DETECTION OF CHOLERA TOXIN-PRODUCING *VIBRIO CHOLERAE* AQUATIC ISOLATES IN KALIMAS RIVER-SURABAYA

Narwati
Health Polytechnic
Indonesian Ministry of Health
Surabaya

ABSTRAK

Tujuan dari penelitian observasional ini adalah untuk mendeteksi toksin kolera memproduksi Vibrio cholerae di lingkungan air dari Sungai Kalimas, Surabaya. Selain untuk menghitung kepadatan bakteri, faktor abiotik, seperti suhu, kekuatan Hidrogen, dan juga diperiksa salinitas di enam poin dari koleksi. Sampel air yang dikumpulkan dari enam titik di sepanjang Sungai Kalimas selama musim hujan (Februari-Maret 2009), dan kemudian dimasukkan ke media pengayaan (air pepton basa), disaring dengan saringan pori ukuran 0,45 pM pori, dan kemudian digoreskan ke TCBS agar. Metode drop plate digunakan untuk menghitung kepadatan bakteri dalam sampel air. Sebanyak 1,5 ml sampel air yang digunakan untuk mempersiapkan DNA template untuk metode PCR. Ekstraksi DNA dilakukan untuk gen ctxA, dan dikonfirmasi dengan metode PCR. Parameter lain yaitu suhu, pH, dan salinitas diukur sesuai. Studi ini menunjukkan bahwa Vibrio cholerae dan Vibrio alginolyticus terdapat di enam titik pengumpulan air sampel, sedangkan pengumpulan titik 4 berisi Vibrio alginolyticus saja. Studi ini menunjukkan tidak satupun dari V. cholerae mengandung gen isolat ctxA. Suhu berkisar antara 27,92 derajat C sampai 28,17 derajat C, kekuatan Hidrogen 6,83-6,95 dan salinitas dari 0,00395% -0,0047% dan tidak ada perbedaan statistik (p-value> 0,05) dalam parameter abiotik diuji. Hal ini menunjukkan bahwa isolat Vibrio cholerae dalam penelitian ini tidak memproduksi toksin kolera Vibrio cholerae. Namun, dipandang perlu untuk mengkarakterisasi faktor penentu dalam ekspresi virulensi gen dan untuk mempelajari penyebaran gen non-negatif CT-01 strain mengandung gen toxR yang mengatur faktor enterotoxic untuk menilai signifikansi CT gen-negatif non-01 strain yang terdistribusi di lingkungan.

ABSTRACT

The purpose of this observational study is to detect cholera toxin producing Vibrio cholerae in aquatic environment of Kalimas River, Surabaya. Besides for calculate the bacterial density, abiotic factor, as temperature, power of Hydrogen, and salinity in the six points of collection were also examined. The water samples were collected from six points along Kalimas River during rainy season (February to March 2009), and then put into an enrichment media (alkaline peptone water), filtered by 0,45 µm pore size filter, and then streaked on to TCBS agar. Drop plate method was used to calculate the bacterial density in the water samples. As many as 1.5 ml of water samples were used for preparing template DNA for PCR method. DNA extraction was done to confirmed ctxA gene by PCR method. Other parameters namely temperature, pH, and salinity were measured accordingly. This study showed that Vibrio cholerae and Vibrio alginolyticus occur in six-point of water sample collection, point 4 of collection, contains Vibrio alginolyticus only. These study, none of the V. cholerae isolates harbours ctxA gene. The temperature ranged from 27.92 degree C to 28.17 degree C, power of Hydrogen from 6.83-6.95 and salinity from 0.00395%-0.0047% and there are no statistically different (p-value > 0,05) in the abiotic parameters tested. It is concluded that Vibrio cholerae isolated in this study are not belong to cholera toxin producing Vibrio cholerae. However, it is considered necessary to characterize determinant factor(s) in expression of virulence gene and to study dissemination of the CT gene-negative non-01 strains but harbour toxR gene regulating the enterotoxic factor(s) to assess the public health significance of the CT gene-negative non-01 strains distributed in the environment.

Keyword : *toxigenic Vibrio cholerae, temperature, pH, salinity, river water*

Correspondence: Narwati, Health Polytechnic, Indonesian Ministry of Health, Surabaya

INTRODUCTION

Vibrio cholerae is the causative agent of cholera in some parts of Asia, Africa and Latin America (Khazaei et al. 2005). Cholera caused by toxigenic *Vibrio cholerae* has occurred in Southeast Asia (Chow et al. 2001). World Health Organization report states that the cholera epidemic has occurred in more than 75 countries in South America, Africa and Asia (WHO 2000). The high mortality of cholera reached 60% if left untreated, and decreased to < 1.0% if treated promptly (Mahalanabis et al. 1992).

Cholera cases reported to WHO in the period of 2006, as many as 236,896 cases from 52 states with the death of a number of 6311 (2.66%) (WHO 2007). From August 2008 until December 1, 2008, in Zimbabwe

there were 11 735 cholera cases with 484 (4.12%) deaths reported to WHO (WHO 2008).

In the province of Papua, Indonesia, there were 105 deaths of 718 (14.6%) cases of cholera from April to August 2008. Cholera cases occurred in two districts namely Nabire much as 666 cases with 97 deaths (14.7%) and Paniai District encountered a number of 52 cases of cholera with 8 deaths (14.4%). Of these cases were obtained laboratory test results that indicate the presence of bacterial infection of *Vibrio cholerae* serotype Ogawa from rectal swabs of patients and families in contact with patients (MOH 2008).

In Surabaya, until now there has been outbreaks of cholera have been reported as occurring in Papua. This raises a presumption, whether in Surabaya has been freed from *Vibrio cholerae* or cholera case data collection system in Surabaya, which is less affordable the local health department. The fact that there is that although no cases of cholera found in Surabaya, but research conducted by Wasito et al in diarrheal stools of 297 pediatric patients under the age of 2 years with clinical diagnosis of acute diarrhea in the hospital dr. Soetomo Surabaya, 27 stool diarrhea (9%) containing *Vibrio cholerae* (Wasito et al. 1995). Another study conducted Wasito et al (Wasito et al. 2000) found intestinal pathogen *Vibrio cholerae* O1 number of 8 (1.82%) of 443 children with diarrhea who are hospitalized in dr. Soetomo Hospital Surabaya. This is also strengthened by the test against clinical isolates collected during the 7 years since 1993 are done, a total of 110 isolates tested positive for *Vibrio cholerae* serotype Ogawa, with 107 (97.3%) and 3 serotype Inaba (2.7%) (Wasito et al. In press). This shows that there still exists the risk of transmission of cholera in Surabaya, primarily through feces-contaminated water with cholera.

Vibrio cholerae toxigenic able to survive and multiply in the natural environment. The presence of *Vibrio cholerae* toxigenic in the absence of cholera cases alleged that the *Vibrio cholerae* provide toxigenic in the environment, particularly the aquatic environment. The fact the presence of *Vibrio cholerae* in the environment shown toxigenic of studies have been conducted at the Louisiana Gulf Coast United States, Hong Kong, and Australia. *Vibrio cholerae* isolates in this study are found in water, waste, and food and *Vibrio cholerae* isolates contain cholera toxin genes (Minami et al. 1991).

River water pollution by waste not only enhances the growth of coliform bacteria but also increase the number of bacterial pathogens such as Salmonella, Shigella and *Vibrio cholerae* (Feliatra 1999). Based on preliminary studies on river water Kalimas conducted on December 23, 2008, Kalimas River water used for bathing, washing, toilet (MCK) for the homeless/bums. Bathing activities conducted in waters thought to contain *Vibrio cholerae* toxigenic is a risk factor when the river water swallowed (eg as a toothbrush). The presence of *Vibrio cholerae* toxigenic in Surabaya River Kalimas potentially causes public health problems. Community Kalimas Surabaya River water users potentially affected by cholera, especially when swallowed in the conduct of activities in Kalimas Surabaya River. Until now, the lack of data on the existence of *Vibrio cholerae* toxigenic Kalimas Surabaya River so as to encourage further research needs to do.

This study aims to find out the presence of cholera toxin producing *Vibrio cholerae*, measure temperature, acidity, and salinity; calculate the density of *Vibrio cholerae* (CFU/mL) at six sampling points Kalimas Surabaya River and isolating cholera toxin producing *Vibrio cholerae* isolates Kalimas Surabaya River waters.

MATERIALS AND METHODS

This type of research included observational studies approach that is analytical studies with ecologic comparison study (Kleinbaum et al. 1982). Fieldwork was conducted at six sampling points on the River Kalimas Surabaya to obtain data on temperature, pH and salinity while the research laboratory to isolate, identify and detect *Vibrio cholerae* containing the ctxA gene. The sample in this study were Kalimas Surabaya river water with the technique of water-extracted samples at each sampling point was repeated 6 times in a different time, with intervals of 1 week. Data collection consisted of field observations and laboratory as well as interview techniques. Research data that was collected was processed and analyzed using One Way ANOVA test. Statistical analysis of these trials used the help of a computerized program SPSS 13.

RESULTS

Information obtained by the utilization of river water that communities along the River Kalimas addition to having a workshop activity, also performed well as fishing activities and worms, washing clothes, watering plants, bathe and defecate. These activities are generally carried out adults, both men and women, although sometimes these activities are done by children.

Information regarding the reasons users River Kalimas conducting continuous washing clothes, bathing and defecation is the river is a strategic place for the implementation of washing clothes, bathing and defecation, especially for the homeless. Convenience factors such as distance of the river near the location of the home or workplace is a major reason for them, because when the study was conducted, yet the existence of public toilets around the outskirts of Surabaya River flow Kalimas.

Water Temperature of Kalimas River Surabaya

Based on temperature measurements made at six sampling points, obtained the following results:

Table 1. Results of Temperature Measurements in the 6 Point Water Sampling Water Surabaya River Kalimas In February - March 2009

N Sample point	Temperature in N replication (˚C)						Mean temperature in N point (˚C)
	1	2	3	4	5	6	
1	28	29	28	28	28	28	28.17 ± 0.408
2	28	29	27.5	27.5	27.5	28	27.92 ± 0.585
3	28.5	29	27.5	28	27	28	28 ± 0.707
4	28.5	28.5	28	28	27	28.5	28.08 ± 0.585
5	28.5	28.5	28	28	27	29	28.17 ± 0.683
6	28.5	28	28	28	28	28	28.08 ± 0.204

From the table, average water temperatures are highest at point 1 and point 5 while the lowest temperature at point 2. The average temperature in 6 Kalimas River water sampling point of water is 28.1 degrees C. One Way Anova statistical test for differences Kalimas River water temperature at each sampling point the results obtained p-value = 0.967 > α (0.05). p-value > α , Then conclude that H0 is accepted, it means there is no difference in average water temperature between the sampling point on the River Kalimas Surabaya.

Water Acidity of Kalimas River Surabaya

Measurement of acidity of water in 6 Surabaya River Kalimas water withdrawal points is obtained as follows: the average acidity of river water is found at the highest point 6 which is 6.95. The lowest acidity found at point 3 is 6.83. Average acidity in 6 point Kalimas Surabaya River is 6.89. The results of acidity measurements at each point on the River Kalimas Surabaya can be seen in Table 2:

Water Salinity of Kalimas River Surabaya

Measurement of river water salinity levels do in Central Health Laboratory, Surabaya. The highest salinity levels in the 6 point Kalimas River water samples obtained at point 4 which is 0.0047% and the lowest at point 3 which is 0.00395%. Overall salinity levels Kalimas Surabaya River water obtained on average 0.0040%. Salinity levels at each sampling point can be seen in Table 3.

Table 2. The results of acidity measurements in 6 Point Water Sampling Water Surabaya River Kalimas In February - March 2009

N sample point	Acidity in n-replication						Mean acidity in n pont
	1	2	3	4	5	6	
1	6.8	7.0	6.9	6.9	6.9	6.8	6.88 ± 0.075
2	6.9	7.0	6.7	6.9	6.9	6.7	6.85 ± 0.122
3	6.8	6.8	6.8	6.9	6.8	6.9	6.83 ± 0.052
4	6.9	7.0	7.0	6.9	6.9	6.9	6.93 ± 0.052
5	7.0	6.7	6.8	6.9	6.9	6.9	6.87 ± 0.103
6	7.0	6.9	6.9	7.0	6.9	7.0	6.95 ± 0.055

One Way Anova statistical tests on river water salinity differences Kalimas at each sampling point, the results obtained p-value = 0.129 > α (0.05). p-value > α, it was concluded H0 is accepted, which means there is no difference in average water salinity between sampling points on the River Kalimas Surabaya.

Identification of *Vibrio cholerae* in Kalimas River Surabaya

A total of 450 mL samples of each sampling point was mixed with 50 ml of alkaline peptone water medium with a concentration 10 times, then filtered through a 0.45 µm membrane filter to isolate the bacterium *Vibrio cholerae*. Membrane filter incorporated into alkaline peptone water; as enrichment media. *Vibrio cholerae* colonies obtained by dropping 50µL sample using a micropipette on selective medium, TCBS, in order to obtain well-separated colonies. Colonies yellow, flat, indicating the ability to ferment sucrose, taken for biochemical tests performed. The results of bacterial colonies of yellow insulation (colony which has the ability to ferment sucrose) that grows flat on TCBS media were inoculated on media "Zero NaCl broth" and other biochemical tests followed. Isolates that did not give positive results (red slant and yellow butt) did not proceed to other biochemical tests because they do not represent the characteristics of *Vibrio cholerae* isolates. Results obtained 14 isolates gave results in the growth media "Zero NaCl broth" indicated the presence of turbidity in the media. After continuing the biochemical tests, obtained 14 isolates suspected as *Vibrio cholerae*. The results of the identification of isolates from Surabaya Kalimas River water samples can be seen in Table 4. Serological test in 14 isolates of *Vibrio cholerae* that allegedly does not produce agglutination reaction with *Vibrio cholerae* O1 polivalent. Biochemical tests using API 20 NE System against 6

isolates that did not grow on media "Zero NaCl broth" species identification results obtained by other vibrio bacteria is *Vibrio alginolyticus*. PCR methods are used to test the fourteenth suspected *Vibrio cholerae* isolates. Test the presence of ctxA genes in 14 isolates suspected *Vibrio cholerae* using the PCR method the results obtained no isolates containing the ctxA gene. CtxA gene detection in river water samples Kalimas directly using the PCR method was also obtained results ctxA genes not found in the sample.

DISCUSSION

Vibrio cholerae is the bacterium that causes cholera, a diarrheal disease epidemic, which becomes a health problem particularly in developing countries that have a socio-economic conditions, sanitation systems, security surveillance systems for drinking water and public health are less well (Preez et al. 2003). Indonesia, including developing countries which cannot be separated from possible breakdown of cholera in the community, especially for those who use water contaminated with *Vibrio cholerae*.

Table 3. Water Salinity Measurement Results in 6 Point Sampling Kalimas Surabaya River Water In February - March 2009

N Sample point	Salinity in N replication (%)						Mean Salinity in N point (%)
	1	2	3	4	5	6	
1	0.0042	0.0039	0.0042	0.0043	0.0035	0.0040	0.0040 ± 0.0003
2	0.0050	0.0042	0.0033	0.0043	0.0039	0.0040	0.0041 ± 0.0006
3	0.0041	0.0044	0.0034	0.0042	0.0036	0.0040	0.00395 ± 0.0004
4	0.0040	0.0054	0.0035	0.0047	0.0046	0.0058	0.0047 ± 0.0008
5	0.0052	0.0045	0.0045	0.0044	0.0038	0.0050	0.0046 ± 0.0005
6	0.0039	0.0034	0.0041	0.0041	0.0040	0.0050	0.0041 ± 0.0005

Table 4. Results Identification of Isolates within Kalimas Surabaya River Water Samples

No	Code	Result	No	Code	Result
1	1.2.a	*Vibrio cholerae* non-O1	19	4.4.a	*Pasteurella pneumotropica*
2	1.1 c	*Aeromonas salmonicida ssp*	20	4.5.a	*Aeromonas salmonicida ssp*
3	1.3.a	*Brevundimonas vesicularis*	21	4.5.c	*Pasteurella pneumotropica*
4	1.3.b	*Vibrio alginolyticus*	22	4.6.b	*Vibrio alginolyticus*
5	1.3.c	*Vibrio cholerae* Non-O1	23	4.6.c	*Vibrio alginolyticus*
6	1.4.a	*Vibrio cholerae* non-O1	24	5.3.b	*Aeromonas salmonicida ssp*
7	2.4.b	*Vibrio cholerae* non-O1	25	5.3.c	*Brevundimonas vesicularis*
8	2.5.b	*Vibrio cholerae* non-O1	26	5.5.a	*Brevundimonas vesicularis*
9	2.5.c	*Vibrio cholerae* non-O1	27	5.5.c	*Vibrio cholerae* non-O1
10	3.2.b	*Vibrio cholerae* non-O1	28	5.6.b	*Vibrio cholerae* non-O1
11	3.4.b	*Vibrio cholerae* non-O1	29	5.6.c	*Vibrio alginolyticus*
12	3.5.b	*Vibrio cholerae* non-O1	30	6.2.a	*Vibrio cholerae* non-O1
13	3.7.c	*Vibrio cholerae* non-O1	31	6.3.c	*Brevundimonas vesicularis*
14	4.2.b	*Vibrio alginolyticus*	32	6.3.a	*Brevundimonas vesicularis*
15	4.2.c	*Pasteurella pneumotropica*	33	6.4.a	*Vibrio cholerae* non-O1
16	4.3.a	*P. pneumotropica*	34	6.4.b	*Pasteurella pneumotropica*
17	4.3.b	*P. pneumotropica*	35	6.5.a	*Vibrio alginolyticus*
18	4.3.c	*Brevundimonas vesicularis*	36	6.6b	*Aeromonas salmonicida ssp*

Details Point Sample Code:
Figures on the first digit: Location of sampling points of river water
Numbers in the second digit: Taking the n
Letter to the third digit: Isolate

The presence of Vibrio that are halophylic in freshwater environments are reported from India and western Germany. A large number of *Vibrio cholerae* can be isolated in estuarine environments and only a small part that can be isolated from freshwater environments (Kasthuri Venkateswaran et al. 1989). *Vibrio cholerae* ecology, abiotic and biotic factors concerned with the survival of bacteria in aquatic environments. Data obtained from laboratory studies that *Vibrio cholerae* had favorable conditions in the aquatic environment that has the characteristics: warm temperatures (30-37 degrees C), acidity neutral or slightly alkaline (7 to 8.5 if the salinity is; 7.5 to 9 if low salinity), salinity was (1.5%-2.5%), containing high nutrients, and the presence of aquatic macrophyte, phytoplankton, zoo-plankton, fish, mollusks and crustaceans (Borroto 1997). This study reviewed the abiotic factors of *Vibrio cholerae* ecology consisting of temperature, acidity and salinity of the River Kalimas Surabaya and the density factor of *Vibrio cholerae*.

The maximum temperatures hasten death of bacteria, because of damage to the protoplasm. That do not fit the temperature is lower than the minimum temperature causes the death of the bacteria and reduced metabolism. Freezing can cause bacterial death. The cause of death of bacteria due to freezing is not known clearly. The speed of freezing is likely to cause the rate of survival of bacteria into nothing. Temperatures close to the minimum or maximum temperature causes morphological changes in various kinds of bacteria. In the culture conditions/pure culture, the influence of temperature on biological reactions in vitro bacterial constantly changing. In nature, bacteria are very difficult environmental conditions can not be observed even. Fluctuations in seasonal changes cause a change in the bacterial population (Waluyo 2005).

The presence of *Vibrio cholerae* in this study is found in the 5 sampling points except the fourth point. The presence of *Vibrio cholerae* is supported by the condition of the average water temperature Kalimas River 28.1 degrees C, where the temperature range allows for *Vibrio cholerae* to grow. Known optimal temperature for growth of *Vibrio cholerae* which is 16 degrees C-42 degrees C (Sedas 2007).

Vibrio cholerae is very sensitive to the acidity of less than 6.0. (Brooks et al. 2001). Known to the average acidity Kalimas River water in this study is 6.89. Average acidity values of Kalimas river water less optimal support on the ability of *Vibrio cholerae* in multiply and attached to the copepod. This is evidenced by research conducted by Huq et al, that the acidity 8.5 provide optimal conditions for the *Vibrio cholerae* in multiply and attach to copepods compared with acidity and acidity 6.5 7.5 (Huq et al. 1984).

Salinity is a major variable affecting the ecology of *Vibrio cholerae*. Salinity also plays a role in the presence of *Vibrio cholerae* in aquatic environments (Sedas 2007). However *Vibrio spp* associated with human diseases (including cholera), is a bacteria that require Na + halophilik 0.5 to 3% for optimal growth (Colwell and Chun 2009).

The results obtained from the culture method of *Vibrio cholerae* in the sampling points of water except on the fourth point. Inoculating isolates on the media "Zero NaCl broth" is intended to reinforce the notion that the observed isolates were *Vibrio cholerae*. This is consistent with the characteristics of *Vibrio cholerae* as written in Bailey & Scott's book, which is able to grow on culture media containing no sodium chloride (NaCl 0%). Although these traits are owned Vibrio mimicus, yet another trait that distinguishes them is the ability to ferment sucrose. Vibrio mimicus do not have the ability to ferment sucrose while *Vibrio cholerae* has the ability to ferment sucrose (Forbes et al. 2002).

River water salinity levels in this study 0.0040%, where the optimal concentration of NaCl for *Vibrio cholerae* for survival is 2%, although concentrations between 0.25%-3.0% are also supported (Borroto 1997). Although salinity is low (0.0040%) and are not optimally support the existence of *Vibrio cholerae* to survive, but *Vibrio cholerae* can be found in the River Kalimas. This study showed that *Vibrio cholerae* frehswater able to survive in the environment, the river water. The study was supported by previous studies that use lake water samples, indicating that *Vibrio cholerae* is not only able to survive but also able to grow in freshwater environments (Vital et al. 2007).

Not found in isolates of *Vibrio cholerae* toxigenic Kalimas Surabaya River because the river salinity Kalimas not support for isolates of *Vibrio cholerae* expressing genes that encode cholera toxin. This is reinforced by the statement that would be optimal expression of cholera toxin in waters that have a salinity of 2% to 2.5%. Studies supporting this claim have occurred in Alvarado who discovered *Vibrio cholerae* O1 Inaba in an aquatic environment that has a salinity of 6.27% (Sedas 2007).

Vibrio cholerae is found if linked between temperature, acidity, and salinity obtained in this study contrasts with a study by Miller et al, that although *Vibrio cholerae* toxigenic tolerant to environmental alkali (base) but very sensitive to the environment that are acidity (Miller et al. 1986). Known acidity optimal for the survival of

Vibrio cholerae in water temperature 25 degrees C, salinity medium (2%) is acidity 7 to 8.5, while acidity from 7.5 to 9 at low salinity (0.05%) (Borroto 1997). This suggests the assumption that the lower levels of water salinity, the higher the acidity necessary for the existence of *Vibrio cholerae*. Conditions are not encountered in this study, the salinity is low (0.004%) is not supported by the acidity (pH = 6.889) aquatic environment. This fact contrasts with the facts that occurred in Austria on Lake Neusiedler See, where the lake has a salinity levels are between 0.1% and 0.35% is supported by acidity between 7.8 and 9.1. In this condition encountered non toxigenic cholerae that cause otitis, and septicemia cases in the region (Kirschner et al. 2008).

Vibrio cholerae density test statistic in this study can not be done because the results of isolation, identification and testing of biochemical diversity of isolates obtained. This study shows that the use of TCBS media do not support the acquisition of the number density *V. cholerae* because they still found the growth of other bacteria, *Aeromonas salmonicida ssp* namely, *Brevun-dimonas vesicularis* and *Pasteurella pneumotropica*.

The presence of *Vibrio cholerae* in points 1, 2, 3, 5 and 6 is possible is supported by the abiotic factors of temperature, acidity, and salinity; where each has the same average in 6 point Kalimas Surabaya River. Moreover, the habit of throwing feces at the site is alleged is a factor that has contributed to the density of *Vibrio cholerae*. Stool content is one of the organic material, which can form carbohydrates, and contain nutrients, typically compounds containing nitrogen. (Zainuddin 2008). Carbohydrate and nitrogen compounds that are needed to support the growth of *Vibrio cholerae* (Fobes et al. 2002).

No discovery of *Vibrio cholerae* on the fourth point of possible existence of other factors that influence the presence of *Vibrio cholerae* in these locations, so that the distribution mainly *Vibrio cholerae* non-O1 *Vibrio cholerae* is uneven, as the bacteria's ability to obtain Assimilable Organic Carbon (AOC). Assimilable Organic Carbon is an important factor in determining the bacterial communities in aquatic ecosystems. Assimilable Organic Carbon is part of the dissolved organic carbon available to be consumed by bacteria to produce cell proliferation. Assimilable Organic Carbon consists of a small component of the molecular mass of amino acids, sugars or organic acids resulting from the process of photosynthesis, biological and chemical hydrolysis of natural organic materials, which in turn consumed bacteria. Bacteria compete for elements Assimilable Organic Carbon (AOC) as a source of energy / carbon (Vital et al. 2007).

Analysis of the results obtained by PCR of *Vibrio cholerae* isolates found not to contain ctxA gene. This indicates that the isolates are not potentially produce cholera toxin. In accordance with the statement that in general the cholera toxin produced by *Vibrio cholerae* O1 and O139 (Nair et al. 2006) as well as most of the environmental strains of *Vibrio cholerae* do not produce cholera toxin (Jiang et al. 2003). This is because most strains of *Vibrio cholerae* from environmental shortcomings of other virulence genes necessary to produce cholera toxin (Sedas 2007).

The presence of *Vibrio cholerae* non-O1 need to watch out, this is because that the transfer of cholera toxin genes in the environment can be facilitated by the CTXΦ phage filament. A survey conducted in west Bengal, India, revealed a wide distribution of genes among *Vibrio cholerae* cholera toxin on the water surface. Studies show that the process of transfer/transduction in the natural environment can result in environmental strains nontoxigenic into strains carrying cholera toxin gene (Gun-Zo et al. 2002). Transfer filamentous bacteriophage CTXΦ among clinical strains requires toxin-coregulated pilus (TCP) as a receptor for CTXΦ, which can infect *Vibrio cholerae* non toxigenic, started initially toxigenic new strain (Chakraborty et al. 2000). The hypothesis that acquired CTXΦ in the human body since known optimal TCP expression occurs in the gastrointestinal tract. The existence of uncertainty about the circulation of ctxA genes in aquatic environments in non-epidemic area lead to the assumption that the possibility of circulation of the genes of *Vibrio cholerae* ctxA through a different mechanism of gene transfer in aquatic environments. This is reinforced by previous studies that the negative *Vibrio cholerae* isolates and strains of Vibrio mimicus TCP and TCP negative susceptible to infection by filamentous phage CTXΦ and form a stable lysogenik (Jiang et al. 2003).

Vibrio cholerae was not found at each sampling point toxigenic Kalimas Surabaya River indicates that there is no relationship between bowel habits, especially cholera that show symtomatic or asymtomatic, Kalimas Surabaya River. This assumption is reinforced by the statement that individuals who showed no symptoms of cholera (asymtomatic) may issue a 102-105 *Vibrio cholerae*/gram of feces while the patient rice water stools of cholera by removing 106-109 *Vibrio cholerae*/ml of feces (Percival et al. 2004).

Although isolates of *Vibrio cholerae* Kalimas River does not contain ctxA gene, but isolates contain genes

toxR. Primers to detect the presence of toxR gene in this study using primers for *Vibrio cholerae* toxR to the size of 779 bp (Singh et al. 2002). This suggests that although V. cholerae is found to not contain ctxA, but this species contain genes toxR, which potentially express other virulence genes. This raises the suspicion whether the strain of *Vibrio cholerae* O1 is the conversion of non-O1 *Vibrio cholerae* filamentous bacteriophage by transfer of CTXΦ. This assumption is strengthened by Maiti et al study which strains of *Vibrio cholerae* non-O1 and non-O139 *Vibrio cholerae* can be simultaneously infected by different CTXΦ that play a role in the emergence of new epidemic clones (Maiti et al. 2006).

CONCLUSION

The conclusion is obtained that the conditions of temperature, acidity, and salinity of the River Kalimas support the existence of non-O1 *Vibrio cholerae*. *Vibrio cholerae* density test can not be done because the results of isolation on TCBS media did not show homogeneity isolates of *Vibrio cholerae* non-O1 cholerae.Vibrio isolated in this study contain genes regulating virulence genes of the other, toxR, so it is considered potentially strain toxigenic and potentially causes community health problems. The presence of *Vibrio cholerae* O1 strains in conversion of non-O1 *Vibrio cholerae* filamentous bacteriophage by transfer of CTXΦ.

Further studies are needed on: the existence of *Vibrio cholerae* isolates of waters in the dry season; *Vibrio cholerae* isolates containing genes ctxA waters; proof that the strain of *Vibrio cholerae* O1 is the conversion of non-O1 *Vibrio cholerae* filamentous bacteriophage by transfer of CTX? for circulation among the ctxA gene of *Vibrio cholerae* isolates clearer waters so that the environmental quality improvement program plan along Kalimas Surabaya, such as: designing public toilets that meet the requirements of environmental health in densely populated locations as well as the need for Health Education to the public that the Surabaya River Kalimas contain *Vibrio cholerae* potential to cause cholera.

REFERENCES

1. Borroto RJ, 1997. Ecology of *Vibrio cholerae* serogroup O1 in aquatic environments. Rev. Panam Salud Publica/ Pan Am J Public Health, 2: 5, 328-33
2. Brooks GF., Butel JS and Morse SA, 2001. Jawetz, Melnick, & Adelberg's Medical Microbiology, 22 eds. USA : McGraw-Hill Companies, Inc. 235-37.
3. Chakraborty S., Mukhopadhyay AK., Bhadra RK et al, 2000. Virulence Genes in Environmental Strains of *Vibrio cholerae*. Appl and Envirn Microbiol, 66 : 9, 4022-28.
4. Chow KH., Ng TK., Yuan KY et al, 2001. Detection of RTX Toxin Gene in *Vibrio cholerae* by PCR. J Clin Microbiol, 39: 2594-7
5. Colwell RR and Chun J. 2009. The Genus Vibrio and Related Genera. In : Practical Handbook of Microbiology, eds 2. United State, CRC Press. 267-73.
6. Depkes, 2008. Pemerintah Serius Tangani Diare-Kolera Di Papua, http://www.depkes.go.id, 1-3. Download: 3 Desember 2008
7. Feliatra, 1999. Identifikasi Bakteri Patogen (Vibrio sp) di Perairan Nongsa Batam Propinsi Riau. Jurnal Natur Indonesia II (1), 28-33.
8. Forbes BA., Sahm DF and Weissfeld, 2002. Diagnostic Microbiology. United States of America, Mosby Inc. 423-34.
9. Gun Zo Y., Rivera ING., Cohen ER., et al. 2002. Genomic profiles of clinical and environmental isolates of *Vibrio cholerae* O1 in cholera-endemic areas of Bangladesh. PNAS, 99: 19, 12409-14.
10. Huq A., West PA., Small EB et al, 1984. Influence of water temperatur, salinity and pH on survival and growth of toxigenic *Vibrio cholerae* serovar O1 associated with live copepods in laboratory microcosms. Appl and Envir Microbiol, 48: 2, 420-24.
11. Jiang S., Chu W and Fu W, 2003. Prevalence of cholera toxin genes (ctxA and zot) among non-O1/non-O139 *Vibrio cholerae* strains from Newport Bay, California. Applied and Environ.Microbiol, 69: 12, 7541-4.
12. Mahalanabis D., Molla A and Sack D, 1992. Clinical Management of Cholera. In (D. Barua and W. Greenough, III eds). Cholera, New York: Plenum Medical Book Company, 253-83.
13. Maiti D., Das B., Saha A., et al, 2006. Genetic organization of pre-CTX and CTX prophages in the genome of an environmental *Vibrio cholerae* non-O1, non-O139 strain. Microbiology, 152 : 3633-41.
14. Miller C., Feachem R., Drasar BS., et al. 1986. The impact of physico-chemical stress on the toxigenicity of *Vibrio cholerae*. London: J Hyg, 96: 49-57.
15. Minami A., Hashimoto S., Abe H et al, 1991. Cholera enterotoxin production in *Vibrio cholerae* O1 strains isolated from the environment and from human in Japan. Appl. Environ. Microbiol. 57: 2152-2157.
16. Nair GB., Qadri F., Holmgren et al, 2006. Cholera Due to Altered El Tor Strains of *Vibrio cholerae* O1. Journal Of Clinical Microbiology, 44: 11, 4211-13

17. Percival SI., Chalmers RM., Embrey M., et al, 2004. *Vibrio cholerae*, In : Microbiology of Waterborne Diseases. California, Elsevier Academic Press. 197-205.
18. Preez Md., Venter SN and Theron J. 2003. Detection of viable toxigenic *Vibrio cholerae* and virulent Shigella spp. in environmental waters by pit-stop seminested polymerase chain reaction assays. ISSN 0378-4738 Water SA. 29: 2, 177-82.
19. Sedas VTP, 2007. Influence of environmental factors on the presence of *Vibrio cholerae* in the marine environment: a climate link. Review Article, J Infect Developing Countries, 1(3):224-41.
20. Singh DV, Isac SR, and Colwell RR, 2002. Development of a hexaplex PCR assay for rapid detection of virulence and regulatory genes in *Vibrio cholerae* and Vibrio mimicus. J. Clin. Microbiol. 40: 4321-4324.
21. Vital M., Füchslin HP., Hammes F et al, 2007. Growth of *Vibrio cholerae* O1 Ogawa Eltor in freshwater. Microbiol, 153 : 1993-2001.
22. Waluyo L, 2005. Mikrobiologi Lingkungan. Universitas Muhammadiyah Malang. p. 12-107.
23. Wasito EB., IchinoseY., Kataoka N., et al, 1995. Isolation Of Non-O1 *Vibrio cholerae* In Surabaya, Indonesia. In Majalah Kedokteran Tropis Indonesia, 2002, 13:1, 7-12.
24. Wasito EB., Rahardjo D and Alimsardjono L, 2000. Patogen Usus Yang Diisolasi Dari Tinja Diare Pada Anak Rawat Inap Di Bawah Umur Dua. Surabaya, Indonesia , Airlangga University, 1-23.
25. Wasito EB., Rahardjo D and De Vries GC, In Press. *Vibrio cholerae* Profile. Surabaya, Indonesia , Airlangga University.
26. World Health Organization, 2000. Report on global surveillance of epidemic prone infectious diseases, communicable diseases and surveillance response. World Health Organization, Geneva, Switzerland. www.who.int/WER. download : 19 September 2008.
27. World Health Organization, 2007. Cholera. http://www.who.int/mediacentre/factsheets/fs107/en/index.html, download: 19 September 2008
28. World Health Organization, 2008. Weekly epidemiological record, 83: 50, 449–60 . http://www.who.int/wer. Download : 12 oktober 2008