

**MINISTRY OF EDUCATION  
AND TRAINING**

**MINISTRY OF  
HEALTH**

**National Institute of Hygiene and Epidemiology**

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**LAI VU KIM**

**CIRCULATION AND LYSING ABILITY OF  
CHOLERA PHAGES (VIBRIOPHAGE) IN THE  
AQUATIC ENVIRONMENT IN SOME NORTHERN  
PROVINCES OF VIETNAM**

**Specialization: Public Health**

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## **PUBLISHED WORKS RELATED TO THE THESIS**

1. **Lai Vu Kim**, Nguyen Dong Tu, Dang Duc Nhu, Le Dang Hai, Ngo Tuan Cuong, Nguyen Hoai Thu, Le Thanh Huong, Dang Duc Anh (2019): Circulation and characteristics of some cholera phages (Vibriophage) isolated in Northern Vietnam. *Journal of Preventive Medicine*, vol. 29(13): 88 – 102.
2. **Lai Vu Kim**, Nguyen Dong Tu, Dang Duc Nhu, Le Thanh Huong, Vu Thi Mai Hien, Ngo Tuan Cuong, Hoang Thi Thu Ha (2022): Characteristics of cholera lytic phage (lytic cholera phage) VP14 isolated in Thai Binh province in 2009. *Journal of Preventive Medicine*, vol. 32(3): 63 – 70.
3. **Lai Vu Kim**, Le Thanh Huong, Dang Duc Nhu, Vu Hai Ha, Ngo Tuan Cuong, Hoang Thi Thu Ha, Nguyen Dong Tu, Vu Thi Mai Hien (2023): Detection of cholera phage in outdoor water environment in some Northern provinces of Vietnam, 2018-2019. *Journal of Preventive Medicine*, vol. 33(2): 29 - 41.

## INTRODUCTION

Cholera is a clinical-epidemiological syndrome caused by cholera bacteria group O1 or O139. Since the discovery of the Cholera, there have been seven pandemics in the world. The prevention and treatment of cholera faces certain difficulties due to the emergence of drug-resistant cholera bacteria.

Research on cholera phage therapy to prevent and treat cholera began in the early 19th century and has recently attracted the attention of many researchers. In Vietnam, there have been a number of studies on cholera phages, but the studies have only stopped at monitoring cholera phages in the external water environment and classifying cholera phages on a small scale. There hasn't been in-depth research on the circulation, isolation, and evaluation of the dissolving ability of cholera phages in the aquatic environment water in the provinces that have experienced plague outbreaks in Northern Vietnam.

For these reasons, we conducted research on the topic: ***"Circulation and lysing ability of cholera phages (Vibriophage) in the aquatic environment in some Northern provinces of Vietnam"***

With the following objectives:

1. Describe the circulation of cholera phages (Vibriophage) in the aquatic environment in some provinces of Northern Vietnam, 2018 -2019.
2. Evaluate the lysing ability of cholera bacteriophages in the laboratory and in the community field under different conditions.

***New scientific points and practical value of this study***

This study is one of the very few studies conducted in Vietnam and has some results as follows:

1. Initially identify and describe the circulation of cholera bacteriophages in a large area of residential geographical community (4 provinces/cities in Northern Vietnam), with a sample size of 800 samples.

2. Evaluate the lysing ability of all 36 cholera bacteriophage strains included in the study under different conditions (temperature, pH, dilution) and are sensitive to cholera bacterial strains of different types. Classical biology (Classical), El tor, O139 Bengal, ...) at the Enteric Pathogens Laboratory, National Institute of Hygiene and Epidemiology. Evaluate the lysing ability and survival time in external environmental conditions of O1 bacteriophage strain with superior characteristics (VP04) in lysing ability among a total of 36 strains.

3. Propose a number of intervention measures to limit cholera outbreaks using a cholera monitoring and warning scheme based on environment water sample testing, thereby contributing to orienting and proposing a selection research strategy Cholera bacteriophages for use in preventing and controlling cholera epidemics, treating contaminated domestic water sources, and progressing to application in cholera treatment; in aquaculture, food industry and preservation.

## **STRUCTURE OF THE THESIS**

The thesis consists of 112 pages, excluding references and appendices, with 17 tables, 8 tables, 12 figures/diagrams. The introduction 2 pages. Overview 36 pages; the subject and research methods cover 16 pages; research results is 30 pages;

the discussion section is 24 pages long; the conclusion is 2 pages, and recommendations is 1 page.

## **Chapter 1. LITERATURE REVIEW**

### **1.1 Circulation of cholera phage**

Bacteriophage are present everywhere on Earth and are found in large numbers in the environments (water, soil, wastewater, etc.), wherever their hosts are present.

Madico, G. et al (1996) studied the presence of cholera O1 bacteria and cholera phage in wastewater in Peru from 1993 to 1996 during a cholera surveillance program in the capital Lima found that cholera outbreaks were 7.6 times more likely when cholera O1 bacteria were present in wastewater in the previous four weeks than when they were not. The likelihood of an outbreak was 2.4 times higher when cholera phages were present in wastewater in the previous 4 weeks compared to when there were none. Thus, the detection of cholera O1 bacteria and cholera phage in wastewater 1 month before an outbreak can be predictive of subsequent cholera outbreaks.

In many countries where cholera is endemic, cholera phages have been detected in wastewater and are considered to indicate the presence of cholera phages and aid in the typing of cholera strains O1 and O139. The presence of cholera phage in water contaminated with cholera O1 depends on the ability of the cholera phage to infect and lyse cholera cells. In many countries where cholera is endemic seasonally, environmental monitoring plays an important role in cholera control.

### **1.2 Phage therapy**

Phage therapy or phage treatment can be described as the use of bacteriophages to control specific pathogens or problem bacteria. In the field of medicine and human health, phage therapy has been practiced in Eastern European regions for more than 60 years. Early phage assays often yielded unreliable results due to inadequate understanding of plant biology and quality control in the preparation of phage therapeutic formulations.

Phage therapy in the water environment has been reported quite early through describing the relationship between cholera bacteria and cholera phages in the environment water. D'Herelle and Malone reported that the end of the cholera epidemic was due to the spread of bacteria from convalescent cases. Pasricha and colleagues (1931) studied the ratio of cholera bacteriophages in the external environment and their correlation with cholera bacteria in Calcutta, India. Scientists found that cholera phages in different external environments: morbidity and mortality rates were high at the beginning of the cholera season, decreasing rapidly when cholera phages were widely distributed in the external environment. Researchers have suggested that bacteriophages played an important role in reducing mortality and ending the outbreak.

About 60 years after Pasricha and colleagues reported the interesting relationship between cholera bacteria and cholera phages, Shah and colleagues reported a similar result, but with a systematic and systematic sampling method. Complete knowledge about cholera bacteria and cholera phages. Over a three-year period, scientists systematically analyzed water samples collected from two major rivers and a lake in Dhaka. The results showed that in the majority of water samples there

was an inverse relationship between the presence of cholera bacteriophages capable of lysing a certain serogroup of cholera bacteria and the presence of a strain of the same group. On the other hand, the number of cholera patients varied seasonally during the study period and frequently coincided with the presence of pathogenic cholera bacterial strains in water samples without detectable cholera phages.

## **Chapter 2. RESEARCH METHODS**

### **2.1 Subject, location and timeline of research**

#### **2.1.1 Research subjects**

- **Objective 1:** Water samples from the external environment (surface water and shrimp bait samples) in canals/ditches, ponds/lakes, and rivers were collected in four provinces in Northern Vietnam, specifically in four provinces/cities (Thai Binh, Hai Phong, Nam Dinh, and Hanoi).

- **Objective 2:**

+ The strains of cholera bacteriophages were isolated from samples in objective 1 and some cholera phage strains from the Enteric Pathogens Laboratory, National Institute of Hygiene and Epidemiology (EPLNIHE).

+ The strains of cholera bacteria isolated from cholera outbreaks in Vietnam, India, Japan, Bangladesh, and Thailand are stored in the EPLNIHE, National Institute of Hygiene and Epidemiology.

+ The other enteric pathogenic bacteria in the strain repository of EPLNIHE.



+ Samples of environmental and domestic water from various sources, including tap water, river/stream water, well water, rainwater, and pond/lake water, were collected in four provinces in Northern Vietnam.

### ***2.1.2 Research location***

- **Objective 1:** Selected 40 locations from the provinces/cities of Nam Dinh, Thai Binh, Hai Phong, and Hanoi (with each province/city choosing 10 sites).

- **Objective 2:**

+ Enteric Pathogens Laboratory, National Institute of Hygiene and Epidemiology.

+ Collected samples from environmental and household water represented for 05 water sources in the community (tap water, river/stream water, Ground well water, rainwater/reservoir water, pond/lake water) in the provinces of Nam Dinh, Thai Binh, Hai Phong, and Hanoi, then transported to a single location in Truc Thai commune, Truc Ninh district, Nam Dinh province, placed in 20 water containers (50 liters each) to introduce cholera bacteriophages over 06 months (from February 2020 to August 2020). Regular monthly sampling was conducted in 06 months.

### ***2.1.3 Research timeline***

- **Objective 1:**

+ Research: From December 2017 to February 2020.

+ Data analysis: From February 2018 to August 2019.

- **Objective 2:** From February 2020 to December 2020.

## **2.2 Research methods**

### ***2.2.1 Research Design***

- **Objective 1:** Cross-sectional descriptive study.

- **Objective 2:** Experimental descriptive study; before-after community intervention research with a control group.

### 2.2.2 Sample size and sampling technique

#### - Objective 1:

External water samples collected are applied using the following formula:

$$N = \frac{Z_{1-\alpha/2}^2 \times p \times (1 - p)}{d^2} \times DEEF$$

In these:

Z: reliability coefficient;  $\alpha = 0,05$ ,  $Z2(1- \alpha/2) = 1,96$ ; p = 0,36 is the proportion of cholera bacteriophage isolation from external water samples in a previous study by EPLNIHE; d: absolute accuracy, d= 0,05. DEEF: design factor. Because it is expected to take 2 types of surface water samples and shrimp swab swab samples to increase the ability to isolate and determine the presence of bacteriophages and take samples at many different locations, the design factor DEEF = 2 is chosen.

+ Apply the formula to calculate the sample size with 13% contingency as n=800. 800 environment water samples including 400 surface water samples and 400 shrimp swab samples collected in 04 provinces/cities (Thai Binh, Hai Phong, Nam Dinh and Hanoi).

+ Sampling method: Select 10 sites collection locations from each province and divide them into 3 types of water sampling points including: places with natural flows (Rivers); Where there is a water source for irrigation/domestic wastewater (Canals/Ditches); where there is a water source that does not circulate regularly (Lake/Pond/swamp), in accordance with criteria such as: near the home of the first cholera patient of cholera outbreaks reported in previous years, in the region

Coastal estuaries, a suitable place for the existence and development of cholera strains and cholera phages. Collect samples periodically every 2 months for 10 consecutive times.

**- Objective 2:**

+ In total of 36 strains of cholera phages: 10 strains isolated in the study at objective 1 and 26 strains of bacteriophages stored in EPLNIHE.

+ Includes 13 strains of cholera bacteria and 07 strains of other intestinal disease-causing bacteria in the strain warehouse of EPLNIHE.

+ Cholera bacteria: H218 O1 Classic and Mak757 O1 El tor (standard strains isolated from the cholera outbreak stored at EPLNIHE.

+ Cholera phage isolated in the study in objective 1, VP04, was used to determine the survival time and lysis ability of cholera phage against cholera bacteria (H218 O1 Classic) in different water sources: tap water; river/stream water; water wells; rainwater and pond/lake water.

**2.2.3 Research variables**

**- Objective 1:** Group of variables determining the circulation of cholera bacteriophages in the external water environment such as:

+ Isolation culture test results detected indicator strains Mak757 O1, El tor H218 O1, Classic AI4450 O139, Bengal distributed according to water sample type, sampling location, and time of month/year.

+ Cholera phage PCR test results (fs1, fs2) are distributed according to water sample type, sampling location, and time of month/year.

- **Objective 2:** Group of variables to evaluate the lysing ability of cholera phage in different environmental conditions such as:

The ability of phage to lyse some strains of cholera bacteria and some different types of intestinal bacteria in the laboratory.

+ Lysis ability of bacteriophages under different dilution, pH, and temperature conditions in the laboratory.

+ Survival time of cholera phage VP04 in domestic and outdoor water sources (tap water; river/stream water; well water; rainwater; pond/lake water) and lysing ability for H218 O1 Classic by week/month.

#### ***2.2.4 Method of Collecting information***

- **Objective 1:** Collect samples in the field, transport them and test samples at EPLNIHE. Using isolation culture and PCR (Polymerase Chain Reaction) methods to detect cholera bacteriophages in surface water samples and shrimp swab samples.

- **Objective 2:** Evaluate the lysing ability of cholera bacteriophages (through quantity and properties of tan streaks/Plaque) under different testing conditions in the laboratory as well as in domestic and environment water samples at EPLNIHE.

#### **2.3 Data analysis**

- Data will be entered into the computer using Epi Data software 3.1 and analyzed using SPSS 12.0 software. Bio-numeric software will be used to analyze the molecular biological characteristics of cholera phage strains.

- Compare the ability to detect cholera phage by testing method (isolation/PCR) according to sample type collected (shrimp swab bait/surface water) estimated by OR and 95%CI.

## 2.4 Ethics in research

The study used water samples from the environment and was allowed to use available strains of cholera phage, cholera bacteria, and intestinal pathogenic bacteria stored at the Enteric Pathogens Laboratory, Department of Bacteriology, National Institute of Hygiene and Hygiene, and not related to humans. The research was carried out according to Decision on detailed outline evaluation No. 1624/ Decision-NIHE dated November 8, 2017 and Decision on adjusting the topic name No. 1478/Decision-NIHE dated October 15, 2018 of the National Institute of Hygiene and Epidemiology. Isolated bacterial strains and bacteriophages are only for research purposes and are handled to ensure biosafety for operators and the environment.

## Chapter 3. RESULTS

### 3.1 Circulation of cholera phages in the external water environment in some northern provinces of Vietnam, 2018-2019

The total number of samples collected during the research period from February 2018 to August 2019 at 40 sampling locations in 4 provinces/cities was 800 samples (400 pairs of samples), including 400 surface water samples and 400 shrimp swab samples.

**Table 3. 1. The distribution ratio of water and shrimp bait samples by sample type, 2018 - 2019**

Collected sample site	Number (n)		Total	
	surface water	shrimp bait	Number (n)	Percentage (%)

Collected	Number (n)		Total	
	Canal/stream	260	260	520
Lagoon/Pond /lake	30	30	60	7,5
River	110	110	220	27,5
Total	400	400	800	100,0

In Table 3.1, out of a total of 800 surface water and shrimp bait samples, the majority were collected from canal (stream) water (n=520; 65%), followed by river samples (n=220; 27.5%), and lake water (n=60; 7.5%).

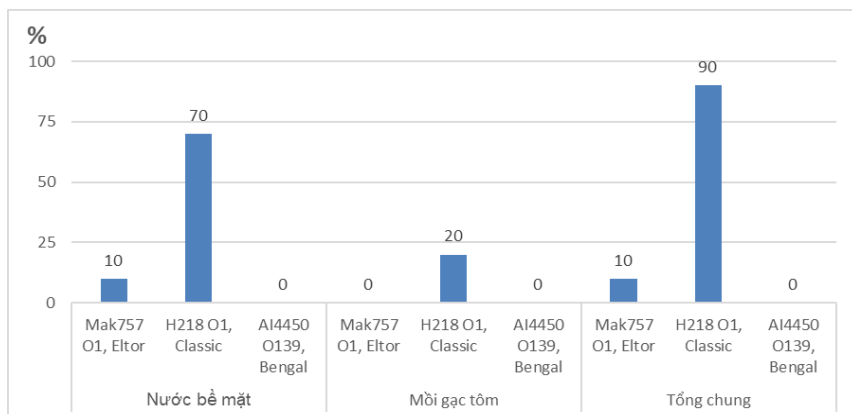
### ***3.1.1 Results of testing surface water samples and shrimp swab samples using the isolation cultivation method***

With surface water samples, the result was that no cholera phages were isolated using indicator strain AI4450 (O139), there was 01 strain isolated using indicator strain Mak757 (O1, El tor) and there were 07 cholera phage strains isolated using indicator strain H218 (O1, classic). Water samples taken in Hanoi and Nam Dinh could not isolate cholera phages; Water samples taken in Hai Phong and Thai Binh isolated 05 and 03 strains of cholera phage, respectively. There were no cholera phage isolated from water samples taken from ponds/lakes, there were 07 cholera phage isolated from water samples taken from canals/ditches and 01 cholera phage isolated from river water sample.

With the shrimp swab sample, the result was that no cholera phages were isolated using indicator strain AI4450 (O139) and indicator strain Mak757 (O1, El tor). There were 02 strains isolated using indicator strain H218 (O1, classic) and both were only isolated in Thai Binh from shrimp swab samples taken from canals/ditches.

According to the sampling time, the isolation results in surface water samples detected 07 cholera phages in samples taken in April, June and August 2018; There was 01 cholera phage detected in the sample taken in August 2019. 12.5% of cholera phage were isolated using indicator strain Mak757 (O1, El tor), 87.5% of cholera phage were isolated. Cholera bacteria were isolated using indicator strain H218 (O1, classical).

According to sampling time, the results of cholera phage isolation in shrimp swab bait samples showed that cholera phage was only detected in 02 samples taken in February and October 2018. 100.0% cholera phage isolated through the use of indicator strain H218 (O1, classical).



**Figure 3.2. Culture results of cholera phage isolation distributed by indicator strain, 2018-2019 (n=10)**

The results in Figure 3.2 show that through the isolation method, up to 90% of cholera phages were detected using indicator strain H218 (O1, classic). The remaining 10% of cholera phages were detected through the use of indicator strain Mak757 (O1, El tor).

### ***3.1.2 Results of testing surface water samples and shrimp swab samples using PCR method***

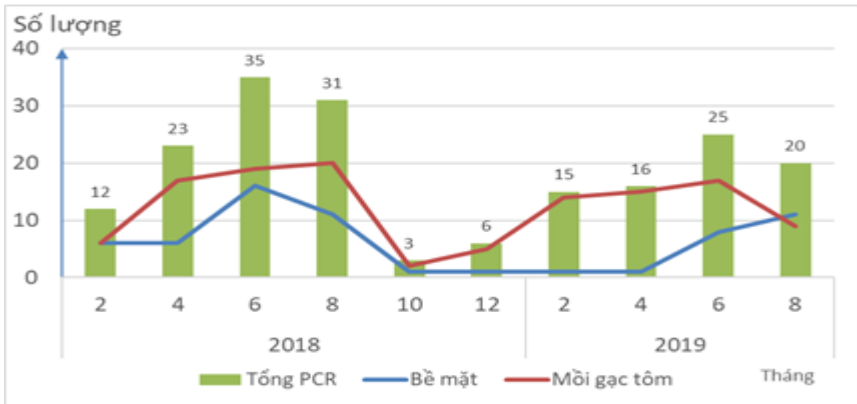
PCR results showed that 62/400 (15.5%) surface water samples detected filamentous cholera phages; 42/400 (10.5%) samples were filamentous cholera phage fs2, 20/400 (5.0%) samples were filamentous cholera phage fs1. There were 124/400 (31.0%) shrimp swab samples that detected cholera phages; 67/400 (16.8%) samples were filamentous cholera phage fs2, 57/400 (14.25%) samples were filamentous cholera phage fs1.

PCR results according to sampling time showed that cholera phages were detected in all months of 2018 and 2019 for surface water samples; fs2 is detected almost monthly; fs1 was only detected in February, April, June, August 2018 and August 2019. Of the total 62 detected samples, fs1 accounted for 32.3%, fs2 accounted for 67.7%.

PCR results according to sampling time showed that cholera phages were detected in all months of 2018 and 2019 for shrimp swab samples. Of the total 124 detected samples, fs1 accounts for 46.0%, fs2 accounts for 54.0%.

PCR results showed that cholera phages were detected in canal/ditch water samples (81.2%), river water samples (17.7%), and Shrimp pond/pond/lake water samples (1.1%); PCR results detected fs2 as 66.7%, fs1 as 33.3%.





**Figure 3.6. Cholera phage PCR results distributed over time, 2018-2019 (n=186)**

Figure 3.6 shows that PCR results detected more cholera phage using shrimp swab samples than surface water samples. Comparison between the two PCR methods and the isolation method shows that, with the PCR method, cholera phages are detected in every month of the year, most frequently in April and June; The isolation method detected cholera phages lower, focusing on April to August 2018. Shrimp swab samples were more valuable, although in the isolation method the difference was not significant, but with the isolation method, the difference was lower. PCR method of this sample type detects 2.4 times more cholera phages (95%CI: 1.7-3.5) than surface water samples. Regarding the testing method, PCR is 23.9 times more valuable in detecting cholera phage (95%CI: 12.6-51.1) than the isolation method.

### **3.2 Lysis ability of cholera bacteriophage**

#### ***3.2.1 Ability to lyse some strains of cholera and other intestinal bacteria***

**Table 3.12. Lysis ability of cholera phage against some strains of cholera bacteria**

STT	Vi khuẩn thử nghiệm													N(%)	
	1	2	3	4	5	6	7	8	9	10	11	12	13	n	%
Chung thực khuẩn thể tả	VP1	+	+	+	-	-	-	-	-	+	+	-	+	7	54,9
	VP2	-	-	+	-	-	-	-	-	-	-	+	-	2	15,4
	VP3	+	-	-	-	-	+	-	-	-	+	-	-	4	30,8
	VP4	+	+	+	-	+	-	-	-	-	+	+	+	8	61,5
	VP5	-	+	+	-	-	-	-	-	-	+	+	-	3	23,1
	VP6	+	-	-	-	-	-	-	-	-	-	-	-	2	15,4
	VP7	+	-	+	-	-	-	-	-	-	+	-	-	3	23,1
	VP8	-	+	-	+	-	+	-	-	-	-	+	-	4	30,8
	VP9	+	-	+	-	+	-	-	-	-	-	-	-	3	23,1
	VP10	-	+	+	-	-	-	-	-	-	-	-	+	3	23,1
	VP11	+	-	+	-	-	-	-	-	-	-	+	-	3	23,1
	VP12	-	+	+	-	-	-	-	-	-	-	+	-	3	23,1
	VP13	-	-	+	-	-	-	-	-	-	-	-	-	2	15,4
	VP14	-	-	+	-	+	+	+	+	+	+	+	+	11	84,6
	VP15	+	+	+	+	-	-	-	-	-	-	+	-	5	38,5
	VP16	+	+	+	+	+	+	-	-	-	-	-	+	7	54,9
	VP17	-	-	+	-	-	-	-	-	-	-	-	-	2	15,4
	VP18	+	-	+	-	-	-	-	-	-	-	-	-	2	15,4
	VP19	+	+	+	+	+	+	+	+	-	-	-	-	8	61,5
	VP20	-	-	-	+	+	+	+	+	+	+	-	-	6	46,2
	VP21	-	-	-	+	+	+	+	+	+	+	-	-	6	46,2
	VP22	+	-	-	-	-	-	-	-	-	-	-	-	2	15,4
	VP23	-	+	+	+	+	+	-	-	-	-	-	-	6	46,2
	VP24	+	-	+	-	-	-	-	-	-	-	-	-	2	15,4
	VP25	-	-	-	+	+	+	+	+	+	-	-	-	5	38,5
	VP26	-	-	+	+	+	+	+	+	-	-	-	-	7	54,9
	VP27	-	-	+	+	+	+	+	+	-	-	-	-	5	38,5
	VP28	+	-	+	+	+	+	+	+	+	+	-	-	9	69,2
	VP29	-	+	+	+	+	+	+	+	-	-	-	-	8	61,5
	VP30	+	-	+	-	-	-	-	-	-	-	-	-	4	30,8
	VP31	-	-	+	+	-	-	-	-	-	-	-	-	4	30,8
	VP32	-	+	+	-	-	-	-	-	-	-	-	-	4	30,8
	VP33	-	+	+	-	-	+	-	-	-	-	-	-	5	38,5
	VP34	-	-	+	-	-	-	-	-	-	+	-	-	3	23,1
	VP35	-	-	+	-	-	+	-	-	-	+	-	-	4	30,8
	VP36	+	-	+	-	+	-	-	-	-	-	-	-	3	23,1
n(%)	24 (66,7)	13 (36,1)	31 (86,1)	15 (41,7)	14 (38,9)	15 (41,7)	6 (16,7)	6 (16,7)	0 (0,0)	11 (30,6)	8 (22,2)	7 (19,4)	12 (33,3)		

Note: (-) No plaque; (+): has plaque

1: *V. cholerae* O1, Classic; Bgd17. 2: *V. cholerae* O1, Classic; VC154. 3: *V. cholerae* O1, Classic; H218. 4: *V. cholerae* O1, El tor; K23. 5: *V. cholerae* O1, El tor; A107. 6: *V. cholerae* O1, El tor; Mak757. 7: *V. cholerae* O139, Bengal; AI1837. 8: *V. cholerae* O139, Bengal; AI1855. 9: *V. cholerae* O139, Bengal; AI4450. 10: *V. cholerae* O1, El tor; VN048p/07. 11: *V. cholerae* O1, El tor; VN29/95. 12: *V. cholerae* O1, El tor; VN293/03VN. 13: *V. cholerae* O1, El tor; VN02P/10.

The results in table 3.12 show that there are no cholera phage strains that lyse cholera strains O139 - Bengal; H218 strain has the highest number of lytic phages at 86.11% (31/36 strains); strain Bgd17 had 66.67% of lytic phages (24/36 strains); El tor strains isolated from Kenya and Indonesia had the number of susceptible/lytic cholera phages from 38% to 41%; Cholera strains isolated in Vietnam had the number of infectious/lytic cholera phages from 19.4% to 33.3%.

Bacteriophages VP14 and VP28 are two cholera test strains capable of lysing all four types of classical cholera bacteria, El tor, Bengal and cholera strains isolated in Vietnam; Of which strain VP14 has the ability to lyse 11/13 strains including 2/3 classical cholera strains O1, 3/3 cholera strains O1 El tor, 2/3 cholera strains O139 Bengal, and 4/4 cholera strains isolated in Vietnam.

No cholera phage strain was able to lyse all 13 cholera strains in the study. VP01, VP04, VP16, VP29 have the ability to lyse some strains of classical cholera, El tor and cholera strains isolated in Vietnam. VP06, VP17, VP22, VP24 only lyse some classical cholera strains, but do not lyse cholera strains O1 El tor, O139 Bengal and cholera strains isolated in Vietnam. VP09, VP26, VP27, VP36 only lyse some classical cholera strains O1, O1 El tor, do not lyse O139 Bengal cholera strains and cholera bacterial strains isolated in Vietnam. VP19, VP20 only lyse some cholera strains O1 classical, O1 El tor, and O139 Bengal, but are not known to lyse cholera strains isolated in Vietnam. VP25 only lyses some cholera strains O1 El tor and O139 Bengal, but is not known to lyse classic O1 cholera strains and cholera strains isolated in Vietnam.

The tested phage strains did not lyse *V. parahemolyticus* strains or other enteric pathogens.

Cholera phage VP04 is capable of lysing groups of classical cholera, El tor and cholera strains isolated in cholera outbreaks in Vietnam. Although it does not lyse with the O139 Bengal cholera strain group (a cholera strain that has not yet occurred in Vietnam), phage strain VP04 along with strain VP14 are the only two strains out of a total of 36 tested phage strains that can lysing ability with 4/4 strains of cholera bacteria isolated from

cholera outbreaks in 1995, 2003, 2007 and 2010 included in the test. This is also an ideal strain model for selecting and using for the purpose of controlling cholera epidemics in Vietnam.

### ***3.2.2 Lysis capacity under different dilution conditions***

Lysing bacteriophages with dilutions from  $10^{-1}$  to  $10^{-6}$  is 100%; Dilute from  $10^{-7}$  to  $10^{-10}$  without lysis.

### ***3.2.3 Ability to lyse with different pH condition***

100% of bacteriophages have the ability to lyse under environmental conditions of pH 4,0 to pH 10,0.

### ***3.2.4 Ability to lyse at different ambient temperatures.***

Cholera phage cannot grow and lyse cholera bacteria at an ambient temperature of 4°C; At 15°C, 25°C, and 30°C lysis occurred but with a slight delay; temperatures of 45°C and 55°C limited and caused actual inactivation of the bacteria; Temperatures of 30°C and 37°C are ideal for phage activity.

### ***3.2.5 The ability to survive and lyse cholera bacteriophages in community water sources***

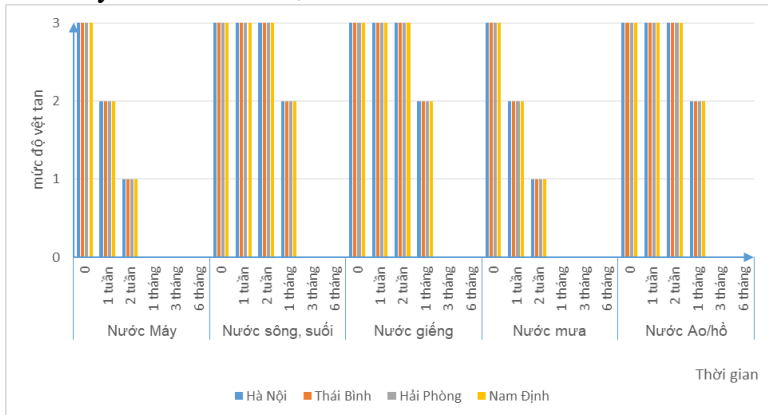
Results of testing the survival time of cholera phage VP04 in different water source conditions show:

- For tap water sources, cholera phages can survive up to 1 month, cholera phages decrease significantly after 2 weeks of testing.
- For river/stream water sources, cholera phage can survive up to 3 months, cholera phage decreases significantly after 1 month of testing.
- For well water sources, cholera phages can survive up to 3 months, cholera phages decrease significantly after 1 month of testing.

- For rainwater sources, cholera phages can survive up to 1 month, cholera phages decrease significantly after 2 weeks of testing.

- For pond/lake water sources, cholera phage can survive up to 3 months, cholera phage decreases significantly after 1 month of testing.

Regarding the lysing ability of bacteriophage VP04 on community water sources, the results in chart 3.8 show:



**Figure 3.8. Results of testing the lysing ability of bacteriophage VP04 on community water sources, 2020**

- For tap water sources, cholera phage has the ability to lyse for up to 02 weeks, cholera phage has a much reduced lysing ability after 01 week of testing.

- For river/stream water sources, cholera phages have the ability to lyse for up to 1 month, cholera phages have a much reduced lysing ability after 02 weeks of testing.

- For well water sources, cholera phage has the ability to lyse for up to 1 month, cholera phage reduces its lysing ability much after 02 weeks of testing.

- For rainwater sources, cholera phage has the ability to lyse

for up to 02 weeks, cholera phage has a much reduced lysing ability after 01 week of testing.

- For pond/lake water sources, cholera phage has the ability to lyse for up to 01 month, cholera phage has a much reduced lysing ability after 02 weeks of testing.

## **Chapter 4 . DISCUSSION**

### **4.1 Circulation of bacteriophages in the external water environment**

As a result of isolation, 08 phages (2.5%) were isolated from surface water samples and 02 phages (0.5%) were isolated from shrimp swab bait samples. Test results using the PCR method detecting cholera phage in 04/04 research sites are consistent with the circulation and cholera epidemic at the research sites; Filamentous bacteriophage fs2 was present in 42 water samples (10.5%) and 67 shrimp swab samples (16.75%); fs1 was present in 20 water samples (9.4%) and 57 shrimp swab samples (20.1%). Culture results showed that cholera bacteriophages were isolated mainly in Hai Phong and Thai Binh from canal/ditch surface water samples. PCR results are concentrated in 3/4 coastal provinces of Hai Phong, Thai Binh and Nam Dinh, which is appropriate because these are provinces/cities with coastal estuaries rich in salt water and brackish water. Previous reports have also shown that cholera bacteria exist mainly in surface water, especially brackish water.

Therefore, the finding of cholera phages regularly every month in cholera endemic areas is appropriate, because cholera

phages have a correlation and influence on the presence of cholera phages in the environment.

## **4.2 Lysis ability of cholera phages under different density, pH, and temperature conditions**

### ***4.2.1 Lysis ability of cholera bacteriophage against cholera bacteria and other intestinal pathogenic bacteria***

For selecting cholera phages for use in phage therapy, phages with a broader host range are better than those with a narrow host range. This characteristic of bacteriophages makes them useful for use alone or in combination with other bacteriophages to control cholera in the field or in the treatment of patients with cholera-induced diarrhea.

Most known bacteriophages only interact with a specific group of bacteria. The narrow host range is a significant challenge for phage therapy. Therefore, there is no cholera phage that can lyse all strains of cholera bacteria. This high host specificity of cholera phages results in the need for phages to inhibit newly isolated cholera bacteria. Bacteriophages are usually isolated from the environment in which the corresponding host exists. Therefore, using cholera bacterial strains isolated from cholera outbreaks is ideal for isolating cholera phages in outdoor water environments.

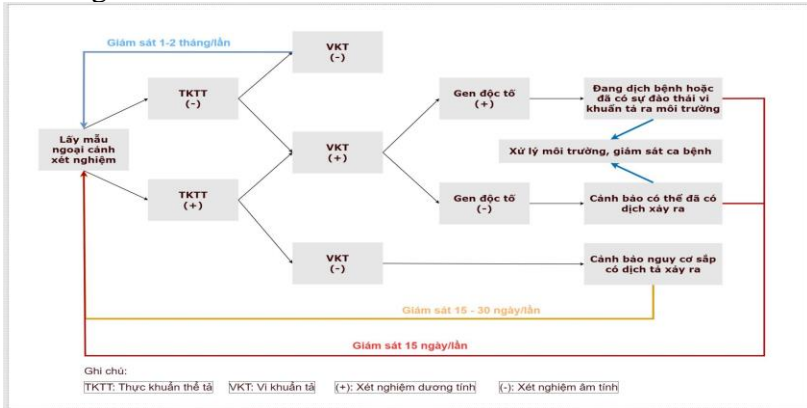
### ***4.2.2 Lysis ability of cholera phages under different environmental conditions***

In this study, we examined phage stability and the ability to lyse cholera bacteria under a number of conditions such as dilution density, environmental pH range, and different environmental temperature ranges. In addition, we also conducted a representative test of 01 VP04 phage on viability

and lysis ability in external environmental conditions. These characteristics are useful for the application of phage therapy.

### 4.2.3 Propose intervention measures to limit cholera outbreaks

Research and propose diagram 4.1. Monitoring cholera warnings:



**Figure 4. 1. Cholera warning monitoring diagram based on outdoor water sample testing**

There are 06 situations that occur with the results of the monitoring sample:

(1) Scenario 1: Cholera phages (-) and Cholera bacteria (-): continue to monitor periodically every 1-2 months.

(2) Situation 2: Cholera phage (-) and Cholera bacteria (+) cholera toxin gene (+): epidemic stage or there has been excretion of cholera bacteria from the patient into the environment. Need to monitor the environment every 15 days + proactively monitor disease cases + treat water sources with phage therapy.

(3) Situation 3: cholera phage (-) and cholera bacteria (+) cholera toxin gene (-): warning that an epidemic may have occurred, need to monitor the environment every 15 days +



main Active case surveillance + water source treatment using phage therapy.

(4) Situation 4: Cholera phage (+) and Cholera bacteria (+) cholera toxin gene (+): are in epidemic stage or there has been excretion of cholera bacteria from the patient into the environment. Need to monitor the environment every 15 days + proactively monitor disease cases + treat water sources with phage therapy.

(5) Situation 5: Cholera phage (+) and Cholera bacteria (+) cholera toxin gene (-): warning that an epidemic may have occurred, need to monitor the environment every 15 days + main Active case surveillance + water source treatment using phage therapy.

(6) Situation 6: Cholera phages (+) and Cholera bacteria (-): warning of the risk of an impending cholera epidemic, monitoring should be performed every 15 - 30 days.

## CONCLUSIONS

### **5.1 Circulation of cholera phages in the external water environment in some Northern provinces of Vietnam, 2018-2019**

The rate of bacteriophages isolated from surface water samples is 8/400 (2%); shrimp swab samples were 2/400 (0.5%). Detected the presence of bacteriophages in Thai Binh and Hai Phong. The presence of cholera phages was not detected in Hanoi and Nam Dinh.

Filamentous phages (filamentous phage 1 - fs1, filamentous phage 2 - fs2) were detected in 4/4/study site, 62/400 (15.5%) in surface water samples and 124/400 (15.5%) in surface water

samples. 31.0%) in shrimp swab samples, appearing commonly in research sites, especially coastal provinces such as Hai Phong, Thai Binh and Nam Dinh.

## **5.2 Lysis ability of cholera phages**

### ***5.2.1 Under different conditions in the laboratory***

Cholera bacteriophage isolated in Northern Vietnam has the ability to lyse cholera strains O1, O139; No lysis was detected with some other intestinal pathogenic bacterial strains.

The lysing ability of cholera phages is seen under the following conditions: dilution of over 1000 phages per 1 ml of solution; pH from 4,0 to 10,0; Ambient temperature from 15°C to 41°C.

### ***5.2.2 For community external water sources***

Viability of cholera phages: Tap water/rainwater group can survive up to 1 month; Natural flowing water (river/stream) can last up to 3 months; Water that does not circulate regularly (wells/ponds/lakes) can last up to 3 months.

Lysis ability of cholera phages against cholera bacteria: Tap water/rainwater group lyses ability for up to 2 weeks; Natural flowing water (river/stream) and irregular water (well/pond/lake) can dissolve for up to 1 month.

## **RECOMMENDATION**

Through research results, we make some recommendations:

1. It is necessary to continue periodic monitoring to determine the presence of cholera bacteria and cholera bacteriophages at research locations to serve proactive forecasting and prevention of cholera epidemics.

2. The Ministry of Health allows research and pilot application of the cholera monitoring and warning scheme based on testing external water samples in this study in practical cholera prevention and control activities.

3. Continue to research and determine the characteristics of cholera phage to build a set of cholera phage strains that can be used in treating water sources contaminated with cholera bacteria, especially testing the lysing ability of cholera bacteria. cholera phages with multidrug-resistant cholera bacterial strains.

