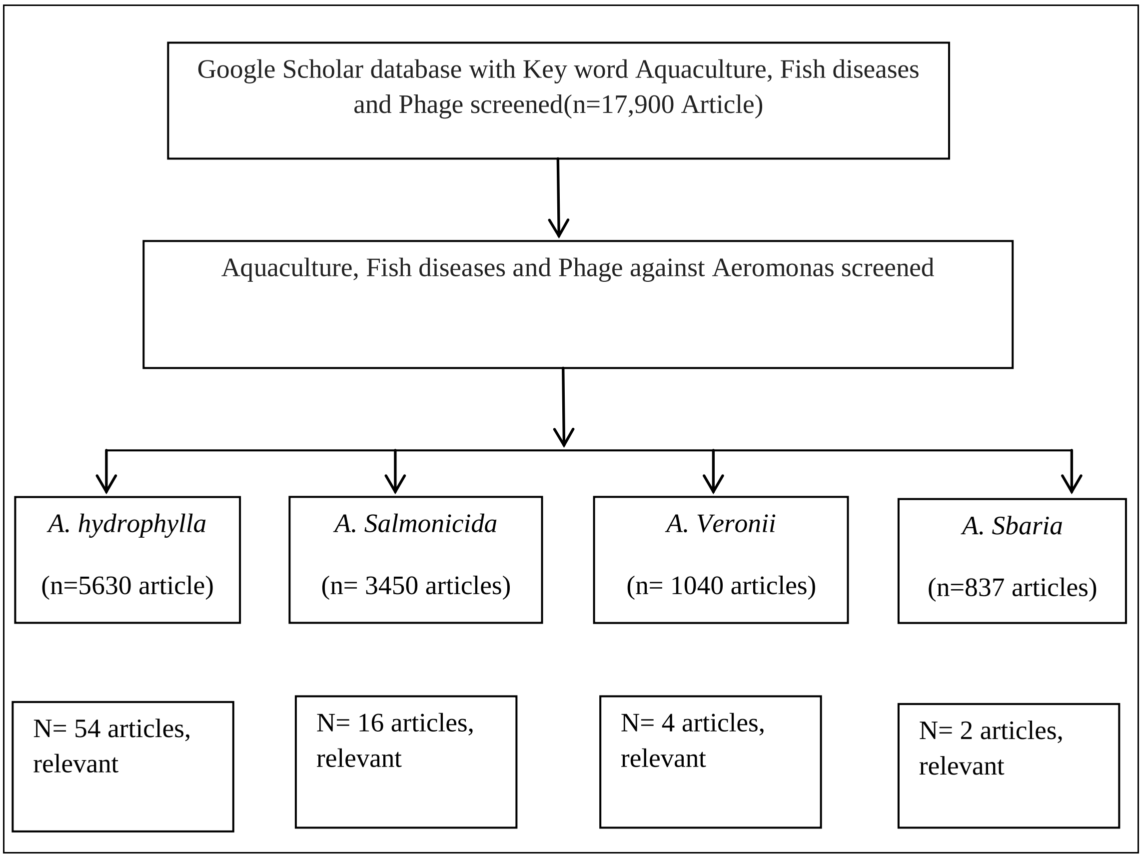
Environmental impact on phage's

Phage treatment may harm the ecosystem by disrupting the aquaculture system's microbiota. By limiting the density of specific bacteria in a given environment, phages alter the ratios of various bacteria in a population. Phages cause bacterial cell lysis, which releases organic molecules, having a profound impact on the organic matter cycle across the whole biosphere.[51] Knowing these traits is essential since the aquatic environment encourages fast proliferation and acts as a phage vector.[53] The likelihood of bacterial populations in the environment being affected can be reduced with lower phage doses. If injected in little concentrations, phages might not be able to effectively suppress harmful microorganisms. Phage growth and reproduction, on the other hand, might occur outside of the planned aquaculture system. Before using them on an industrial scale, it is crucial to look at their effects on the treated microbial community, even if they are harmless.[54] Previous researcher[80] discovered that phage AS-1 (*A. salmonicida* phage), despite having a wide range of hosts, had little impact on the composition of the bacterial population in an aquaculture system. Not the organic makeup of the bacterial community, but the bacterial population present in the fish's digestive tract.[55]

Phage research on Aeromonas infection in aquaculture

There have been conflicting outcomes in studies on the use of phages for preventing diseases in aquaculture, with some bacteria proving to be easier to control than others. It is evident that research in this field is still in its earliest phases when one takes into account the overall number of fish diseases and the large variety of fish species used in aquaculture.

Fig 4: A flow diagram showing research on Bacteriophage against Fish pathogen Aeromonas from 2000 to 2022 in systematic review. (Google scholar and Pubmed).



Utilization of *Aeromonas sp.*-infecting Phages in Aquaculture

Numerous studies have now examined and demonstrated the benefits that various Aeromonas phages provide as replacement biological control agents and as therapeutics in aquaculture. All investigations employing phages to prevent or eradicate *Aeromonas spp*. in aquaculture in to this point have concentrated on both of the pathogenic bacterial species *A. salmonicida* and *A. hydrophila*.[48]

Table 2: Involving research on phage therapy in fish and shellfish.

Sr.no	Country	Bacteria	Bacterial Strain	Phage	Fish	Author
1	China	*A.hydrophylla*	A18	Ahy-Yong1	Brocade Carp	Lingting Pan et al.,(2022) [56]
2	Georgia	*A.hydrophylla*	GW3-10.	AhMtk13a	Zebra fish (Danio rerio)	Nino Janelidze et al.,(2022)[57]
3	Spain	*A. molluscorum*	JF3328	Ah-7	Bivalve mollusk	Gabrielle.R Leduc et al.,(2021)[58]
4	Spain	*A. veronii biovar sobria*	JF3071 (ATCC 51106)	Ah-7	Infected fish	Gabrielle.R Leduc et al.,(2021)[58]
5	Iceland	*A. salmonicida subsp. Achrom o genes*	JF2997	Ah-7	Atlantic salmon	Gabrielle.R Leduc et al.,(2021)[58]
6	Iceland	*A. salmonicida subsp. Achrom o genes*	JF3115 (ATCC 19261)	Ah-7	Brown trout	Gabrielle.R Leduc et al.,(2021)[58]

7	Scotland	*A. salmonicida subsp. Achrom o genes*	JF3116	Ah-7	Trout	Gabrielle.R Leduc et a.,(202l)[58]
8	Iceland	*A. salmonicida subsp. Achrom o genes*	JF3499	Ah-7	Atlantic cod (Gadus morhua)	Gabrielle.R Leduc et al.,(2021)[58]
9	Japan	*A. salmonicida subsp.masouci da*	JF3118 (ATCC 27013T	Ah-7	Salmon	Gabrielle.R Leduc et al.,(2021)[58]
10	Canada	*A. salmonicida subsp. salmonicida*	SHY16-3432	Ah-7	Brook trout	Gabrielle.R Leduc et al.,(2021)[58]
11	Canada	*A. salmonicida subsp. salmonicida*	01-B522	Ah-7	Brook trout	Gabrielle.R Leduc et al.,(2021)[58]
12	Canada	*A. salmonicida subsp. Salmonicida*	01-B526	Ah-7	Brook trout	Gabrielle.R Leduc et al.,(2021)[58]
13	Canada	*Aeromonas sp. probiotic candidate*	CPB5	Ah-7	Fish Gill	Gabrielle.R Leduc et al.,(2021)[58]
14	China	*A.salmonicida sub sp. Masoucida*	AS01	vB_AsM_ZH F	Turbot (Scophthalmus maximus)	Zhenhe Xu et.al.,(2021)[59]
15	India	*A.veronii*	-	Ø265		Alka Nokhwal et al.,(2021)[60]
16	India	*A.hydrophylla*	AhZ1K	AhFM4, AhFM5	Tillapia,craps,salmon	Nithin.M et al.,(2021)[61]
17	Vietnam	*A.hydrophylla*	-	PVN02	Striped catfish (Plotosus lineatus)	Dang,T.H.O et al.,(2021)[62]
18	Taiwan	*A.hydrophylla*	-	vB_AhyM_A hp2	Infected fish	Wang,J.B et al.,(2021)[63]
19	Thailand	*A.hydrophylla*	-	pAh62TG	Tilapia	Dien L.T et al.,(2021)[64]
20	India	*A.hydrophilla*	-	VB-AhyM-AP1	Infected fish	B.Pallavi et al.,(2020)[65]
21	Korea	*A.hydrophylla*	-	AHP1,APH	Carassius carassius	Chandrarathna,H. P.S.V et al.,(2020)[66]
22	Pakistan	*A.hydrophylla*	KTCC2358	Akh2	Misguns anguillicaudals(Loac h)	Akmal et.al.,(2020)[67]
23	China	*A.hydrophylla*	4572	AhyVDH1	Infected fish	Yahui cheng et al.,(2020)[68]
24	Vietnam	*A.hydrophylla*		PVN02	Striped fish	Vinh Q.Tu et al.,(2020)[69]
25	China	*A.hydrophylla*	NJ35,XY16	N21,W3,G65 ,Y71,Y81	Infected fish	Jin Liu et al.,(2020)[70]
26	China	*A.hydrophylla*	-	MJG	Rainbow trout	Yongsheng Cao et al.,(2020)[71]
27	Poland	*A.hydrophylla*	-	50Ahyd13pp, 60AhydR15p p,25AhydR2 pp	Anguilla anguilla, Oncorhynchus mykiss	Schulz et al.,(2019)[72]
28	India	*A.hydrophylla*	-	CF7	Infected fish	Anuj Tyagi et al.,(2019)[73]
29	India	*A.salmonicida*	-	ASP-1	Goldfish (carassius auratus)	Nikapitiya C et al.,(2019)[74]
30	Vietnam	*A.hydrophylla*	-	CT45, TG25p	Striped Catfish	Hoang,H.A et al.,(2019)[75]
31	Egypt	*A.veronii*	-	AvFo7	Nile Tilapia	Nahed A. El-Wafai et al.,(2019)[76]
32	Egypt	*A.hydrophylla*	-	AP1,AP2,AP 3, AP4	Nile Tilapia	Hassan, s.w.m et al.,(2018)[77]
33	Vietnam	*A.hydophylla*	-	TG25p	Striped Catfish	Xuan,T et al.,(2018)[78]
34	China	*A.salmonicida*	-	AS-yj,AS-gz, AS-zj, AS-szw, AS-sw	Infected fish	Chen .L et al.,(2018)[79]

35	Portugul	*A.salmonicida*	Cefas 78027	O,R and B,ASP1	Atlantic salmon (Salmo salar)	Duarate J et al.,(2018)[80]
36	Canada	*A.salmonicida*	ATCC27013	PAS-1		Vincent A T et al.,(2018)[46]
37	Vietnam	*A.hydrophylla*	-	Ø2,Ø5	Catfish	Le,T.S et al.,(2018)[49]
38	India	*A.veronii*	-	T7 like phage	Infected fish	Taruna Anand et al.,(2017)[81]
39	Korea	*A.hydrophylla*	KCTC12487	pAh-1	Zebra fish (Danio rerio)	Easwaran.M et al.,(2016)[82]
40	Korea	*A.hydrophylla*	-	AHP-1	Zebra fish(Danio rerio)	Wang,J.B et al.,(2016)[83]
41	Egypt	*A.hydrophylla*	-	ΨZH, ΨZH 2	Tilapia	El.Araby.D et al.,(2016)[84]
42	Taiwan	*A.hydrophylla*	-	AHP1	Infected fish	Silva YJ et al.,(2016)[85]
43	Portugal	*A.salmonicida*	CECT894	AS-D,AS-A	Senegalese sole	Silva YJ et al.,(2015)[85]
44	India	*A. hydrophyla*	-	T4	-	Taruna Anand et al.,(2015)[86]

Country-specific Insights on the Availability of Bacteriophage Products

Table 3: Bacteriophage products in Global Market (Carla Pereira et al., 2022)[48]

Year	Country	Commercial product	Application
2021	Germany's Ludwigshafen	BASF New Business GmbH	Treatments for diseases causing Aeromonas , Rickettsia, Pseudomonas, Vibrio, Yersinia, Moritella, Lactococcus, Piscirickettsia, Flavobacterium covalently attach phages to food particles.
2021	Poland's Lódz	Proteon Pharmaceuticals S.A.	Using the natural feed supplement BAFADOR® will shield commercial aquaculture from bacterial diseases caused by *Aeromonas spp.*and *Pseudomonas spp*. serotypes.
2021	Glasgow, Scotland	Fixed Phage Ltd	Fish and crustacean feed can be supplemented with phage particles immobilised in pellets in order to cure bacterial infections in aquaculture, such as salmonids with flavobacteria infections and prawns with early mortality syndrome.
2021	Oslo Norway's	ACD Pharma	Many diseases that impact aquaculture are treated with phage-based medicines; one such product is CUSTUS® YRS, which lowers the *Y. ruckeri* infective strain in aquaculture water.
2021	Karnataka, India	Mangalore Biotech Lab	Luminous vibriosis caused by *Vibrio harveyi* can be prevented and treated using a product called LUMI-NIL MBL.
2021	Greece	Aquatic Biologicals	Treatment using phages against a number of infections linked to aquaculture fatalities.
2020	Baltimore, MD, USA	Intralytix Inc	To treat infections caused by *V. coralliilyticus* and *V. tubiashii* in oysters grown in hatcheries, use a phage-based treatment
2016	Australia	Biologix	Phage therapy for *Vibrio sp*. linked to aquaculture mortality
2014	Rehovot , Israel's	Phage biotech Laboratory	Treatment for a phage that causes *V. harveyi* in farmed shrimp

II. Future Prospect

Only one phage has been demonstrated to be effective against the Aeromonas strain on commercial platforms, and there have been few attempts to combine two or more phages. On the other hand, phage cocktail items, such as phage with antibiotics, probiotics, or any feed additives, etc., should be used to make phage therapy successful. In order to stop the growth of mutant bacteria, researchers advised for future studies utilising a variety of phages in phage treatments, acknowledging that doing so would boost their efficacy. As a result, even if the target pathogens develop resistance to one or more of them, the other phage variants will continue to be effective in killing bacteria over time.

III. Discussion and Conclusion

The aquaculture sector is a rapidly expanding global business that is constantly infected by a wide range of harmful viruses. Bacterial infections, such as *Aeromonas, Edwardsiella, Flavobacterium*, and *Pseudomonas*, are mostly to blame for the extreme morbidity and mortality of Indian major carps. Despite substantial monetary loss, pathogens are resistant to many antibiotics. After studying biotic and abiotic stress loads, additional research on bacteriophage treatment is needed. Only in vitro phage research has been conducted thus far; in vivo phage therapy experiment development is required. India is a major producer of Catla *(Catla catla*), rohu (*Labeo rohita*), and mrigal (*Cirrhinus mrigla*); While bacteriophage therapy is less frequently studied and used on particular main crop products in India, it is widely used to treat their fish diseases there. Because India is the second-largest producer of freshwater fish in the world, with *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* being the most widely cultivated carps and are also sold alongside untreated or raw seafood caught by fishermen from freshwater sources, more study on application of phage therapy on edible fish is necessary.

Overcome the challenges with phage before applying commercially

The efficiency of phages to control Aeromonas spp. in aquaculture systems is affected by a number of variables, including phage selection, MOI, and environmental variables that affect lytic phage viability, such as pH, salinity, temperature, UV delivery methods, radiation, and bacterial resistance to phages. Furthermore, neither the in vivo results for one phage nor the in vitro results for another phage can be extrapolated to represent the in vivo results for in vivo experiments[48]. Phage efficacy testing is necessary to confirm the effectiveness and safety of phages before this method is used commercially. The cost-effectiveness, delivery strategy, optimal MOI for bacterial inactivation, and stability of phage preparations are a few of the variables that need to be standardised and taken into account.

An increase in consumer knowledge about bacteriophage treatment

Consumer interest in bio control products is unbalanced nowadays. As a result, there has been a trend toward more traditional therapies, which has increased demand for bacteriophages. By making bacteriophage-based goods accessible through internet channels, producers will be able to contact more consumers and their demand will rise as a result of the products' increased worldwide awareness. Additionally, customers have the option to choose from a range of choices based on their needs. Over the next few years, demand for products will significantly rise as a result of their ubiquitous availability across all platforms for sales. Vaccination against *A.hydrophila* is essential to the aquaculture sector. By the end of 2032, the bacteriophage market is projected to increase from its present value according to Worlds FAO report 2020.

IV. Acknowledgment

The authors would like to thank the Principal and Head of Department of Annasaheb Kulkarni Department of Biodiversity, M.E.S Abasaheb Garware College, Maharashtra India.

References

[1]. Figueras Salvat MJ, Ashbolt N. Aeromonas. In: Michigan State University, Rose JB, Jiménez Cisneros B, UNESCO - International Hydrological Programme, Eds. Water And Sanitation For The 21st Century: Health And Microbiological Aspects Of Excreta And Wastewater Management (Global Water Pathogen Project).Michiganstateuniversity; 2019. Doi:10.14321/Waterpathogens.21

[2]. HUDSON EB, BUCKE D, FORREST A. Isolation Of Infectious Pancreatic Necrosis Virus From Eels, Anguilla Anguilla L., In The United Kingdom. Journal Of Fish Diseases. 1981;4(5):429-431. Doi:10.1111/J.1365-2761.1981.Tb01153.X

[3]. Vethaak AD, Ap Rheinallt T. Fish Disease As A Monitor For Marine Pollution: The Case Of The North Sea. Rev Fish Biol Fisheries. 1992;2(1):1-32. Doi:10.1007/BF00042915

[4]. Zhang J, Shen H, Wang X, Wu J, Xue Y. Effects Of Chronic Exposure Of 2,4-Dichlorophenol On The Antioxidant System In Liver Of Freshwater Fish Carassius Auratus. Chemosphere. 2004;55(2):167-174. Doi:10.1016/J.Chemosphere.2003.10.048

[5]. Jyotirmayee P, Sachidananda D, Basanta KD. Antibacterial Activity Of Freshwater Microalgae: A Review. Afr J Pharm Pharmacol. 2014;8(32):809-818. Doi:10.5897/AJPP2013.0002

[6]. Zheng Z, Aweya JJ, Wang F, Et Al. Acute Hepatopancreatic Necrosis Disease (AHPND) Related Micrornas In Litopenaeus Vannamei Infected With AHPND-Causing Strain Of Vibrio Parahemolyticus. BMC Genomics. 2018;19(1). Doi:10.1186/S12864-018-4728-4

[7]. Kotloff KL, Nataro JP, Blackwelder WC, Et Al. Burden And Aetiology Of Diarrhoeal Disease In Infants And Young Children In Developing Countries (The Global Enteric Multicenter Study, GEMS): A Prospective, Case-Control Study. The Lancet. 2013;382(9888):209-222. Doi:10.1016/S0140-6736(13)60844-2

[8]. Nair AV, Vijayan KK, Chakraborty K, Leo Antony M. Diversity And Characterization Of Antagonistic Bacteria From Tropical Estuarine Habitats Of Cochin, India For Fish Health Management. World J Microbiol Biotechnol. 2012;28(7):2581-2592. Doi:10.1007/S11274-012-1067-5

[9]. Mishra A, Nam GH, Gim JA, Lee HE, Jo A, Kim HS. Current Challenges Of Streptococcus Infection And Effective Molecular, Cellular, And Environmental Control Methods In Aquaculture. Mol Cells. 2018;41(6):495-505. Doi:10.14348/Molcells.2018.2154

[10]. Hodgson K. Bacteriophage Therapy. Microbiol Aust. 2013;34(1):28. Doi:10.1071/MA13009

[11]. ICAR-Central Institute Of Freshwater Aquaculture, Ss M. Present Status Of Fish Disease Management In Freshwater Aquaculture In India: State-Of-The-Art-Review. AAF. 2017;1(1):1-9. Doi:10.24966/AAF-5523/100003

[12]. Mohan B, Sethuraman N, Verma R, Taneja N. Speciation, Clinical Profile & Antibiotic Resistance In Aeromonas Species Isolated From Cholera-Like Illnesses In A Tertiary Care Hospital In North India. Indian J Med Res. 2017;146(7):53. Doi:10.4103/Ijmr.IJMR_378_15

[13]. Beaz-Hidalgo R, Latif-Eugenín F, Hossain MJ, Et Al. Aeromonas Aquatica Sp. Nov., Aeromonas Finlandiensis Sp. Nov. And Aeromonas Lacus Sp. Nov. Isolated From Finnish Waters Associated With Cyanobacterial Blooms. Systematic And Applied Microbiology. 2015;38(3):161-168. Doi:10.1016/J.Syapm.2015.02.005

[14]. Nzeako B, Okafor N. Bacterial Enteropathogens And Factors Associated With Seasonal Episodes Of Gastroenteritis In Nsukka, Nigeria. British Journal Of Biomedical Science. 2002;59(2):76-79. Doi:10.1080/09674845.2002.11783638

[15]. Ferguson AS, Layton AC, Mailloux BJ, Et Al. Comparison Of Fecal Indicators With Pathogenic Bacteria And Rotavirus In Groundwater. Science Of The Total Environment. 2012;431:314-322. Doi:10.1016/J.Scitotenv.2012.05.060

[16]. Soltan Dallal MM, Moezardalan K. Aeromonas Spp Associated With Children's Diarrhoea In Tehran: A Case–Control Study. Annals Of Tropical Paediatrics. 2004;24(1):45-51. Doi:10.1179/027249304225013231
[17]. Bravo JA, Julio-Pieper M, Forsythe P, Et Al. Communication Between Gastrointestinal Bacteria And The Nervous System. Current Opinion In Pharmacology. 2012;12(6):667-672. Doi:10.1016/J.Coph.2012.09.010
[18]. Janda JM, Abbott SL. The Genus Aeromonas : Taxonomy, Pathogenicity, And Infection. Clin Microbiol Rev. 2010;23(1):35-73. Doi:10.1128/CMR.00039-09
[19]. Austin B, Austin DA. Vibrionaceae Representatives. In: Bacterial Fish Pathogens. Springer Netherlands; 2012:357-411. Doi:10.1007/978-94-007-4884-2_11
[20]. Pk S, A P, Mk S, S P, Kumar P R, Bk D. Incidences Of Infectious Diseases In Freshwater Aquaculture Farms Of Eastern India: A Passive Surveillance Based Study From 2014-2018. J Aquac Res Development. 2020;11(1). Doi:10.35248/2155-9546.20.11.579
[21]. Nakano H, Kameyama T, Venkateswaran K, Kawakami H, Hashimoto H. Distribution And Characterization Of Hemolytic, And Enteropathogenic Motile AeromonasIn Aquatic Environment. Microbiology And Immunology. 1990;34(5):447-458. Doi:10.1111/J.1348-0421.1990.Tb01027.X
[22]. Popoff M, Lallier R. 4 Biochemical And Serological Characteristics Of Aeromonas. In: Methods In Microbiology. Vol 16. Elsevier; 1984:127-145. Doi:10.1016/S0580-9517(08)70389-1
[23]. Namdari H, Bottone EJ. Microbiologic And Clinical Evidence Supporting The Role Of Aeromonas Caviae As A Pediatric Enteric Pathogen. J Clin Microbiol. 1990;28(5):837-840. Doi:10.1128/Jcm.28.5.837-840.1990
[24]. Richards GP. Bacteriophage Remediation Of Bacterial Pathogens In Aquaculture: A Review Of The Technology. Bacteriophage. 2014;4(4):E975540. Doi:10.4161/21597081.2014.975540
[25]. Imbeault S, Parent S, Blais JF, Lagacé M, Uhland C. Utilisation De Bactériophages Pour Contrôler Les Populations De Aeromonas Salmonicida Résistantes Aux Antibiotiques. Rseau. 2007;19(4):275-282. Doi:10.7202/014415ar
[26]. Assefa A, Abunna F. Maintenance Of Fish Health In Aquaculture: Review Of Epidemiological Approaches For Prevention And Control Of Infectious Disease Of Fish. Veterinary Medicine International. 2018;2018:1-10. Doi:10.1155/2018/5432497
[27]. Gram L. Inhibition Of Mesophilic Spoilage Aeromonas Spp. On Fish By Salt, Potassium Sorbate, Liquid Smoke, And Chilling. Journal Of Food Protection. 1991;54(6):436-442. Doi:10.4315/0362-028X-54.6.436
[28]. Barnhart HM, Pancorbo OC, Dreesen DW, Shotts EB. Recovery Of Aeromonas Hydrophila From Carcasses And Processing Water In A Broiler Processing Operation. Journal Of Food Protection. 1989;52(9):646-650. Doi:10.4315/0362-028X-52.9.646
[29]. Huys G, Coopman R, Janssen P, Kersters K. High-Resolution Genotypic Analysis Of The Genus Aeromonas By AFLP Fingerprinting. International Journal Of Systematic Bacteriology. 1996;46(2):572-580. Doi:10.1099/00207713-46-2-572
[30]. Sofos JN, Maga JA, Boyle DL. Effect Of Ether Extracts From Condensed Wood Smokes On The Growth Of Aeromonas Hydrophila And Staphylococcus Aureus. J Food Science. 1988;53(6):1840-1843. Doi:10.1111/J.1365-2621.1988.Tb07856.X
[31]. Anis K. Growth And Survival Of Aeromonas Hydrophila In Rice Pudding (Milk Rice) During Its Storage At 4°C And 12°C. Food Microbiology. 2003;20(4):385-390. Doi:10.1016/S0740-0020(03)00022-4