Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 119 (2019) 192-203 EISSN 2392-2192

Vibrio cholerae Test on Fishery Products at Cirebon Test and Application of Fishery Products Technical Unit Agency, West Java, Indonesia

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ABSTRACT

Fiheries products that will be exported or marketed domestically, must have the specified food safety criteria. One of the criteria that the products is safe to be marketed and consumed is free from the presence of pathogenic bacteria. One of the common pathogenic bacteria found in food is *Vibrio cholerae*. The purpose of this research aims to examine, isolate and confirm the presence of dangerous pathogenic bacteria *V. cholerae* which shouldn't exist in fisheries products with sample Frozen cuttle fish, frozen baby octopus small cut and Pasteurized crab meat. This research was held from July-August 2018 at Cirebon Test and Application of Fishery Products Technical Unit Agency. Data is collected through direct observation or testing in a microbiology laboratory with conventional methods using special media for *V. cholerae* such as Alkaline Peptone Water (APW) and Thiosulfate Citrate Bile Salts Sucrose (TCBS). The testing is qualitative, so the results obtained are either positive or negative. The results of the research showed that there's no pathogenic bacteria *V. cholerae* were found in tested fisheries products so that they were safe to consume and satisfied the standards to be marketed.

***Keywords*:** bacteria, fisheries product, microbiology test, seafood, *Vibrio cholerae*

(Received 13 January 2019; Accepted 28 January 2019; Date of Publication 29 January 2019)

1. INTRODUCTION

Indonesia has the potential of fisheries, marine and fishery products. Fishery as one sub sector supporting Indonesia's economy also needs to be developed to improve the international trade in Indonesia (Rachmawati et al., 2017). Fishery products that will be exported or marketed must meet predetermined safety criteria, including fishery products which are products for consumption. One of the criteria that the product is safe to be marketed and consumed is free of any pathogenic bacteria. Among some of the pathogens that are often found in food products, especially fishery products is *Vibrio* spp (Osunla & Okoh, 2017). One of the causative agent of cholera is the species *Vibrio cholerae*. Based on Novoslavskij et al. (2016) the presence of human pathogenic microorganisms in fish and fish products may be affected by various factors, including cultural practices, environmental conditions, processing, and distribution of products. The most important fish pathogens can be generally divided into two groups: those native to natural freshwater habitats and those associated with water pollution.

Vibrio cholerae is highly motile, gram-negative, curved or comma-shaped rods with a single polar flagellum (Kumari et al., 2014). According Faruque et al. (1998), states that *V. cholerae* can move very actively through one smooth flagella (monothric) and is aerobic or facultative anaerobes. These bacteria can grow rapidly when grown on solid media Thiosulfate-citrate-bile-sucrose or commonly known as TCBS. In this medium, *Vibrio cholerae* is yellow so that it can be distinguished from other bacterial colonies to facilitate the isolation process. *V. cholerae* can survive in an environment with pH 6-11 (Thomas et al., 2006) and is found in the surrounding environment such as river water, sea water, well water, reservoir water and animals that are commonly consumed by humans. It is believed that there is a type of *Vibrio* sp. originating from Indonesia that probably does not exist in other countries, because the diversity of *Vibrio* species in Indonesian waters is still very little studied and analyzed (Felix et al., 2011).

Vibrios of seafood origin have attracted increasing attention from time to time as it is found to be one of the most important causes of human food poisoning (Chakraborty et al., 2008). When these bacteria and contaminate food consumed by humans in a certain amount, it can cause a gastrointestinal infection that causes cholera (Moreno & Taylor, 2013). The most common causes of infections related to seafood, most hospitalizations and deaths are caused by bacterial agents. Various types of viruses, bacteria, and parasites have been involved in seafood related outbreaks, which are reported worldwide (Butt et al., 2004). The spread of Vibrio cholerae bacteria can occur through water or animals that live in water contaminated by the bacteria *Vibrio cholerae* (Cabral, 2010). The purpose of this research aims to examine, isolate and confirm the presence of dangerous pathogenic bacteria *V. cholerae* which shouldn't exist in fisheries products with sample frozen cuttle fish, frozen baby octopus and pasteurized crab.

2. MATERIALS AND METHODS

The study was conducted in Cirebon Test and Application of Fishery Products Technical Unit Agency, West Java in July - August 2018. The method used is the exploration method using two data, namely primary data and secondary data. Primary data is obtained by conducting direct observation or testing including examining, isolating and confirming the type of pathogenic bacteria Vibrio cholerae in the sample of fishery products used (frozen cuttle fish,

frozen baby octopus and pasteurized crab). The secondary data is obtained by collecting literature related to testing *Vibrio cholerae* bacteria.

The procedure for testing V. cholerae uses the National Standardization Agency of Indonesia (Tapotubun, 2016) principle, namely Indonesia National Standar (INS) 01-2332.4-2006. Based on Sanders (2012), the testing process is applied aseptic technique. Before testing, the media and reagents used for biochemical tests were prepared by weighing as needed and dissolved with distilled water according to each procedure. The samples tested were first grown on enrichment media and detected by growing on the agar selective medium. Colonies suspected of *V. cholerae* in the agar selective medium isolation was followed by confirmation through biochemical tests to convince the presence or absence of *V. cholerae* (Huq et al., 2012).

Sample weighing: The testing procedure begins by weighing samples of frozen cuttle fish, frozen baby octopus small cut and crab pasteurized cans. Each weigh as much as 25 g and then put in a petri dish, cover to avoid contamination with bacteria in the vicinity. The weighed sample was put in a sterile plastic, 225 ml of an Alkaline Peptone Water (APW) solution was added. Homogenization for 1-2 minutes with a stomatcher. Then put it back into the bottle of Scott and incubate for 12-24 hours at 36 ºC ± 1 ºC.

Isolation of Vibrio cholerae: 1 ose needle inserted as deep as 1 cm from the surface of the liquid sample, then scratched on TCBS agar media. Incubated TCBS agar at 36 ºC ± 1 ºC for 18-24 hours. After incubation, observe the presence of *V. cholerae* with the characteristics of large V. cholerae colonies, yellow, smooth surface, opaque center and bright edge.

Purification: Carefully taken 3 or more suspected colonies from each TCBS agar, etched into the T_1N_1 agar. Incubated for 18-24 hours at 36 ºC ± 1 ºC.

Biochemical Test Introduction: If after checking TCBS agar to obtain a positive or unexpected result containing *V. cholerae*, then proceed with a biochemical test to confirm whether the bacteria include *V. cholerae* or other types of Vibrio bacteria.

1) Oxidase Test

Oxidation test was carried out by scraping 1 ose of T1N1 agar or Tryptic Soy Agar (TSA) + 1.5% NaCl into a petri dish containing TSA agar. Incubate at 36 ºC ± 1 ºC for 18-24 hours. Add 2 - 3 drops of oxidase to the bacterial colonies. Positive reactions are marked in blue.

2) Sensitivity Test for 0/29 vibriostat

Etch 1 ose of the T_1N_1 agar, place the disk 0/129 10 µg and 150 µg on the most tightly etched and incubated at 36 ºC ± 1 ºC for 18-24 hours. Observe growth around the disc. Sensitive reactions are indicated by the formation of a zone around the disk (S) and a resistance reaction characterized by growth around the disk (R). *Vibrio cholerae* is sensitive to 0/129 10 µg and 150 µg.

3) Triple Sugar Iron (TSI) Agar and Kliger Iron Agar (KIA)

Colonies of TSA + 1.5% NaCl were inoculated with scratches on agar slant on TSI and KIA agar to use an ose needle, then incubated at 36 ºC ± 1 ºC for 18-24 hours. V. cholerae produces acid (yellow) in agar slant, acid (yellow) in agar straight and not produce H_2S gas.

4) Tolerance Test on Salt

Inoculated cultures from TSB into 3 tubes each containing Tryptone Broth 1% which added 0%, 1% and 3% NaCl (T_1N_0, T_1N_1, T_1N_3). Incubated at 36 ºC ± 1 ºC for 18-24 hours. Positive reactions are characterized by turbidity, V.cholerae grows on T_1N_0 and T_1N_3 media.

Advanced Biochemical Test: advanced biochemical test was conducted when the preliminary biochemical tests found that the typical reaction V.cholerae

1) Urea Hydrolysis Test

Inoculate 1 ose of TSA + 1.5% NaCl into Urea media. Incubate at 36 ºC ± 1 ºC for 18 hours. Positive reactions are indicated by discoloration of the media from orange to pink. V. cholerae does not have the ability to hydrolyze Urea.

2) Arginine, Lysine and Ornithin tests

Inocullate of TSA + 1.5% NaCl into 3 basic media tubes containing Arginine, Lysine and Ornithin and into 1 control tube that does not contain amino acids. Incubate at 36 ºC ± 1 ºC for 4 days. V.cholerae produces a negative Arginine dihydrolase reaction, Lysine and Ornithin are positive.

3) Carbohydrate Fermentation Test

Inoculated 1 ose of TSA + 1.5% NaCl into each Purple Broth Base tube containing sucrose, lactose, D-mannitol, D-mannose, D-cellobiose and arabinose. Incubate at 36 ºC ± 1 ºC for 4-5 days. A positive reaction produces acid and converts the media to yellow.

Interpretation of Results

After testing, the test results are adjusted to the minimum characteristics of biochemical tests for identification of *V. cholerae*. Table 1 is a minimum characteristics of the biochemical test. Oxidative-Fermentative Medium

Table 1. Minimum characteristics of the biochemical test *Vibrio cholera.*

	Type of test	Interpretation of results
1	Morphology	Gram-negative, rod shape or coma
2	TSA	Agar slant : acid (yellow) Agar straight : acid (yellow)
3	Oxidative-Fermentative Medium	Positive oxidative and positive fermentative
4	*Oksidase*	Positive
5	*Arginin dehidrolase*	Negative
6	*Lysine dekarboksilase*	Positive
7	VP	Variable

8	Growth at temperature 42 °C	Positive
9	*Halophilik*	T_1N_0 = positive; T_1N_1 = positive; T_1N_3 = positive; T_1N_6 : negative
10	*Sucrose* fermentation	Positive
11	ONPG	Positive
12	*Arabinose* fermentation	Negative
13	Sensitivity to 0/129	Sensitive (S) terhadap 10 µ g dan 150 µg

3. RESULT

Based on the results of testing of 3 types of fishery products in Table 2, namely frozen cuttle fish, frozen baby octopus and pasteurized crab, the results were the presence of colonies of *V. cholerae* bacteria in small cut frozen baby octopus samples. This can be seen based on macroscopic observations by looking at the signs or characteristics of *V. cholerae* that appear, so that further biochemical tests are carried out (Table 3).

Table 2. Test Results of Fishery Product Samples

No	Fishery product	Code	Results	
			Vibrio group bacteria	Pathogenic Bacteria Vibrio cholerae
1	Frozen cuttle fish	a	negative	negative
2	Frozen Baby Octopus	b	positive	negative
3	Pasteurized crab	c	negative	negative

Tabele 3. Biochemical Characteristics of *V. cholerae* Sample Test Results.

Test	**Results**
TCBS agar	Yellow
Oxidase	+
Arginine dihiydrolase	-
Ornithine Decarboxylase	+

Lysine *Decarboxylase*		+
Growth:	0% NaCl	+
	1% NaCl	-
	3% NaCl	+
Acid:	*Sucrose*	+
	D-*Cellobiose*	+
	Lactose	+
	Arabinose	+
	D-*Mannose*	+
	D-*Mannitol*	+
Sensitivity of:	10 µg 0/129	Sensitive
	150 µg 0/129	Sensitive
	Urea	-

(a) **(b)** **(c)**

Figure 1. Test Result: **(a)** Frozen cuttle fish, **(b)** Frozen baby octopus, **(c)** Pasteurized crab

4. DISCUSSION

Based on the test results, the frozen cuttle fish samples showed a negative result, where the bacteria that grew on TCBS media did not show characteristics of *V. cholerae* bacteria because the colonies were green and there was black which was the result of H2S gas formation. Then for canned pasteurized crab samples showed negative results, in the absence of yellow colonies that showed no growth on TCBS media. As for the results of testing of frozen baby octopus small cut samples, there are signs of colonies of pathogenic bacteria *V. cholerae.*

The suspected colonies are bright yellow, smooth surfaces, bright edges that show growth in TCBS agar media. Thus it is necessary to carry out further testing.

Biochemical Test Introduction

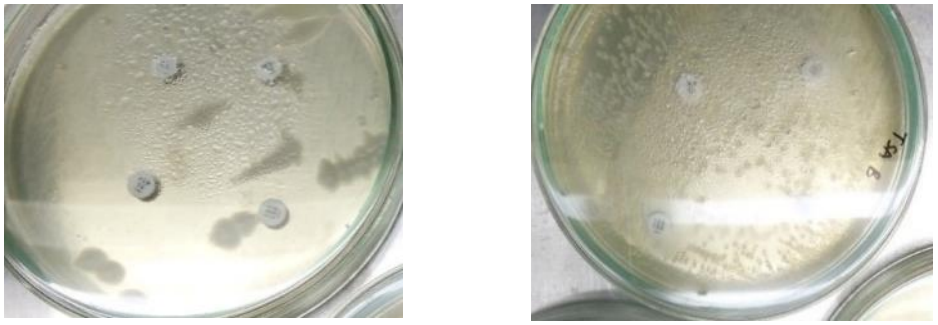
1) Oxidase test

Oxidase test serves to determine the presence of cytochrome oxidase that can be found in certain microorganisms, including *V. cholerae*. The oxidase test results show a positive reaction with a change in dark blue (Figure 2).

Figure 2. Result of Oxidase Test

2) Sensitivity test for 0/29 vibriostat

The test results showed that bacteria tested sensitive to 10 mg and 150 mg were characterized by the formation of zones around the disk (Figure 3). Sensitivity test for 0/129 vibriostats was used to see suspected colonies sensitive or resistant to 0/129 vibriostat 10µg and 150 µg. The *Vibrio cholerae* species is sensitive to a mixture of 0/129 (2,4-diamino-6,7-diisopropylpteridine phosphate), which distinguishes them from Aeromonas species, which are resistant to 0/129 (Abbott et al., 1998).

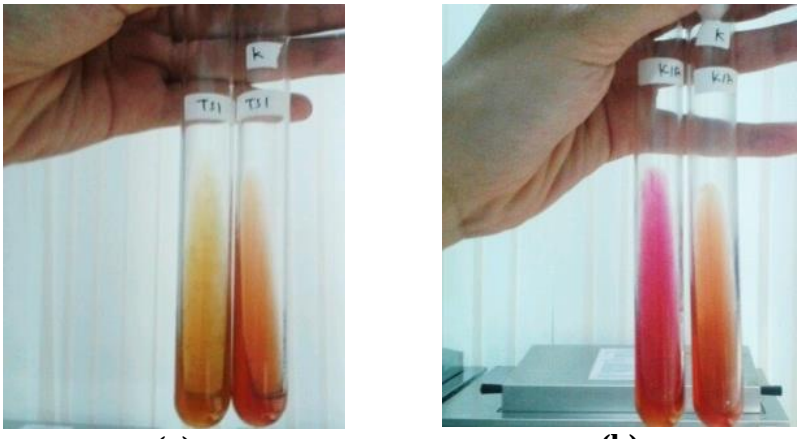


(a) **(b)**

Figure 3. Result of Sensitivity test **(a)** sensitivity 10 µg, **(b)** sensitivity 150 µg

3) TSI and KIA test

Triple Sugar Iron (TSI) and Kligger Iron Agar (KIA) which have been incubated for 18-24 hours can be observed by looking at the color changes (Figure 4). Based on the test results, the TSI agar slant and straight become yellow (acid). While in the KIA test the agar slant is yellow (acidic) and agar slant is red (alkaline). KIA contains sugar which will be reacted by bacteria and form an acidic atmosphere which is marked in yellow, while the alkaline atmosphere is marked in red.

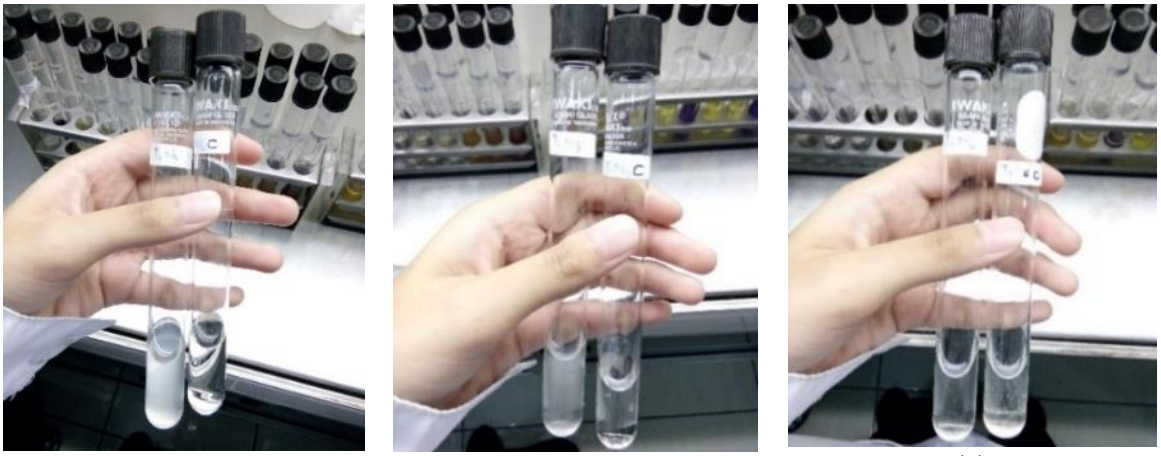


(a) **(b)**

Figure 4. Result: (a) TSI, (b) KIA

4) Tolerance Test on Salt

This test aims to see the suspected colonies can live at a predetermined salinity according to the characteristics of *V. cholerae.* Based on the results of the test, a positive reaction was characterized by turbidity on the T_1N_0 and T_1N_3 media which showed the suspected colony of V. cholerae growing on the medium.



(a) **(b)** **(c)**

Figure 5. Result of Tolerance Test on Salt: **(a)** T_1N_0, **(b)** T_1N_3, **(c)** T_1N_6

Advanced Biochemical Test

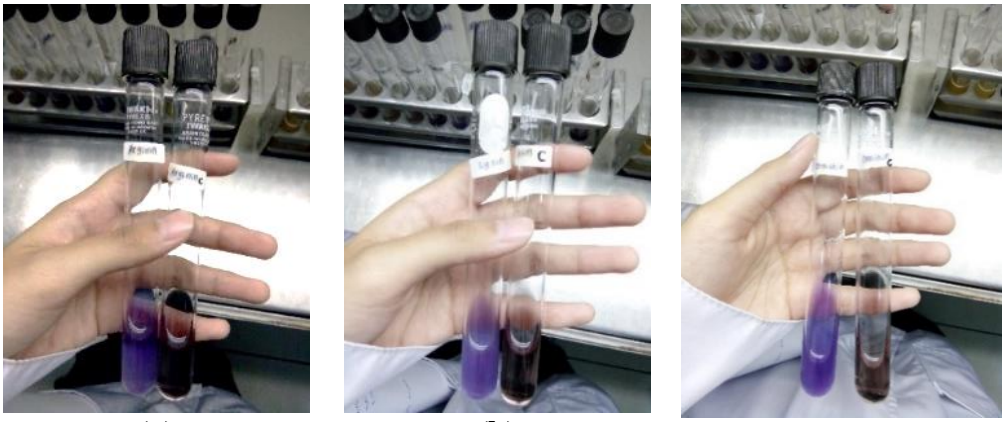
1) Urea Hydrolysis Test

The test results showed a positive reaction, indicated by the change in color of the media from orange to pink, this indicates that *V. cholerae* does not have the ability to hydrolyze Urea (Carr, 2017).

Figure 6. Hasil Uji Hidrolisis *Urea*

2) Arginine, Lysine and Ornithin tests

Decarboxylase reaction to amino acids produces alkaline pH and converts the media to bright purple (positive reaction) (Mah et al., 2003). While the glucose fermentation reaction produces acid and converts the media to yellow (negative reaction). Control tubes that do not contain amino acids turn yellow. V. cholerae produces a positive arginine reaction, lysine and Ornithin are positive (Choopun et al., 2002). The test results showed a negative reaction on arginine media and positive reactions in the media of Lysine and Ornithin.

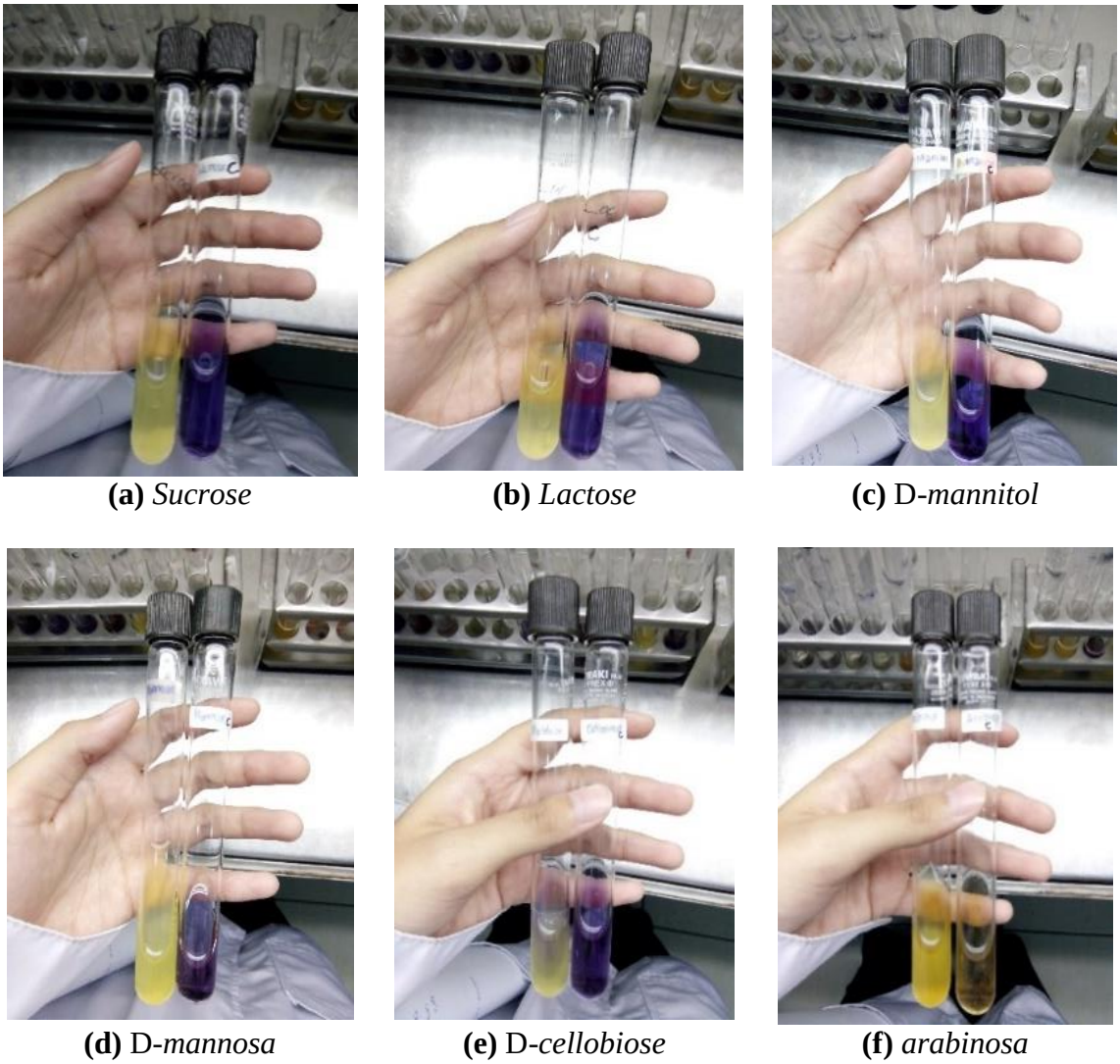


(a) **(b)** **(c)**

Figure 7. Test Results: **(a)** Arginin, **(b)** Lysine, **(c)** Ornithin

3) Carbohydrate Fermentation Test

In this carbohydrate fermentation test, the sugars used were Sucrose, Lactose, D-mannitol, D-mannose, D-cellobiose and Arabinose (Abbott et al., 2003). Based on the results of carbohydrate fermentation testing, all the tested media showed a positive reaction in which there was a yellow change in color indicating a change in pH to acid. The test results showed a discrepancy with the BAM (Bacteriological Analytical Manual) FDA 1998 quality standard, where Vibrio cholerae should not be able to ferment D-cellobiose, Lactose and Arabinose (Sariadji et al., 2015). Some studies show that there are various sources of transmission from the bacterium *V. cholerae*. Food sources derived from fishery products are one of the most common sources of transmission. This is closely related to the theory that water with high salinity content such as sea water is a natural place of life for *Vibrio* sp. thus facilitating the contamination process (Vernberg et al., 1992).



(a) *Sucrose* **(b)** *Lactose* **(c)** D-*mannitol*

(d) D-*mannosa* **(e)** D-*cellobiose* **(f)** *arabinosa*

Figure 8(a-f). Results of Carbohydrate Fermentation Test

5. CONCLUSIONS

The results showed that the three samples of fisheries products tested (frozen cuttle fish, frozen baby octopus and pasteurized crab) it was not indicated to contain pathogenic bacteria Vibrio cholerae in both qualitative and biochemical tests. The frozen baby octopus samples had a chance of contaminated *V. cholerae*. The results of the minimal characteristic test of V.cholerae biochemical test on frozen baby octopus samples obtained 3 tests that were not in accordance with the characteristics of *V. cholerae*, namely carbohydrate fermentation test (D-cellobiose, Lactose and Arabinose) where *Vibrio cholerae* should not be able to ferment D-cellobiose , Lactose and Arabinose. Thus it was concluded that the bacteria were Vibrio bacteria but not *Vibrio cholerae*, given the variety of Vibrio bacteria.

References

[1] Rachmawati L, Mursinto D, & Istifadah N. 2017. Fishery's Potential in Indonesia. *International Journal of Humanities and Social Science Invention*, 6 (2), 58-64

[2] Osunla CA, & Okoh AI. 2017. *Vibrio* Pathogens: A Public Health Concern in Rural Water Resources in Sub-Saharan Africa. *International Journal of Environmental Research and Public Health*, 14 (10), 1188

[3] Novoslavskij A, Terentjeva M, Eizenberga I, Valciņa O, Bartkevičs V, Bērziņš A. 2016. Major foodborne pathogens in fish and fish products: a review. *Annals of Microbiology*, 66 (1), 1–15

[4] Kumari CK, Diwedi K, Singh SK, Shukla S, & Singh M. 2014. Isolation, Partial Purification and Immobilization of Neuraminidase enzyme from Vibrio cholera. *International Journal of Research in BioSciences*, 3 (2), 53-57

[5] Faruque SM, Albert MJ, Mekalanos JJ. 1998. Epidemiology, Genetics, and Ecology of Toxigenic *Vibrio cholerae*. *Microbiology and Molecular Biology Reviews*, 62 (4), 1301-1314

[6] Thomas KU, Joseph N, Raveendran O, & Shanta Nair. 2006. Salinity-induced survival strategy of Vibrio cholerae associated with copepods in Cochin backwaters. *Marine Pollution Bulletin*, 52 (11), 1425-1430

[7] Felix F, Nugroho T, Silalahi S, and Octavia Y. 2011. Molecular characteristics of Vibrio sp causing Black Tiger Prawn (*Penaeus monodon*) disease in Sumatra and Java shrimp ponds by 16S rDNA sequencing. *Journal of Agricultural Technology*, 7 (3), 679-694

[8] Moreno SA, & Taylor RK. 2013. Cholera: Environmental Reservoirs and Impact on Disease Transmission. *Microbiology Spectrum*, 1 (2), 1-12

[9] Chakraborty RD, Surendran PK, & Joseph TC. 2008. Isolation and characterization of Vibrio parahaemolyticus from seafoods along the southwest coast of India. *World Journal Microbiol Biotechnol*, 24, 2045-2054

[10] Butt AA, Aldridge KE, & Sanders CV. 2004. Infections related to the ingestion of seafood Part I: viral and bacterial infections. *The Lancet Infectious Disease*, 4 (4), 201-212

[11] Cabral JPS. 2010. Water Microbiology, Bacterial Pathogens and Water. *International Journal of Environmental Research and Public Health*, 7 (10), 3657-3703.

[12] Tapotubun AM, Savitri IKE, & Matrutty TEAA. 2016. The Inhibitor Pathogen Bacteria's of Sea Grape Caulerpa Lentillifera Applies on Fresh Fish. *Indonesian Fisheries Processing Journal* 19 (3), 299-309

[13] Sanders ER. 2012. Aseptic Laboratory Techniques: Plating Methods. *Journal of Visualized Experiments*, 63, 3064

[14] Huq A, Haley BJ, Taviani E, Chen A, Hasan NA, and Colwell RR. 2012. Detection, Isolation, and Identification of Vibrio cholerae from the Environment. *Current Protocol in Microbiology*, 26 (1), 6A.5.1-6A.5.51

[15] Abbott SL, Lourdes S. Seli, Michael Catino, Jr., Michael A. Hartley, and J. Michael Janda. 1998. Misidentification of Unusual Aeromonas Species as Members of the Genus Vibrio: a Continuing Problem. *Journal of Clinical Microbiology*, 36(4), 1103-1104

[16] Carr FJ. 2017. Microbiology: A Fundamental Introduction Second Edition. *EC Microbiology*, 8 (3), 123-183

[17] Mah, Hyung J, Ahn JB, Park JH, Sung HC, & Hwang HJ. 2003. Characterization of Biogenic Amine-Producing Microorganisms Isolated from Myeolchi-Jeot, Korean Salted and Fermented Anchovy. *Journal of Microbiology and Biotechnology*, 13 (5), 692-699

[18] Choopun N, Louis V, Huq A, & Colwell RR. 2002. Simple Procedure for Rapid Identification of Vibrio cholerae from the Aquatic Environment. *Applied and Environmental Microbiology*, 68 (2), 995-998

[19] Abbott SL, Cheung WKW, and Janda JM. 2003. The Genus Aeromonas: Biochemical Characteristics, Atypical Reactions, and Phenotypic Identification Schemes. *Journal of Clinical Microbiology*, 41 (6): 2348-2357

[20] Sariadji K, Sunarno, & Putranto RH. 2015. Fast Diagnostic Test as an Alternative Method for Diagnosing Kholera Caused by Agent Vibrio Cholera. *Indonesian Journal of Biotechnology Medicine*, 4 (1), 1-7

[21] Vernberg FJ, W.B. Vernberg, E. Blood, A. Fortner, M. Fulton, H. Mc Kellar, W. Michener, G. Scott, T. Siewicki, & K. El Figi. 1992. Impact of urbanization on high-salinity estuaries in the southeastern United States. *Netherlands Journal of Sea Research*, 30, 239-248