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Isolation and Genomic Characterization of phiVibrioH1 a Myoviridae Phage for Controlling Pathogenic *Vibrio parahaemolyticus* from Seafood and Human

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VIBRIO parahaemolyticus is a zoonotic pathogen causing vibriosis in marine fish and associated with food poisoning outbreaks in humans. This study aimed to isolate and characterize bacteriophage against multidrug resistant (MDR) *Vibrio parahaemolyticus* from seafood and humans, and also to assess the lytic efficacy of phage on growth. *Vibrio Parahaemolyticus* was isolated from flesh of 80 white shrimp, 70 blue crabs and 50 mullets, and from 50 hand swabs of humans (fish handlers) on Thiosulphate Citrate bile salts sucrose agar media. The suspected colonies were biochemically identified. Pathogenic *V. parahaemolyticus* carrying thermostable direct hemolysin (tdh+) gene was molecularly detected. The pathogenic tdh+*V. parahaemolyticus* (n= 25) isolates were explored for antimicrobial vulnerability resistant to 12 antimicrobials employing the disc diffusion method. Fifteen isolates of tdh+V. parahaemolyticus were 100% resistant to five antimicrobials. Bacteriophage was isolated from sewage water using spot test and double over layer agar assay. The phiVibrioH1 was belonged to family Myoviridae according to transmission electron microscopy. This study revealed a polyvalent phage infecting wide host range of MDR *V. parahaemolyticus, V. vulnificus, V. fluvialis, V. alginolyticus*, *Pseudomonas aeruginosa, E. coli* O26 and *Proteus vulgaris*. The genome of phage was sensitive to digestion with BamH1 and ECoR1 restriction enzymes indicating double stranded DNA. From 24 to 42hrs post-treatment of pathogenic *V. parahaemolyticus* with the PhiVibrioH1 phage showed complete lysis of bacterial cells. This study confirmed that the phiVibrioH1 is a lytic phage and has a high potential to control pathogenic *V. parahaemolyticus* strains recovered from seafood and humans.

Keywords: Biocontrol, Human, phiVibrioH1, Seafood, *V. parahaemolyticus*.

Introduction

In seafood particularly shellfish, *Vibrio parahaemolyticus* is presented naturally in the marine environment and has the ability to tolerate highly extremely habitat conditions as increasing in temperature, alkalinity, and high salt concentrations, which is found marine organism (Wong et al., 1999). In the last 20 years, human intestinal infections, blood toxicity and incision infection occurred via eating shellfish and insinuation to seawater contaminated with Vibrio species (Thomspon et al., 2004). This pathogenic bacterium is mostly responsible for early mortality. Acute destroy to liver and pancreas, colonization in the stomach of prawn and discharges a vigorous toxin that disperses over the aquaculture production takes place by *V. parahaemolyticus*. (Lai et al., 2015). Gastrointestinal infections occurred via exhaustion of foods infected with huge intensity of total *V. parahaemolyticus* and/or pathogenic *V. parahaemolyticus*. The infection by *V. parahaemolyticus* is proposed to occur via skin wounds. Gastroenteritis, wound infection and blood toxicity are the main syndromes caused by *V. parahaemolyticus*. Microbe resistance perceived

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noteworthy worldwide risk concern to worldwide nation validity and bread safety (FAO, 2016). The emergence and prevalence of microbe resistance in aquaculture occurred due to broad use and abuse of antibiotics. Via chromosomal DNA mutations, enzymatic suppression, genetic recombination multidrug-resistant (MDR) bacterial strains are developed (Van Hoek et al., 2011). Promotion of aquatic organism's prosperity and controlling the pathogenic bacterial infection even tuated through antibiotics addition in water and feed. It was found that respectable appearance of MDR*Vibrio* species is due to inclusive utilization of antibiotics in fish farming (Letchumananet al., 2015b). *Vibrio* spp. infections therapy occurs via utilization some widely suggested clinical antibiotics as quinolones, cephalosporins, tetracycline, cefotaxime, ceftazidime and penicillins (Han et al., 2007; Wong et al., 2015). It was found that *V. parahaemolyticus* strains isolated from shellfish and environmental origins in Malaysia are reluctant to cephalexin and ciprofloxacin (Al-Othrubi et al., 2011). In India, isolation of *V. parahaemolyticus* strains, which resist antibiotics, takes place from clinical and ecological samples (Sudha et al., 2014). Multidrug resistance outline on the way to ampicillin, oxytetracycline and chloramphenicol is showed by the bacterial strains (Dang et al., 2007). Virulence factors in *V. parahaemolyticus* which are thermostable direct hemolysin and thermostable direct-related hemolysinand play an important role in pathogenicity. TDH is a heat stable protein, which resists boiling at 100°C for 10min and has the ability to make pores (Yanagihara et al., 2010). Hemolysis and cytotoxicity are elicited via TDH (Raimondi et al., 2000). In Maldives, gastroenteritis outbreak in 1985 occurred due to the heat labile TRH (Honda et al., 1988). It was found a resemblance between TRH and TDH in biological, immunological, and physicochemical properties as termed by Honda & Iida (1993). Via chloride secretion, both hemolysins affect the epithelial cells of human colon (Takahashi et al., 2000). The appearance of genes responsible for antibiotic resistant in bacteria is due to extensive usage of several antibiotics in aqua farming. Penicillin and B-lactam resistant genes are types of numerous various ARGs found in bacteria in the habitat (Zhang et al., 2009). Cat and floR genes are responsible for chloramphenicol resistance (Dang et al., 2008). Tet genes and many more are considered genes for tetracycline resistance (Kim et al., 2013). Encoding of heat stable hemolysin gene (*tdh*) of *V. parahaemolyticus* causes serious health hazards (Richards, 1988). In most clinical isolates of *V. parahaemolyticus* have *tdh* gene is utilized as pathogenicity marker (Nordstrom et al., 2007). Unfortunately; most commercially available antibiotics are resisted by Vibrio species (Letchumanan et al., 2015b). There was lack of novel antibacterial drugs in formative productivity line (Rice, 2008; Freire-Moran et al., 2011). As of late, there has been restored enthusiasm for utilization of bacteriophage as alternative way to deal with the bacterial diseases (Wittebole et al., 2014). Therefore, this study was aimed to isolate and characterize bacteriophage against multidrug resistant (MDR) *V. parahaemolyticus* isolated from seafood (shrimps, crabs and mullets) and hand swabs of humans, and also to determine the lytic performance of recovered phage on the growth of pathogenic *V. parahaemolyticus* isolate.

Materials and Methods

Sample collection and isolation of V. parahaemolyticus strains

Two hundred sea food samples including [white shrimp (n= 80), blue crabs (n= 70) and mullets (n= 50)] and fifty hand swabs of humans handling fish were collected from Zagazig fish market during period of February to May, 2018. The muscles of mullets, shrimps and crabs as well as the hand swabs of humans (fish sellers) were subjected to enrichment; then inoculated on Thiosulphatecitrate bile salts sucrose agar media (TCBSA) and incubated at 37°C for 18hrs **(**as previously explained Pinto et al. (2008) and Colakoglu et al. (2006).

Identification of bacterial strains

The isolated bacterial colonies were identified using morphological and biochemical tests according to Farmer et al. (2005).

Molecular detection of tdh gene in recovered V. parahaemolyticus

Genomic DNA extraction of bacterial isolates

The pure culture of biochemically identified *V. parahaemolyticus* isolate was subjected to centrifugation at 13000g for 5min at room temperature. Extraction of genomic DNA of *V. parahaemolyticus* was performed using QIAamp DNA Mini Kit (QIAGEN, Germany). The elution of DNA was done from QIA amp spin column into the elution buffer.

Primer design and PCR cycling conditions

The sequences of the primers of thermostable direct hemolysin gene including TDH-L: 5′GTA AAG GTC TCT GAC TTT TGG AC 3′ and TDH-R: 5′TGG AAT AGA ACC TTC ATC TTC ACC 3′ was designed according to Nishibuchi & Kaper (1985).

PCR reaction was done in a total volume of 25µl as a reaction mixture including template DNA (5µl), 20pmol of each primer and 1X of PCR master mix (Taq Master/, Jena Bioscience) which that contains 2.5 units per reaction of DNA polymerase, 0.2mM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, dTTP), 1 X PCR buffer (with 1.5mM–$MgCl_2$). The program of PCR conditions to amplify *tdh* gene in *V. parahaemolyticus* isolates was carried out according to Bej et al. (1999). The PCR cycling program included initial denaturation (95°C/15min), followed by denaturation for 30 cycles at 94°C for 30sec, annealing (60°C for 30sec) and extension at 72°C/ 45 seconds and a final extension at 72°C for 10min.

Antibiotic susceptibility test

Antibiotic susceptibility testing of *V. parahaemolyticus* was done by disc diffusion test using different 12 antibiotic discs (Oxoid, UK) including 12 antibiotics: amikacin (30µg), cefotaxime (30µg), ceftazidime (30µg), ampicillin (10µg), chloramphenicol (30µg), gentamicin (30µg), kanamycin (30µg), imipenem (10µg), sulfamethoxazole/trimethoprim (25µg), oxytetracycline (30µg), nalidixic acid (30µg) and levofloxacin (5µg). The antibiotic susceptibility of *V. parahaemolyticus* isolates were achieved using the disc diffusion test according to Yano et al. (2014). Results of the inhibition zone were detected and deciphered using the guidelines of Clinical and Laboratory Standards Institute (CLSI) M45-A2 (Clinical Laboratory Standard Institute (CLSI, 2010).

Phage isolation

The bacteriophage was isolated from sewage water samples at Zagazig City, Egypt using the enrichment method (Adams, 1959). Briefly, a sewage sample was exposed to centrifugation at 10,000xg for 15min, and then passed via a sterile 0.45µm Millipore filter (Steradisc, Kurabo Industries LTD., Japan). Fifty milliliters of the filtrate was appended to an equivalent volume of Luria-Bertani (LB,Oxoid,UK) broth, which was inoculated with 1ml of *V. parahaemolyticus*, then subjected to incubation with shaker at 120rpm at a temperature of 37°C/24hrs. The centrifugation of bacterial cultures was performed at 10,000xg/ 10min. The supernatant was percolated using a sterile 0.45µm Millipore strainer, then used as a phage source to be found on the propagative *V. parahaemolyticus* isolates. The bacterial lawns of *V. parahaemolyticus* were subjected to propagation on LB plates using the double agar overlay method (Adams, 1959) Two hundred µl of *V. parahaemolyticus* (OD of 0.4 at 600nm) were added to semi-solid LB agar (4ml), then poured over nutrient agar plates. The droplets of formerly prepared bacteriophage source (10µl) were spotted on the bacterial lawns, and then were left for drying. Incubation of plates was carried out at 37°C stay lasting one night and examined for existence of the lytic areas.

Purification and proliferation of phage

The bacteriophage was purified by three single plaque isolation assays using sterile pasture pipette (Adams, 1959). In brief, a single plaque was picked up, then placed in 0.5ml of nutrient broth harboring 100µl of *V. parahemolyticus*, then subjected to incubation at 37ºC with shaking at 1200rpm. After wards, centrifugation of phage-bacteria mixture was done at 10,000xg for 10min. The supernatant was percolated via a sterile 0.45µm Millipore strainer to exclude any bacteria. Storage of purified phages was done at 4ºC.

Determination of phage host range

The isolated bacteriophage was tested against 40 isolates of pathogenic bacteria including *V. parahaemolyticus* (n= 12), *V. vulnificus* (n= 5), *V. fluvialis* (n= 4), *V. alginolyticus* (n= 3), *Pseudomonas aeruginosa* (n= 5), *E. coli* O26 (n= 3) and *Proteus vulgaris* (n= 4), *Bacillus cereus* (n= 2) and *Staphylococcus aureus* (n= 2) to detect the host range as previously explained (Carvalho et al., 2010). The propagation of bacterial lawns was achieved on LB agar plates, followed by addition of 10µl of phage droplets (1 X 10 plague forming unit, PFU/ ml). These plates were incubated at 37ºC/ 24hrs then tested for existence of plaques (lytic zones). Efficient phage was selected on the bases of lysis profile, clarity of plaque and size.

Transmission electron microscopy analysis

Phage droplet (10^7 PFU/ml) was located on grids which coated with the carbon films, and over abundant was removed with a filter paper. The uranyl acetate as a saturated solution was placed

on grids, and the excess was removed by filter paper. The purified phage particle was stained with uranyl acetate, and then was investigatedviaEM (Hitachi H600A, Japan) at Mansoura University, Egypt (Abdel-Haliem & Askora, 2013).

One -step growth experiment

Determination of the bacteriophage one step growth curve was done according to Pajunen et al. (2000). The phage was added at a multiplicity of infection (MOI)= 1.0 to *V. parahaemolyticus* cells then the phage was allowed to be adsorbed on host cells at 37ºC/ 10min. The phage-host blend was centrifuged at 10,000 xg for 10min, and the precipitate was annotated in 10ml of nutrient broth then incubated at 25ºC. The specimens were possessed twice at 15min interval for 160min. The titer of phage was estimated by the double-layer plague assay. The first sample set was subjected to dilution before titration; while, 1% chloroform (v/v) was used to treat the second sample set for the release of intracellular phages and determination of the eclipse period. Three independent assays were carried out.

Nucleic acids characterization of phage particle

The genomic DNA of isolated phage (10^7 PFU/ml) was released using the phenol/chloroform procedure as previously described (Sambrook & Russell, 2001). Two restriction enzymes (*Eco*R1, and *Bam* H1) were used to digest phage DNA according to the manufacturer's instructions (Takara Bio Inc., Japan). The restriction enzymes required a time ranging from 2-4hrs. The investigation of DNA digestion bands was performed via electrophoresis (at 100V) in agarose gel (1.0%) embedded with the ethidium bromide.

Effects of phage on the growth of V. parahaemolyticus

The efficacy of isolated phage were evaluated on the growth of MDR *V. paraheamolyticus* isolate in a liquid media over a time period from zero time to 42hrs by measurement of the optical density (O.D. 600). The freshly prepared bacterial culture of *V. parahaemolyticus* (0.1ml) and phage filtrate (0.1ml) were added to sterile nutrient broth (3ml) at MOI equals to 1.0 starting from zero time to 42hrs. The optical density (OD_{600}) was measured each 6hrs interval for the bacterial culture alone and for the treated bacterial broth (bacteria+ recovered phage) as previouslyexplained (Mahmoud et al., 2018). This experiment was performed for independently three times.

Results

Prevalence and distribution of pathogenic V. parahaemolyticus strains from seafood and humans

The prevalence of *V. parahaemolyticus* was determined among samples of shrimp, blue crabs, and mullets and also in hand swabs of humans selling these fish species based on the isolation and biochemical identification of isolates as illustrated in Table 1. The prevalence of *V. parahaemolyticus* was higher (28%, 14 out of 50) in muscles of mullets followed by 25.7% (18/70) for muscles of blue crabs, then 18.8% (15 out of 80) in white shrimps muscles and the lowest isolation rate (14%, 7 out of 50) was detected in hand swabs of humans (Table 1).

Polymerase chain reaction (PCR) was used to identify thermostable direct hemolysin (*tdh*) gene that determines the pathogenicity of recovered *V. parahaemolyticus* strains. It was found that the distribution of *tdh* gene in *V. parahemolyticus* isolates (tdh^+ isolates) was 60% (9 out of 15) in white shrimps followed by 44.4% (8 out of 18) in blue crabs, then 42.8% (3 out of 7) in humans; while the lowest distribution of *tdh*+ *V. parahemolyticus* (35.7%, 5/14) in muscles of mullets (Table 1).

TABLE 1. Prevalence and distribution of pathogenic tdh^+ and tdh^- *V. parahaemolticus* isolates from seafood and humans.

Source of samples	Number of examined samples	Number of positive samples (%)	**tdh*+ No.(%)	*tdh*- No. (%)
Muscles of white shrimp	80	15(18.8)	9(60)	6(40)
Muscles of blue crabs	70	18(25.7)	8(44.4)	10(55.5)
Muscles of mullets	50	14(28)	5(35.7)	9(64.3)
Hand swabs of humans	50	7(14)	3(42.8)	4(57.1)

tdh+*:* Positive isolate of *V. parahaemolyticus* for thermostable direct hemolysin gene (*tdh*).

Antibiotics susceptability of V. parahaemolyticus

Results of antibiotic susceptibility of *V. parahaemolyticus* were summarized in Table 2. The isolated bacteria were highly resistant to most of antibiotics where the peak level of resistance was detected to ampicillin (10µg) (100%), chloramphenicol (30µg) (100%), amikacin (30µg) (100%), oxytetracycline (30µg) (100%) and sulfamethoxazole/trimethoprim (25µg) (100%). The results showed variability in percentages of resistance to kanamycin (30µg), gentamicin (30µg) and imipenem (10µg) were 76%, 72% and 52%, respectively. For cefotaxime (30µg), 36% of isolates were resistant, 40% were intermediate and 24% were sensitive. Forty four percent of tested isolates were sensitive to ceftazidime (30µg), 32% were intermediate and 28% were resistant. The resistance of bacterial isolates was 28% and 40% toward levofloxacin (5µg) and nalidixic acid (30µg), respectively.

Isolation of bacteriophages and morphological characterization (electron microscopy)

The phage isolation against multidrug resistant and Pathogenic *V. parahaemolyticus* was detected in sewage from Zagazig City using the spot test and the double over layer agar techniques. Phage phiVibrioH1 was able to form clear pen point circular plaques on the host lawn; with diameter about 2mm (Photo 1). Single plaques were selected and elected for moreover purification, amplification and characterization. A single plaque was picked up for the propagation and characterization. The used phage concentration in the techniques of propagation and purification was 10^7 PFU/ml. Three phage particles were noticed during electron micrograph. The recovered phage was designated as phiVibrioH1. The phage phiVibrioH1 was belonged to the family Myoviridae due to the presence of a contractile tail (Fig. 1). PhiVibrioH1 had a mean head diameter of 60x36.6nm, a tail length of 103.3nm.

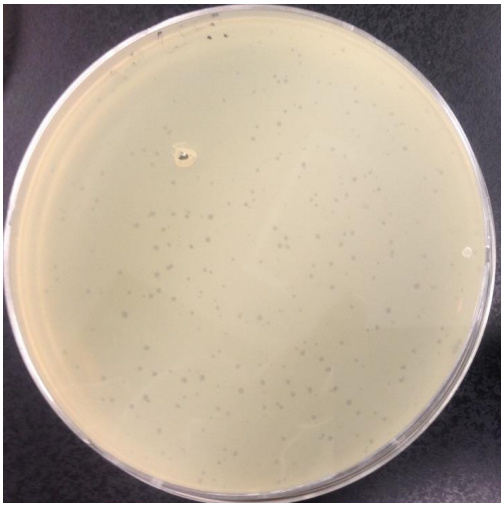

Photo 1. Plaques of bacteriophage phiVibrioH1 appeared as a clear pen point circular plaques using single plaque assay.

TABLE 2. Antimicrobial resistance patterns of *V. parahaemolyticus* isolated from seafood and humans by the disc diffusion method.

Antimicrobials (disc content/µg)	Pathogenic *V. parahaemolyticus* (no= 25)		
	R* No. (%)	I No. (%)	S No. (%)
Ampicillin (10)	25(100)	0 (0.00)	0 (0.00)
Amikacin (30)	25(100)	0 (0.00)	0 (0.00)
Cefotaxime (30)	9 (36)	10 (40)	6 (24)
Ceftazidime (30)	6 (28)	8 (32)	11(44)
Chloramphenicol (30)	25 (100)	0 (0.00)	0 (0.00)
Gentamicin (30)	18 (72)	6 (24)	1 (4)
Imipenem (10)	13 (52)	9 (36)	3 (12)
Kanamycin (30)	19 (76)	3 (12)	3 (12)
Levofloxacin (5)	7 (28)	9 (36)	9 (36)
Nalidixic acid (30)	10 (40)	11 (44)	4 (16)
Oxytetracycline (30)	25 (100)	0 (0.00)	0 (0.00)
Sulfamethoxazole/trimethoprim (25)	25 (100)	0 (0.00)	0 (0.00)

*R: Resistant, I: Intermediate susceptibility, S: sensitive.

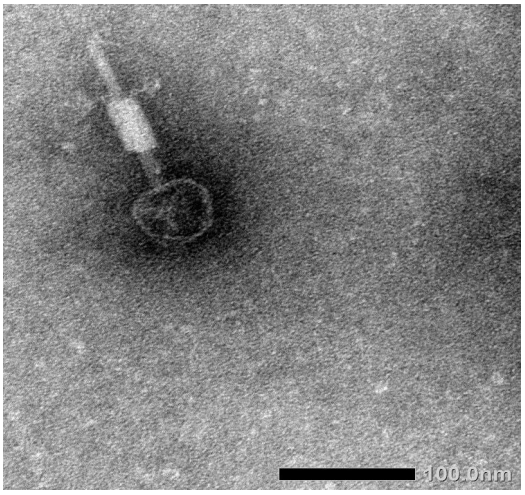

Fig. 1. Electron micrograph of a Myoviridae phage (phiVibrioH1) [The phage had icoshedral head and a contractile tail].

Host range of PhiVibrioH1 phage

Determination of phiVibrioH1 host range was done via using twelve different strains of *V. parahaemolyticus*, five *V. vulnificus*, four different *V. fluvialis* strains, three *V. alginolyticus*, five *P. aeruginosa*, two *Bacillus cereus* strains, two *S. aureus*, four *P. vulgaris* strains and three *E. coli* O26. (Table 3). The results in Table 3 revealed that our isolated phiVibrioH1 phage had the capability to induce infection and lysis for MDR *Vibrio parahaemolyticus* isolates, *V. vulnificus*, *V. fluvialis*, *V. alginolyticus*, *P. aeruginosa, P. vulgaris* strains and *E. coli* serotype O26; while the bacteriophage could not infect Gram positive bacteria (*B. cereus* and *S. aureus* strains) as shown in Table 3. Our results revealed that phiVibrioH1 had a lytic effect on all tested bacterial strains; and this phage had a wide host range (Table 3).

Characteristics of single step growth curve

In Fig. 2, the one step growth curve of phiVibrioH1 phage showed a latent period of 35min then followed by a rise period of 75-80min. The average burst size was 90PFU/ infected bacterial cell.

Genomic characterization of Vibrio parahaemolyticus bacteriophage

Two restriction enzymes (*Eco*RI, and *Bam* H1) were utilized for digestion of DNA isolated from phage (i.e., phage concentration 10^7PFU/ ml). The genomic DNA of phage was sensitive to the digestion with the restriction enzymes (Fig. 3). The enzymes *Eco*R1 and *Bam* H1 digested the genomic DNA of PhiVibrioH1 into 10 and 7 bands, respectively (Fig. 3). Our results indicated that the genome of PhiVibrioH1 phage was a linear double-stranded DNA owing to its susceptibility to digestion.

TABLE 3. The host range of isolated phage phiVibrioH1.

Bacterial strains	No. of used isolates	phiVibrioH1	Source of strains
V. parahaemolyticus	12	+	This study
V. vulnificus	5	+	ZVZU
V. fluvialis	4	+	ZVZU
V. alginolyticus	3	+	MSZU
Pseudomonas aeruginosa	5	+	MSZU
Bacillus cereus	2	-	MSZU
Staphylococcus aureus	2	-	ZVZU
Proteus vulagaris	4	+	MSZU
Escherichia coli O26	3	+	MSZU

(+): Bacterial strain was susceptible to the bacteriophage phiVibrioH1 and the plaques were produced.
(-): Bacterial strain was resistant to the bacteriophage phiVibrioH1 and the plaques were not produced.
MSZU: Microbiology Department, Faculty of Science, Zagazig University, Egypt.
ZVZU: Zoonoses Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

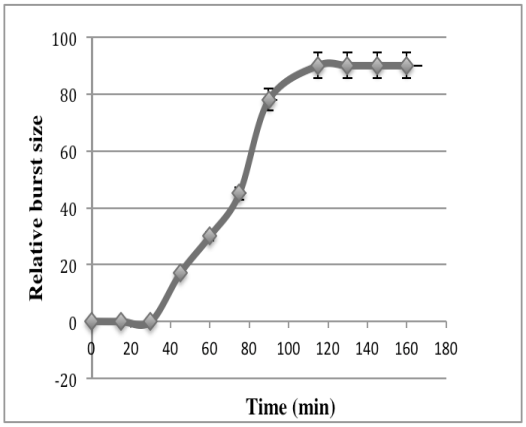

Fig. 2. Single step growth curve of isolated phiVibrioH1 [The plaque forming Units per infected cell in *Vibrio parahaemolyticus* cultures were calculated at various times post-treatment. The samples were taken after an interval of 15min. The means of relative burst size were calculated ± standard deviation].

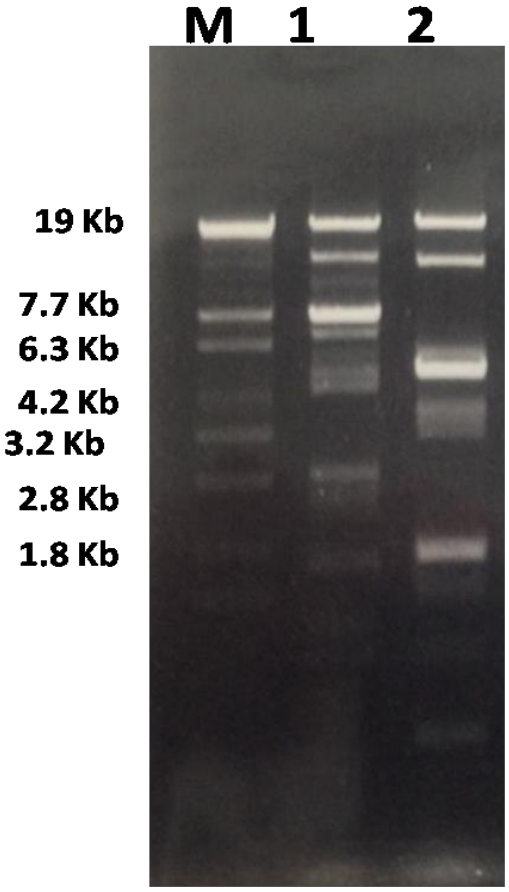

Fig. 3. Digestion patterns of phiVibrioH1 phage with two restriction enzymes [M: Lambda DNA marker, Lane 1: phiVibrioH1 phage subjected to digestion with EcoRI, Lane 2: phiVibrioH1 phage subjected to digestion with Bam H1].

Effect of phiVibrioH1 phage on the growth of pathogenic V. parahaemolyticus

The lytic effects of phiVibrioH1 phage on the growth of pathogenic *V. parahaemolyticus* isolate (positive for *tdh* gene and revealing multidrug resistance) was determined by measuring the optical density (OD600) of bacterial culture infected with this myoviridae phage at MOI of 1.0 and followed by incubation at 37ºC (Fig. 4). From 6-12hrs post infection, the growth of bacterial hosts were increased by phage infection (OD values were much higher compared with uninfected control) and after 12-18hrs post-treatment, the growth of bacterial cells were reduced by bacteriophage infection (values of OD were much lower when compared with the untreated control) (Fig.4). After 18-24hrs post infection, the lysis of bacterial cells was apparent. From 24 to 42hrs post-treatment of bacteria with the phiVibrioH1 phage showed complete lysis of bacterial cells (Fig.4).

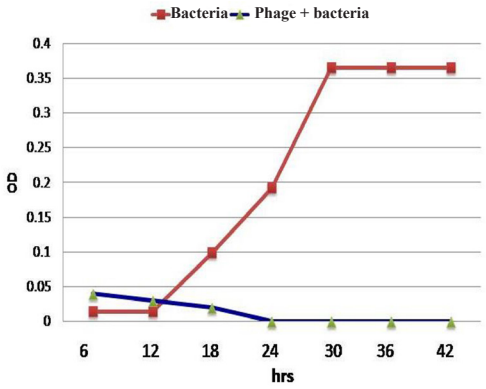

Fig. 4. Effect of phiVibrioH1 phage on the growth of pathogenic *V. parahaemolyticus* at 37ºC at MOI of 1.0 [Each data point was referring to means of bacterial OD± standard deviation].

Discussion

Moderately predominance and microbial capacities of pathogenic *V. parahaemolyticus* were detected in 28% (14/50) of blue crabs samples. Comparable results were found in Baffone et al. (2000) detected the existence of *V. parahaemolyticus* in the collected samples in percentage 2.6% (3/114). Comparative investigation discoveries detailed by Letchumanan et al. (2015a) additionally found an abnormal increase in the number of *V. parahaemolyticus* bought from sodden markets compared to supermarkets. To preserve the freshness of seafood and control the propagation of hurtful

microorganisms, the maintenance of storage temperature must be required due to seafood is easily to decay. In Egypt, there is no norm security boarder or minimum permissible grade of *V. parahaemolyticus* in shellfish. Security systems and scales for fish and seafood yield confirmed from different nations, for example, the United States is eluded. Limits for *V. parahaemolyticus* in prepared to-eat fish output must be <1 × 104 for each gram as revealedvia the FDA microbiological wellbeing (FDA, 2011). Fish looting, washing, flushing, and cooking process done by food handlers amid food readiness is recommended equipped for accomplishing certain decreases of *V. parahaemolyticus*. For precedents, Watanabe et al. (1994) exhibited adequacy cleaning of the horse mackerel with water decreasing *V. parahaemolyticus* about 1.99 logs. Ye et al. (2012) announced gentle warmth handling at 50◦C for 20min was fit for diminishing the quantity of *V. parahaemolyticus* to underneath revelation scale (<3 MPN/g).

Our results revealed that 60%, 44.4%, 42.8% and 35.71% of the 54 positive *V. parahaemolyticus* samples isolated from Flesh of white shrimp, Blue crabs, Fish sellers and Mullets, respectively were carried *tdh* gene and these results are considered a high prevalence if compared with that obtained by Tan et al. (2017) found that *V. parahaemolyticus* strains with *tdh* gene were found in low level 8.5% in short mackerels. Rodriguez-Castro et al. (2010) reported that *tdh*+ *V. parahaemolyticus* tends to expand in the cozy water. Suthienkul et al. (1995) reported that, 27 *V. parahaemolyticus* isolates abroad of 489 (6%)taken from patients with acute gastroenteritis had the *trh* and *tdh* genes. Bhoopong et al. (2007) reported that in Thailand *tdh* gene was detected in 87.4% of the clinical *V. parahaemolyticus* isolates isolated from sixty three patients. However, there are many factors affecting the allocations of *tdh*+ and/or *trh*+ strains as the geographical area, sample origin, and revelation process (Raghunath, 2015). Our results showed that *V. parahaemolyticus* isolates obtained from seafood and humans exhibited a high level of resistance to ampicillin (10µg) (100%), amikacin (30µg) (100%), chloramphenicol (30µg) (100%), oxytetracycline (30µg) (100%) and sulfamethoxazole/ trimethoprim (25µg) (100%). The results obtained by Elexson et al. (2014) revealed that seafood products harbored *V. parahaemolyticus* isolates all of which were resistant to ampicillin. Additionally our results revealed variability in percentage of resistance to kanamycin (30µg), gentamicin (30µg) and imipenem (10µg) were 76%, 72% and 52%, respectively. For cefotaxime (30µg), 36% of isolates were resistant, 40% were intermediate and 24% were sensitive. Forty four percent of tested isolates were sensitive to ceftazidime (30µg), 32% were intermediate and 28% were resistant. The resistance of bacterial isolates were 28% and 40% toward levofloxacin (5µg) and nalidixic acid (30µg), respectively. It was found that Fluoroquinolones, tetracyclines, third-generation cephalosporins, aminoglycosides and folate pathway inhibitors (trimethoprim-sulfamethoxazole) are considered the proposed antibiotics against *Vibrios* (Daniels & Shafaie, 2000). However, as reported by the United States Centers for Disease Control and Prevention (CDC), since 2001, infections via *Vibrio* have increased dramatically (CDC, 2016). Gram-negative bacteria have created disparate techniques to sidestep the preventing impacts of antibiotics like (1) Drug deactivation/devastation (2) Lowering antibiotic permeation and passing (3) Target site alteration and (4) Secular cell modifications (Kumar & Singh, 2013; Munita & Arias, 2016; Miller, 2016). The evolution and predominance of microbe resistance in aquaculture occurred due to broad use and abuse of antibiotics. (Van Hoek et al., 2011). Promotion of aquatic organism's prosperity and controlling the pathogenic bacterial infection eventuated through antibiotics addition in water and feed. It was found that respectable appearance of AMR *Vibrio* species is due to excessive usage of antibiotics in fish farming (Letchumanan et al., 2015b). Due to persistent evolution of MDR bacterial strains in the environment, it was required an alternative tool to antibiotics (Tan et al., 2017). For biological control of bacterial diseases in marine aquaculture, it was suggested to utilize bacteriophages as charming substitutional to antibiotics (Almeida et al., 2009). To control infectious diseases caused by bacteria, usage of phage therapy has some novel favorable circumstances over antibiotics, as high host particularity, self-propagation, and low ecological effect (Efrony et al., 2007; Almeida et al., 2009). Utilization of phage considered as an alternative solution to multi-drug resistant bacteria. The investigations of Jun et al. (2014) recommend that phage treatment is a hopeful agent to commute antibiotics on

biological control of overwhelming disorders in marine habitats. In our study, a lytic phage specific against *V. parahaemolyticus* strain was isolated and characterized. This phage is tailed virus and belongs to the family *Siphoviridae*. Previous studies had indicated that ultimate of phages particular averse to *Vibrio* spp. related to *Siphoviridae* (Phumkhachorn & Rattanachaikunsopon, 2010; Thiyagarajan et al., 2011). These confirmations showed that members of the family *Siphoviridae* are pervasive in aquaculture habitat and may be successful to control *Vibrios* infections in aquaculture systems.

Conclusion

There was a reduction in OD of *V. parahaemolyticus* co-treated with the bacteriophage from 18- 24hrs post infection compared to the controls and induced complete lysis from 24-42hrs post-treatment. Our study confirmed that the phiVibrioH1 is a lytic phage, wide host range and has a high potential to control pathogenic MDR *V. parahaemolyticus* strains recovered from seafood and humans handling fish.

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العزل والتوصيف الجيني لل Myoviridae فاج (phiVibrioH1) للتحكم في سلالة *Vibrio parahaemolyticus* الممرضة من الكائنات البحرية والإنسان

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إن سلالة *Vibrio parahaemolyticus* هو كائن ممرض للفصيلة الحيوانية ويسبب مرض vibriosis في الأسماك البحرية وهو مرتبط بتفشي التسمم الغذائي في البشر. هذه الدراسة هدفت إلى عزل وتوصيف البكتيريوفاج ضد *Vibrio parahaemolyticus* المقاومة للأدوية المتعددة (MDR) من الكائنات البحرية والبشر ، وكذلك لتقييم الفعالية التحللية للفاج على النموالبكتيرى. تم عزل *Vibrio parahaemolyticus* من لحم 80 جمبري أبيض و 70 سلطعون أزرق و 50 من أسماك البورى ، ومن 50 مسحة يد بشرية (بائعين الأسماك) على وسط Thiosulphate Citrate bile salts sucrose agar. تم تعريف المستعمرات البكتيرية بيوكيميائيا وتم الكشف عن الجين الممرض (+ tdh) جزيئيًا. وتم إختبار خمسة و عشرون عزلة من البكتيريا الحاملة للجين (+ tdh) على إثنى عشر مضاد حيوى بإستخدام طريقة الإنتشار بالقرص. وقد وجد خمسة عشر عزلة من *V. parhaemolyticus* (+tdh) مقاومة بنسبة 100% لخمسة مضادات حيوية. تم عزل البكتيروفاج من مياه الصرف الصحي باستخدام تحليلات إختبار البقعة وطبقة الأجار المزدوجة. وقد وجد أن عزلة الفاج phiVibrioH1 ينتمي إلى عائلة Myoviridae وفقًا للفحص المجهري الإلكتروني. هذا وقد كشفت هذه الدراسة عن أن هذا الفاج متعدد الإصابة لمدى واسع من العوائل المقاومة للمضادات الحيوية (MDR) مثل *V. vulnificus* و *V. fluvialis* و *V. alginolyticus* و *Pseudomonas aeruginosa* و *E. coli* O26 و *Proteus vulgaris*. وكان الجينوم الخاص بالفاج حساسًا للتكسير بإستخدام إنزيمات القطع BamH1 و ECoR1 والتي تشير إلى إزدواجية الحامض النووي. وقد تحللت الخلايا البكتيرية تحللًا كاملاً فى الفترة من 24 إلى 42 ساعة بعد التعرض لتأثير الفاج المعزول. أكدت هذه الدراسة أن الفاج phiVibrioH1 من الفاجات المحللة ولديه قدرة عالية للسيطرة على سلالات بكتيريا *V. parahaemolyticus* المسببة للأمراض المستردة من الكائنات البحرية والبشر.