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Bacteriophages for aquaculture disease control

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Abstract
The administration of phage therapy for aquaculture disease has been anticipated by the researchers over a decade as an effective and an alternative control mechanism, though the application of phages as a disease control agent in aquaculture projects various beneficial aspects, critical limitations, and negative influence on production. This present scenario made a pressure to review the possible disclosure of phage therapy with its critical boundaries and limiting influences towards the disease control management of aquaculture (fish, shrimps, lobsters, bivalve mollusks, etc.). The phage therapy has proven its efficacy as a biocontrol agent towards aquaculture disease, although the sustainability of the phage therapy needs further investigation on the following: commercial application, formulation of bacteriophage for layman usage, and development of protocol for various diseases with consistent results. The marginal space existing between the inventors and the end user must be fulfilled by the awareness program and the government policies. The administration of the phage therapy could be effective for long-term safety and negatively influence the development of multidrug-resistant bacteria pathogens in the future.

Keywords Bacteriophage therapy · Aquaculture · Multidrug resistant · Bacteriolysis

Introduction

The government authorities have permitted the antibiotics towards the common bacterial infections of the fish, like tail and fin rot, epizootic skin erosion, ulcerative syndrome, and gill damage for-off. It seems to be an economically viable and sensible remedy than the critical financial defeat caused by the bacterial infections; hence, it has its own limitation due to

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multidrug resistance (MDR) of bacteria, microbial changeover, and accumulation of residual chemicals, in the environment, and general public (Park et al. 2000; Perreten 2005; Oliveira et al. 2012). Noteworthy, this current problem has been effectively initiated to resolve by using the bacteriophages, since it is being a natural, low cost, viable, and an alternative biomedicine for antibiotics and the chemicals used as an antibacterial substance, to control the dispersal of multidrug-resistant bacteria in the aquatic fish cultivation (Nakai and Park 2002; Oliveira et al. 2012; Subharthi 2015).

Bacterial viruses are actually viruses that may possibly survive in the bacterial host through infections and gradually consume the entire host known as bacteriophages or phages. Phages are as similar as virus in obligate parasitic feature; hence, it does not have any basic or specific metabolic function to survive its life rather it depends the metabolic support of the host (Al-Sum and Al-Dhabi 2014). As the nature omnipresent, bacteriophages are generally occurring in soil, fresh and marine environment, and in particular mammal intestinal tract. In the period of 1919–1920, scientist Félix d'Herelle, who was the discoverer of phages, has first introduced the phage therapy. In this method, the bacterial infectious diseases were treated with phages (Citorik et al. 2014). This phage therapy has become the booming solution to protect the fish population from the pathogens and effectively applied in the real cultivation (Rao and Lalitha 2015) The effect of bacteriophage therapy and its potential application in aquaculture practices has been documented by Oliveira et al. (2012). The crucial achievement of this technique using phage is more environmental friendly, and it has an uninterrupted host phage mechanism. This review has extensively highlighted the research finding of the phages and their potential mechanism against vast bacterial pathogens of aquaculture (Richards 2014).

Efficacy of phage therapy

The efficacy of the phage therapy is comparably high than the chemotherapy: (1) the reports are evidenced with effective mechanisms of phages towards MDR pathogenic bacteria because it induces bacteriolysis through a completely different mechanism from antibiotics (Matsuzaki et al. 2003); (2) the phage has high host-specific nature towards the bacterial pathogens, and the replacement of microbes could not be possible with this; (3) the immediate counter action like a mutation of phages could take place against the phage-resistant mutant bacteria; (4) in the economical point of view, the expenditure required for the phage therapy is considerably very less than the antibiotic dosage; and (5) still no reports stated on the side effects of the phage and phage products particularly lysin against eukaryotic cells (Hagens and Offerhaus 2014; Ly-Chatain 2014).

Mechanism of phage therapy

The phage mechanisms involved in the pathogenic bacteriolysis is well defined with following steps and is primarily started with the phage infection, which can be initiated by the adsorption of phages on the particular receptors of proteins or sugars (peptidoglycan (PG), teichoic acids, oligosaccharides, lipopolysaccharide (LPS), capsule, flagellum, type IV fimbriae, and sex pilus) of the pathogens cell wall. In general, the phages have the potential to attract the exact targeted bacterial pathogens in a species level or even in strain level, in very few studies reported with cross bacterial pathogens at species or genus level and named as polyvalent

phages (Ross et al. 2016). The phage therapy finally achieved to destroy the complete bacterial infections caused by various sources of the aquaculture with the notable advantage of not harming natural flora of the host (Loc-Carrillo and Abedon 2011). In the phage life cycle, the insertion of phage genetic material into the pathogen cytoplasm is being the most important mechanism. The cell wall lysis of the pathogen particularly mediated by the phage depolymerases enzyme which includes a peptidoglycan hydrolases, endosialidases, endorhamnosidases, alginate lyases, and hyaluronate lyases (Drulis-Kawa et al. 2015) Then, the phage pre genes have been expressed and the related proteins (holing, endolysin, and spanins) are synthesized which would act as a main part in the control/destroy mechanism of the targeted bacterial pathogen system. The protein holin mean to make a hole in the cell wall of the pathogenic bacteria and give a way to the lysin enter into the peptidioglycan layer; it was known as a peptidoglycan hydrolase which could effectively degrade the peptidoglycans. As the same as a phage, the enzyme lysin has also been used as an alternative agent in the therapy. At the end of the phage life cycle, the new progeny phages released from the bacterial host and which could be mainly mediated by the protein endolysin (Young et al. 2000). The enzymes spanins (i-spanin and o-spanin) specially needed for the phages those who infect through disrupt the outer cell wall of Gram-negative pathogens (Kongari et al. 2018). As a subsequent process, the new progeny of the phage potentially infect the nearby pathogen. The basic mechanism of the phage to kill the bacteria pathogen has been effectively applied as a therapy against both Gram-positive and Gram-negative pathogens. Hence, it would be possible when all the infectious steps stated in the phage life cycle should entirely finish. Lack of studies on the mechanism of bacteriophages infecting aquaculture pathogens further limits the development of phage therapy as an alternative to antibiotics.

Active role of receptors and resistance to phages

In the phage infectious cycle, adsorption of the phage on the bacterial cell wall receptors has been considered as one of the vital stage and it could be possible by the exploitation of bacterial surface proteins (as receptors), host parts like flagella, capsules, fimbriae, and lipopolysaccharide (LPS) (Drulis-Kawa et al. 2015). In addition to this, the phages has the potential enzymes, which significantly works on the cell wall puncture mechanism for the effective entry to the bacterium. The well-known phage reported was PK1A with endosialidase secretion in its tail and deconstruct the polysialc acid capsule of *E. coli* K1 (Pelkonen et al. 1992; Skurnik and Strauch 2006). The *Escherichia coli* PK1A2 bacteriophage was also reported for effective mechanism (Lehti et al. 2017). Literature showed the role of microtubules in cargo trafficking which has been demonstrated in eukaryotic viruses. Particularly Pseudomonas phage capsid assembles on the membrane to move along tubulin-like protein, PhuZ on the phage nucleus facilitates DNA packaging (Chaikeeratisak et al. 2019). This study demonstrated that "a transport and distribution mechanism in which capsids attached to the sides of filaments are trafficked to the nucleus by PhuZ polymerization at the poles indicates the phage cytoskeleton evolved cargo-trafficking capabilities in bacteria".

The bacteria might have been anti-phage resistance at various situations: one when a mutation occurs and another one reported was loss/modification of receptors (Labrie et al. 2010). Even the bacterial host system expresses anti-phage mechanism to phage due to the action of lysogeny, in some circumstances the *Escherichia coli* trigger restriction-modification system which can destroy the genetic materials of the T4 phage (Manning and Kuehn 2011).

The genetic mutation of the phage in the bacterial system may interrupt the reproduction and assembly mechanism of the phage. Though this condition has not been reported as worse because the resistance of the bacteria could change or decrease the physical strength of it, and in case the receptor represents the virulence factor used by the phage, then it could be declined by the phage significantly (Levin and Bull 2004; Azam and Tanji 2019).

Basics of phage therapy

The expectation of phage therapy has been limited to control certain number of infectious bacteria in the aquaculture animals, the rest can be taken care by the defense mechanism of the animals (Levin and Bull 2004). The mechanism of phage multiplication in targeted bacteria was well defined, though due to the unpredictable factors, it is still in unsuccessful stage. To meet the success end point in the phage therapy set of repeatable, multidimensional and quantitative efforts have to be taken in particularly in vivo state.

The phage therapy should come in practice once it has done in vivo state with complete revealing of the mechanism because both bacterial infectious mechanisms as the same way the phage controlling mechanisms are intricate. The formulation of phage might fulfill the complete protection from bacterial and other infectious contaminants. The healthy population of phages must be a main fraction of the commercial formulation that should be comply with product certification. The phage formulation should be specified with their respective receptor. The probability of occurring incomplete receptors/altered receptors and impulsive phage-resistant mutants would be possible with the bacterial population of 10^6–10^8. The predictable mutation in the receptor with virulence nature of the bacteria could end up with the bacteria with avirulent bacteria that promote the immune system to eradicate with no trouble. The successful technology with animal model testing should be making them efficient with phage unique character.

Aquaculture vs phage therapy

The phage therapy has been reported for its significant application in the fields of medicine, both human and veterinary, agricultural, and food sectors. The phage has extended its application towards aquaculture, especially to vast aquaculture organisms (Table 1) (Oliveira et al. 2012).

The vibrio species are normal ubiquitous bacteria of the aquatic system responsible for most of the bacterial outbreaks recorded across the countries (Kiran et al. 2016). The phage therapy against fish bacterial control was first reached in the application stage at 1981 and was demonstrated first in Japanese eel Anguilla japonica (Wu et al. 1981; Wu and Chao 1982; Oliveira et al. 2012). Further, the continuation of research in the field of control mechanism towards pathogens with field trials in fish aquaculture experimented at 1977. The phage therapy with positive note on protective mechanism was reported successfully in yellow tail (*Seriola quinqueradiata*) against the pathogenic effect of *L. garvieae* infections (Nakai et al. 1999) when there was no remedy for the drug-resistant strains. Those phages with virulent effect against *L. garvieae* were denoted as PLgY and known as the family of Siphoviridae (Park et al. 1997). In accordance with the sensitivity to phages, the pathogenic strains *L. garvieae* of fish and their ecosystem were classified into various groups (Park et al. 1998).

The phage verified to have significant effects on cultured ayu fish (*Plecoglossus altivelis*) against the bacterial pathogen of *P. plecoglossicida* at the time the licensed chemotherapeutic

Table 1 Bacteriophages used to control disease in aquaculture

Name of bacteriophage	Fish/shellfish/shrimp species involved	Caused disease	Disease controlled	Key references
DNA bacteriophage *Siphoviridae*	Tiger shrimp (*Penaeus monodon*)	Luminous vibriosis (*Vibrio harveyi*)	80% mortalities of *Vibrio harveyi*	Vinod et al. (2006)
Viha10,Viha8 *Siphoviridae*	Shrimp larvae (*Penaeus monodon*)	*Vibrio harveyi*	Lysed 55–70% of 100 *V. harveyi*, 85% survival of host	Karunasagar et al. (2007)
Phage VhCCS-06 *Siphoviridae*	Tropical rock lobster (*Panulirus ornatus*)	*Vibrio harveyi*	1.2×10^7 CFU *Vibrio harveyi* reduced compared to control 9.3×10^7 CFU	Stomps et al. (2010)
Phage AS-1 Phage VP-1 *Myoviridae*	Fish disease	*V. anguillarium* *V. parahaemolyticus* *A. salmonicida*	Efficacy of plating AS-1 (98, 96, 100) VP-1 (83, 100, 64)	Pereira et al. (2011)
Phage FCL-2 *Myoviridae*	Fish, rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Danio rerio*)	*Flavobacterium columnare* strain B185	Zebrafish 100% survival, rainbow trout 50% survival	Laanto et al. (2015)
pSs-1 bacteriophage *Myoviridae*	–	Waterborne, foodborne pathogens (*Shigella flexneri*, *S. sonnei*)	–	Jun et al. (2016)
Phage pVp-1 *Demerecviridae*	Marine shrimp (*Penaeus vannamei*)	Acute hepatopancreatic necrosis disease (AHPND) (*Vibrio parahaemolyticus*)	100% cumulative mortalities *Vibrio parahaemolyticus*	Jun et al. (2018)
phT4A, ECA2 ***Adenoviridae***	Bivalves	Multiplicity of infection (MOI) (*E. coli*)	0.6 log CFU/g reduction of *E. coli*	Pereira et al. (2017)
vB_VhaS-tm phage *Siphoviridae*	Abalone (snail) (*Haliotis laevigata*)	Vibriosis (*Vibrio harveyi*)	Survival of 70% host	Wang et al. (2017)
Phages AS-yj, AS-gz *Myoviridae*	Fish disease	Furunculosis (*Aeromonas salmonicida*)	Highest burst size (145 PFU/host)	Chen et al. (2018)

agents were not permitted for aquaculture application. The key points were investigated from the research reports of phage therapy as follows: (1) the food prepared with the infused phage has given optimistic results against experimental infection, and it positively correlated with massive phage activity at in vivo state (Park et al. 2000; Park and Nakai 2003); (2) in addition, application of phage suspension in open water system has effectively controlled the further spreading over of the pathogen for secondary (Oliveira et al. 2012; Almeida et al. 2019); (3) noteworthy, the research extensively reported no evidence for the presence of phage-resistant organisms and nullifying compounds released by the immune system of the both healthy as well as infected fish (Oliveira et al. 2012; Rao and Lalitha 2015); (4) it is evidently proved with the application of phage leads with very less percentile of infectious and fish mortality rate (Park and Nakai 2003).

The research findings of the model studies are a benchmark for biocontrol mechanism that could be effectively implemented for numerous fish cultivation models. Since, it serves up a significant and sustainable biocontrol therapy against various uncontrollable infectious diseases in aquaculture industry (Nakai and Park 2002). The combined mechanism with bacteriophage has given proven results in the fish farms. Hence, it is highly suitable for prophylactic use and not recommend for to treat furunculosis caused by *Aeromonas salmonicida* and in Atlantic salmon (Imbeault et al. 2006; Verner-Jeffreys et al. 2007).

The vibrio species are normal ubiquitous bacteria of the aquatic system responsible for most of the bacterial outbreaks recorded across the countries (Austin and Zhang 2006; Kiran et al. 2016). The use of lytic phages on the control of V. harveyi infection in Penaeusmonodon larvae was demonstrated by Karunasagar et al. (2007). In this report, the bacteriophages isolated from oyster and P. monodon hatchery water were testedagainst V. harveyi in vitro and the results showed lysis 55–70% V. harveyi tested. The effective phages belonged to Siphoviridae family. Significant findings of this studyinclude effective elimination of V. harveyi cells from the biofilm formed on high density polyethylene (HDPE) surface, the tanks used in the P. monodon hatchery.Another report by Vinod et al (2006), showed a double stranded DNA phage belong to Siphoviridae was isolated from farm water P. monodon ponds. The lytic phageeffectively controlled luminous vibrios in microcosm experiments.

Based on the reports from the aquaculture industry, the success of phagetherapy depends on delivery methods which ensure delivery of phages in the infected site and a titre required to eliminate the pathogenic bacteria (Kalatzis et al. 2018). Most of the successful phage therapy reports showed injection of diseased fish, however, this could not be a reliable method for treatment of a population. Application ofphage therapy to contain a well-known zoonotic pathogen Vibrio parahaemolyticus was demonstrated by Jun et al. (2014). The commercialization of phage therapy inaquaculture production systems requires economic and consistent phage production methods. The production method that could reduce the chances of emerging resistant bacteriophages need to be preferred include cellstat and a two-stage self-cycling process (García et al. 2019).

Disease control management in shrimps

Antibiotics

The use of antibiotics in shrimp aquaculture pose potential threat include residual antibiotics in the food systems (Selvin and Lipton 2003, 2004) and possible emergence of resistantstrains. In aquaculture industry 36 types of antibiotics were used in seven major aquaculture production

countries (Rico et al. 2012). In marine invertebrates, especially in penaeid shrimp (economically important shrimp), a serious disease was caused by *Vibrio harveyi* (Karunasagar et al. 2004; Austin and Zhang 2006). Antibiotics are frequently used in some of hatcheries to control the diseases; it sometimes leads failure to control the luminous bacteria. The growth of antibiotic-resistant *V. harveyi* increased in larval tanks causing serious mortality in *P. monodon* larvae (Lavilla-Pitogo et al. 1990; Karunasagar et al. 1994). Due to these effects, some of the shrimp farmers used to apply diverse group of antibiotics on a daily basis as a precautionary measure (Holmstrom et al. 2003) Antibiotics are used as both prophylactic and reactive mode oftreatments. Among the antibiotics used in the aquaculture, oxytetracycline and chloramphenicol were most prevalent in many production countries (Selvin and Lipton 2003, 2004). Considering the impacts of antibiotics, the use of chloramphenicol was banned in aquaculture (Rico et al. 2012).

Bacteriophage therapy

The use of bacteriophage based treatments obviously known as 'phagetherapy' become a green alternative to the use of antibiotics in aquaculture. The effect of phage therapy can be improved with a combination of lytic phages and/orphages with antimicrobial substances, which ultimately prevents the emergence of phage resistance (Karunasagar et al. 2005; Shivu et al. 2007; Rao and Lalitha 2015). This report also showed the possible threat of lysogenic phages might transform the virulent gent into the non-virulent strains. Almeida et al. (2019) demonstrated the effect of phage therapy in the control of Flavobacterium columnare infection in rainbow trout Oncorhynchus mykiss cultured in recirculating aquaculture systems (RAS). This study demonstrated the persistence of exposed phage for three weeks in a RAS, evidenced the potential application of phage therapy in RAS. Further this reportrevealed the highest concentration of phage in biofilm layers over plastic surfaces indicating the possibility of developing immobilization techniques for sustained releaseof phase in RAS. Several literatures have reported the usage of the bacteriophages for the biocontrol of luminous vibriosis with complete characterization of bacteriophages (Fig. 1). The consistent results needed to certify the effective application of bacteriophage to control luminous vibriosis in shrimps, and it is necessary to extend the application to other organisms to which this disease is associated.

Disease control management in lobsters

Bacteriophage therapy is one of the alternative techniques that potentially control and remove the pathogenic *Vibrio* spp. in the larval culture of the tropical rock lobster, *Panulirus ornatus* (Payne 2007). In spiny lobster, has been identified with diseases caused by *V. harveyi* (Vinod et al. 2006). Among eight bacteriophages investigated for lytic activity against *V. harveyi*, six come under Siphoviridae and two from Myoviridae, among them one from the Siphoviridae family had noticed in clear and no apparent transducing property. Crothers-Stomps et al. (2010) demonstrated the use of bacteriophage on the control of *V. harveyi* strains infectingphyllosoma larvae of tropical rock lobster Panulirus ornatus. The bacteriophage strains showed effectively controlled vibrio strains such as *V. rotiferianus*, *V. harveyi*, *V. campbellii*, and *V. parahaemolyticus* belong to the family Siphoviridae and Myoviridae. Significant outcome of this study by Crothers-Stomps et al. (2010) include demonstration of phage induced bacteriocin production.

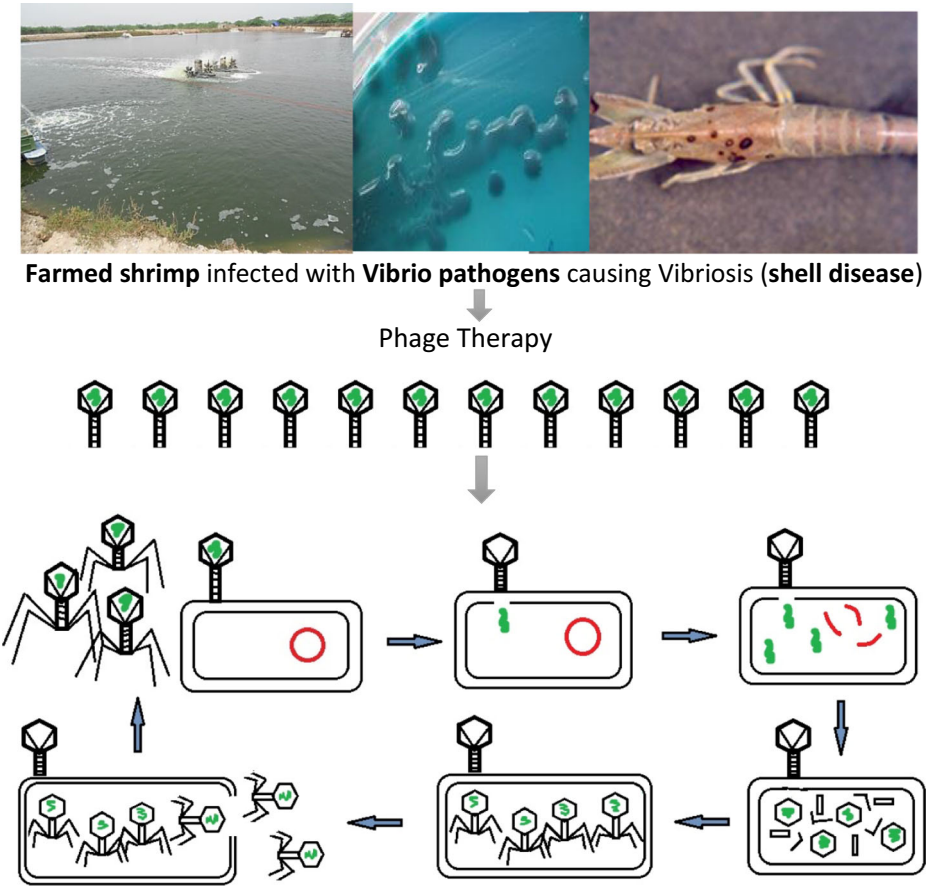

Fig. 1 Bacteriophage therapy effectively kills bacterial pathogens through lytic cycle. In lytic cycle, the phages replicate and lyse the pathogenic bacterial cell; thereby, the host (pathogenic bacteria) cell completely eradicated from the aquaculture farms

Disease control management in bivalve mollusks

The bacteriophage treatment has been used as a control mechanism towards the infection of *Vibrio splendidus* in a cultured larvae stage of the Pacific oyster, *Crassostrea gigas* (Sugumar et al. 1998; Park and Nakai 2003), and often used for bacterial infections of molluscan aquaculture (Berthe 2005). The pathogens *V. harveyi* and *Vibrio alginolyticus* have been found to be a main disease causing agent in pearl oysters, hence, the application of bacteriophages as an antimicrobial strategy to overcome this disease was not yet to be proved. In present scenario, the available research reports are very less on biocontrol of microbial disease with bacteriophages for any other bivalve species.

Enzybiotics

The diverse group of bacteriophage products (lysins and bacteriophage tail-like bacteriocins) has been isolated in purified form, and the alternative therapy results revealed its anti-infective nature (Skurnik and Strauch 2006; Oliveira et al. 2018). The enzyme lysin has efficiently worked on Gram-positive bacteria and effectively destroys the peptidoglycan layer from outside of the cell wall. It has the potential to degrade the cell walls by the bacteriolytic effects on non-growing bacteria within a few seconds after applying the lysins (Drulis-Kawa et al. 2015). On the other hand, cell wall-degrading antibiotics like penicillin and cephalosporin requires a channel to reach within the cell and inhibit peptidoglycan synthesis of bacteria in division phase only. The instantaneous administration of two lysins that have different peptidoglycan cutting sites has been reported to possess a synergistic effect. The bacteriophage lysin was also been reported to be fairly specific for bacterial species, eliminating the specific bacteria without disturbing the natural habitats. However, the lysins studied so far are inactive against Gram-negative cell wall, since lysin does not have the ability to enter the outer membrane of Gram-negative bacteria (Parisien et al. 2008).

Bacteriophage tail-like bacteriocins are one of the groups of bacteriophage products with high molecular weight fragments of bacteriophages, synthesized by a number of Enterobacteriaceae and other Gram-negative bacteria (Bradley 1967; Daw and Falkiner 1996). These special groups of bacteriocins have been reported to eliminate the target bacterial cell by pore formation in the cell wall and lead to a rapid loss of ions. Phage-specific lysins and phage tail-like bacteriocins isolated and analyzed by many researchers as an alternative potential therapy than to use whole bacteriophage (Inal 2003).

Phage therapy vs hindrance

The following challenges should be addressed in the phage therapy: (1) development of phage resistance, (2) risk of anaphylaxis reactions, (3) capture and transfer of bacterial toxin gene, and (4) utility against intracellular pathogens.

Concern to the phage resistant, it has been reported and proved that the development of mutants occurred at laboratory investigations which holds the resistant to bacteriophages. Hence, to implement the bacteriophage therapy, the above state one should be a core concern, and also act as a limiting factor to the positive approach of the bacteriophage therapy on multidrug resistance pathogens (Tang et al. 2019). The resistance mechanism of bacteria to bacteriophages could induce by a variety of means; for example, it happens through a change in the phage-receptors molecules in Gram-negative bacteria or via CRISPR-Cas systems (Ormala and Jalasvuori 2013). This problem should be addressed by re-isolation of a new phage from the environment. Since the phage and bacterial host exist together in the same environment, a phage species might attack mutated/resistant bacteria. The bacteriophage being a more significant finding than the discovery of many chemical antibiotics since it needs prolonged time to develop a new antibiotic. Though, the bacteriophage resistance has not been considered as a bigger challenge than the drug-resistant mechanism (Haq et al. 2012).

The administration of high dosage of bacteriophage as a treatment may induce an extreme response such as anaphylaxis, sometimes they reflect the opposite side effects, though it has not been reported yet (Sulakvelidze et al. 2001). To the above stated issue, the bacteriophage therapy has exhibited the potential recovery of endotoxin when causesing the death of the bacteria especially in

Gram-negative bacteria. This may negatively impact the efficacy of the bacteriophage therapy as same as the exploitation of antibiotics (Wittebole et al. 2014). The warning has been given by the researchers that the excess amount of endotoxin released either by the phage therapy or antibiotics could cause fever, septic shock, and finally ends up with death (Nobrega et al. 2015).

The capability of the phage to carry the genetic material by horizontal gene transfer was an important factor that being a challenge to practice the bacteriophage therapy. In addition, in the time of deletion of the bacteriophage DNA from the host chromosome, the host DNA may also assimilate with the bacteriophage DNA. Thus, lysogenic bacteriophages could initiate the horizontal transmission of bacterial genes from one bacterium to another bacterium to improve the bacterial virulence or genes for antibiotic resistance. The CTX ϕ prophage infection has been considered as a classical example to improve the virulence factor of *V. cholerae* (Meaden and Koskella 2013). The few other bacteriophages also possess the ability to hold the genetic materials by means of horizontal gene transfer. Due to this, the bacteriophage has been routed to generate toxins (enterotoxins and exfoliating toxins) of bacteria (Mohammed-Ali and Jamalludeen 2015). In addition to the above stated, the toxins CTX cholera toxin, botulinum toxin, Shiga toxin, and diphtheria toxin were also being reported as a toxin of bacteriophage. To overcome this issue and develop the successful bacteriophage therapy, the bacteriophages are anticipated to refuse the host DNA (Wittebole et al. 2014). The complete understanding of bacteriophage genome sequence paws the way to defeat the barriers of phage therapy. Lytic phages since do not assimilate into the host's DNA and do not influence the host's virulence factor, and the features made them an ideal candidate for therapeutic use.

The bacteriophage therapy has not been reported as potential one to kill the intracellular pathogens particularly *Salmonella* species. Because of the lack of ability of the bacteriophage to reach the intracellular pathogens, especially eukaryotic cell, they are still being a challenge to the researchers to completely destroy this intracellular pathogen through the phage therapy. Although the bacteriophages are not a direct pathogens of eukaryotic cells, the human immune system may identify bacteriophage as foreign antigens and respond by producing bacteriophage-neutralizing antibodies (Sulakvelidze et al. 2001).

Conclusions and future prospective

The production of different species of fish, crustaceans, and mollusks made the aquaculture industry as one of the significant elements of Indian economy. The disease control management in aquaculture has been updated constantly by the researchers to overcome the next-generation pathogenic diseases. Though, somewhere the bacterial infectious disease leaves the economic lags. The administration of antibiotics has existed as the only route to control the pathogenic effect and be a protective mechanism of aquaculture farming so far. Though the intelligence of the pathogenic microbes has been upgraded and introduced, the novel character of multidrug resistant (MDR) against almost all antibiotics.

Several research attempts have been initiated to target the multidrug-resistant pathogens through the development of new antibiotics or novel compounds/drugs against MDR pathogens. The government bodies have rolling announcement to submit proposals to discover the viable and new drugs with industrial collaboration. In addition to the above, it is also essential to create awareness programs about the recent research and inventions of aqua farming among the aquaculture producers. Natural compounds from the resource like flora and fauna of terrestrial and marine habitats have been focused on the implementation of new drugs. It is

noteworthy that the bacteriophage therapy has a unique approach towards infectious disease control management in aquaculture farming and does not comparably hazardous as antibiotics. The bacteriophage therapy has been tried well in many aquaculture farming for the past 38 years especially fish (Japanese eel, yellow tail, aya), shrimps, lobsters (rock lobster), and bivalve mollusks. The promising phage therapy seems to be an economical, eco-friendly, biologically safe than the regular chemotherapy.

The main hindrance of the phage therapy was noticed recently as the phage-resistant mechanism developed by the bacterial defense system. Though it was evidently proved, the strategy of co-evaluation between the bacteriophage and pathogenic bacteria would preserve the phage therapy for certain promising extant. The phage therapy on the phage-resistant bacteria impacts the structural change which is responsible for the virulence mechanism and limits the virulence though it does not completely destroy the bacteria. To extend to this, the above discussed apertures are still needed to be fulfilled only through the impending research areas like application of bacteriophage against intracellular pathogens and in all taxonomic groups of aquaculture (fish, shrimp, etc.) disease control management, etc. with effective cocktail formulation of bacteriophage for commercial application as an anti-pathogenic agent.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by anyof the authors.

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