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FULL PAPER

Detection of *Vibrio cholerae* in street food (*satar* and *otak-otak*) by Loop-Mediated Isothermal Amplification (LAMP), multiplex Polymerase Chain Reaction (mPCR) and plating methods

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Abstract

This goal of this study was to investigate the presence of *Vibrio cholerae* in street food, namely *satar* and *otak-otak*, using Loop-Mediated Isothermal Amplification (LAMP), multiplex Polymerase Chain Reaction (mPCR) and conventional plating on Thiosulphate Citrate Bile-Salt Sucrose (TCBS) agar methods. A total of 78 *satar* and 35 *otak-otak* were purchased from different districts of Terengganu (Besut, Setiu, Kuala Terengganu and Kemaman). *V. cholerae* was found in *satar* with LAMP (10.3%), mPCR (10.3%) and plating (0%). No *V. cholerae* was found in *otak-otak* using the three methods. This might be due to *V. cholerae* able to survive in *satar* after grilling due to its thickness which may contribute to undercooking. This study concluded that low presence of *V. cholerae* in *satar* and *otak-otak* can be detected by molecular methods but not the conventional plating method. LAMP assay is a useful tool for rapid detection of pathogens in food due to its simplicity, highly sensitive and visual interpretation capability. Though the prevalence of *V. cholerae* was low in the samples, proper handling of this food will help in reducing the risk of acquiring infection from *V. cholerae* in contaminated samples.

1. Introduction

Vibrio cholerae, a member of the family *Vibrionaceae*, is a facultatively anaerobic, Gram-negative, and non-spore-forming curved rod about 1.4–2.6 mm long (Baumann *et al.,* 1984). *V. cholerae* live naturally in both marine and freshwater habitats and in association with aquatic animals (Feldhusen, 2000). The genus *Vibrio* of the family *Vibrionaceae* embraces more than 60 species, mostly marine in origin and its taxonomy is continuously being revised due to the addition of new species (Etinosa *et al.,* 2008). The natural biota of fish might contain some *Vibrio* species (Colwell, 1996; Supungul *et al*., 2004).

Satar and *otak-otak* are popular street food in Terengganu that is usually consumed as snack or side dish. It is easy to find these foods in East Coast of Peninsular Malaysia, namely Terengganu and Kelantan due to the abundance of fish in these states. *Satar* and *otak-otak* normally used round scad fish (*selayang*) or chub mackerel fish (*kembung*) as their main ingredient because these types of fish are cheaper than other fish. In Malaysia, *satar* and *otak-otak* are fish-based street food products that are usually prepared and grilled in a large amount before being served to customers. They are exposed to the ambient temperature for a long period of time which might cause multiplication of microorganism. The grilling process for *satar* and *otak-otak* using charcoal require skills and doneness of the products are difficult to be determine. This is because if the grilling process is not handled carefully will result in the *satar* and *otak-otak* burnt on the outside but not cooked or undercooked inside. Thus, the microorganism or pathogen present in the sample will grow and cause

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foodborne illness.

Oliver (2003) reported that, foods implicated in the spread of *Vibrio cholerae* include seafood (raw fish, crabs, shrimp, mussels, cockles, squid, oysters, clams), rice, raw pork, street vendor food, frozen coconut milk, and raw fruits and vegetables, with greater survival occurring in cooked foods. *V. cholerae* is the etiological agent of cholera which is transmitted to human by ingestion of contaminated food or water, eating raw or improperly cooked seafood or seafood products, and also direct fecal contact with food handlers. *V. cholerae* serogroups O1 and O139 are usually found as the main causes of this disease (Kaper *et al.,* 1995; Fraga *et al.,* 2007; Suzita *et al.,* 2009). This is due to more than 95% of these strains produce the cholera toxin (CT). The symptoms of cholera disease are characterized by abdominal cramps, nausea, vomiting, as well as massive acute diarrhoea. These symptoms cause the depletion of body fluids and electrolytes which are essential for life, causing dehydration to the infected person which might lead to death in severe and untreated cases (Rabbani and Greenough, 1999; Weinke *et al*. 2008; Shrestha *et al*., 2010). The aim of this paper is, therefore, to determine the presence of *V. cholera* in local popular street food, *satar,* and *otak-otak,* using novel molecular method LAMP, multiplex PCR and the conventional plating method.

2. Materials and methods

2.1 Sample collection

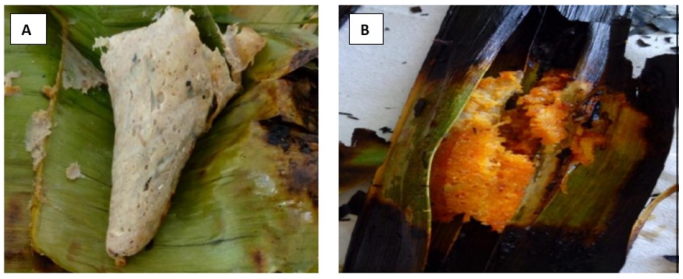

Figure 1. Fish-based product sold as street food. A) *Satar* and B) *Otak-otak*

Satar and *otak-otak* were chosen as popular fish-based street food products as shown in Figure 1. A total of 113 samples which consist of seventy-eight *satar* and thirty-five *otak-otak* were purchased from different stalls in districts of Terengganu such as Besut, Setiu, Kuala Terengganu, Marang and Kemaman. Each sample was labeled with an identification number to differentiate their place of origin.

2.2 Enrichment of Vibrio cholerae in satar and otak-otak

For enrichment method, 10 g of sample was weighed into a sterile stomacher bag and added with 90 mL of alkaline peptone water (APW) (Merck, Germany). The mixture was homogenized in the stomacher (Seward, UK) for 1 min at 250 rpm and incubated at 37°C for 24 h.

2.3 Isolation of Vibrio cholerae from satar and otak-otak

A loopful of the incubated samples was taken from the top pellicle and streaked onto duplicate selective medium TCBS agar plates (Merck, Germany). The agar plates were then incubated for 18−24 h at 37°C. The presumptive colonies of *V. cholerae* (yellow colonies with 2-3 mm diameter) on TCBS agar plate were selected for further confirmation using PCR.

2.4 DNA extraction

V. cholerae DNA from vegetable samples will be extracted using boiled-cell method as described in Tang *et al.* (2014) with slight modification. A portion of 500 µL enriched sample was centrifuged at 10,000 x *g* to pellet the cells. The supernatant was discarded, and the pellet will be re-suspended with 500 µL of sterile deionised water. The tube containing the re-suspended cells pellet was boiled at 100˚C for 10 mins using digital dry bath (Corning, Japan). The boiled mixture was cooled at -20˚C for 10 mins before undergone centrifugation for 10 mins at 10,000 x *g*. The supernatant containing DNA was used for *V. cholerae* detection by multiplex PCR or LAMP.

2.5 Molecular detection method

2.5.1 Multiplex PCR assay

A multiplex PCR was used to detect the presence and virulence gene (*hly*A, *tcp*I, *ctx*B) of *Vibrio cholerae* in *satar* and *otak-otak*. Table 1 shows the sequence of specific primers used for detection of *V. cholerae*.

Table 1. Sequences of oligonucleotide primers used to target specific genes in *Vibrio cholerae* and their respective amplicons sizes.

Target genes	Nucleotide sequences 5'−3'	Tm (°C)	Amplicon size (bp)	References
ctxB-F	ATG AGG CGT TTT ATT CCA TAC AC	57.5	128	Imani *et al*. (2013)
ctxB-R	TAC CAG GTA GTC AAC ATA TAG ATT CA			
tcpI-F	TGC GTG ATG CTA ATT GGA CT	60.4	444	Imani *et al*. (2013)
tcpI-R	TTC GGT TTG TTT GCT TGA TG			
hlyA-F	GGC AAA CAG CGA AAC AAA TAC C	59.0	738	Panicker *et al*. (2004)
hlyA-R	CTC AGC GGG CTA ATA CGG TTT A			

Multiplex PCR was performed in a 25 uL reaction mixture containing 12.1 µL of sterile ultra-pure water, 5 µL of 5X Green Go Taq Flexi Buffer (Promega, USA), 2 µL of 25 mM $MgCl_2$ solution (Promega, USA), 0.5 µL of 10 mM deoxynucleotide triphosphate (dNTP) (Promega, USA), 0.5 µL of 10 uM *ctx*B forward and reverse primers (IDT, Singapore), 0.5 µL of 10 uM *tcp*I forward and reverse primers (IDT, Singapore), 0.5 µL of 10 uM *hly*A forward and reverse primers (IDT, Singapore), 0.4 µL of 5U/µL Go *Taq* DNA polymerase (Promega, USA), and 2 µL of DNA template.

The PCR assay was performed with the Veriti 96-Well Thermal Cycler (Applied Biosystems, Singapore). The PCR reaction initiated with pre-denaturation at 95ºC for 5 mins, 35 cycles of denaturation at 95ºC (45 s), annealing at 55ºC (1 min) and extension at 72ºC (1 min 30s), followed by a final extension at 72ºC for 5 mins.

PCR products were run through electrophoresis using 1.0% of an agarose gel, 70V for 60 mins. DNA-molecular ladder, 100 bp, (Promega Madison, USA) was included for each electrophoresis gel run. The result was visualized using the Fujifilm LAS-3000 Imager (Fujifilm, Japan).

2.5.2 LAMP assay

LAMP assay was carried out in a total of 25 µL of reagents mixture containing 7.2 µL of sterile ultra-pure water, 12.5 µL of 2x Reaction Mix (RM) (Loopamp, Japan), 2 µL of 30.77 uM *V. cholerae* Primer Mix (PM) (Loopamp, Japan), 1 µL of Fluorescent Detection Reagent (FD) (Loopamp, Japan), 1 µL of Bst DNA polymerase (Loopamp, Japan), and 2 µL of DNA template. The reagents mixture was then be heated in a thermal cycler at 63°C for 60 mins (predetermined time) and terminated at 80°C for 10 mins. Finally, the LAMP products were observed for colour changes. The changed in colour from yellowish orange to green fluorescent indicated the LAMP product was positive, but if the colour remains unchanged, it indicated negative result.

3. Results and discussion

3.1 Prevalence of Vibrio cholerae in Samples

Vibrio spp. infection has been found to be one of the most common foodborne infections in Asia (Sutherland and Varnam, 2000). The infections are usually initiated by consumption of raw or undercooked seafood (Ottaviani *et al.,* 2009; Baker-Austin, 2015), which may be contaminated by the presence of the pathogen in the marine environment (Sutherland and Varnam, 2000). Poor hygiene practices by food handlers during processing and post-processing of food may cause the pathogens to multiply and re-contaminated the food under favourable conditions which might pose a significant risk of food poisoning. Table 2 and Table 3 summarizes the prevalence of *V. cholerae* in *satar* and *otak-otak* obtained throughout this study.

The present study showed 7.96% of 113 samples fish

Table 2. Number of samples positive for *Vibrio cholerae* in *satar* detected using multiplex PCR, LAMP methods and plating.

District	Stall	N	Multiplex PCR				LAMP	Plating
			n	*ctxB*	*tcpI*	*hlyA*		
Besut	PB (1)	10	0	0	0	0	0	0
	PB (2)	5	0	0	0	0	0	0
	PR	5	0	0	0	0	0	0
	T	10	0	0	0	0	0	0
Setiu	S	10	0	0	0	0	0	0
Kuala Terengganu	BR (1)	16	4	0	4	3	4	0
	BR (2)	12	4	0	0	4	4	0
Marang	M	5	0	0	0	0	0	0
Kemaman	K	5	0	0	0	0	0	0
Total:		78	8	0	4	7	8	0

Table 3. Number of samples positive for *Vibrio cholerae* in *otak-otak* detected using multiplex PCR, LAMP methods, and plating.

District	Stall	N	Multiplex PCR				LAMP	Plating
			n	*ctxB*	*tcpI*	*hlyA*		
Besut	PB (1)	10	0	0	0	0	0	0
	PB (2)	10	0	0	0	0	0	0
Setiu	S	5	0	0	0	0	0	0
Kuala Terengganu	BR (1)	10	0	0	0	0	0	0
Total:		35	0	0	0	0	0	0

-based street food products including 78 *satar* and 35 *otak-otak* which obtained from different districts in Terengganu were detected for *V. cholerae.* From the data collected, it showed that *V. cholerae* was only present in *satar* (8 *satar*), and not *otak-otak.* This means the prevalence of *V. cholerae* in *satar* (11.5%) was higher than *otak-otak* (0%). All the positive samples were purchased in Kuala Terengganu from two different stalls, BR (1) and BR (2).

This study revealed that *V. cholerae* was found in certain fish-based street food products commonly found in Malaysia, even though the detection is low. In accordance to Hong Kong, Food and Environmental Hygiene Department (FEHD) (2005), indigenous microflora in the live seafood might harbor *Vibrio* spp. at the time of seafood capture or harvest. Meanwhile, previous studies showed positive detection of *V. cholerae* in raw seafood samples such as shrimp, squid, crab, cockles, mussels, mackerel, clam, prawn, and fish (Elhadi *et al.,* 2004; Ottaviani *et al.,* 2009; Mrityunjoy *et al.,* 2013). Differ with raw seafood, the presence of high salt content as one of the ingredients in the seafood products may reduce the growth of *V. cholerae* as it can grow in the salt range of 0.1−4.0% NaCl, while optimum is 0.5% NaCl (Rabbani and Greenough, 1999).

Besides that, *V. cholerae* grow optimally at 37°C within the temperature range of 10°C to 43°C (FEHD, 2005) and they are very sensitive to both heat and radiation, as well as dry condition (ICMSF, 1996), hence, its prevalence in seafood products especially in *satar* and *otak-otak* may be lowered as both of these street foods used traditional grilling method in their preparation process. This type of cooking method can accelerate the reduction of moisture content of the foods. However, if the process is not properly controlled, *satar* and *otak-otak* may be burnt on the outside but undercooked inside. As such, the contaminated *satar* and *otak-otak* will pose a risk of food poisoning due to *Vibrios* capable of surviving for up to 6 hours (Tang *et al.*, 2017).

Generally, microorganisms need water for their growth. The range of water activity for *V. cholerae* is between 0.940−0.988, with an optimum water activity of 0.984 (Rabbani and Greenough, 1999). In addition, *V. cholerae* may survive for several days and multiply in food that is alkaline and moist (European Commission, 1998). Heating shellfish for several minutes until the internal temperature of at least 60°C is sufficient to kill the pathogenic *Vibrios* (Anon, 1991). *Vibrios* were found to be able to grow well in a variety of cooked foods with alkaline pH at 22°C, 30°C and 37°C, such as cooked prawns, hard boiled eggs and cooked mussels (Kolvin and Roberts, 1982).

The prevalence of *V. cholerae* in *satar* was higher than those found in *otak-otak* might result from the undercooked thicker and moist *satar* in certain samples. In contrast, *otak-otak* is less moist than *satar* because of its lower in size and thickness. Therefore, *otak-otak* may be cooked evenly and achieved adequate heat or temperature to eliminate the growth of *V. cholerae* in a shorter time than *satar* needed.

Besides that, it has been commonly known that water is an important factor in the transmission of cholera which has been implicated with six pandemics. Generally, *V. cholerae* is widely distributed in tropical aquatic environments and shows the highest numbers in temperate water. According to Wong and Desmarchelier (1999), the optimum water temperatures for the isolation of *V. cholerae* are 20-35°C, and culturable cells may no longer be detectable when the temperature drops below 16°C.

Both molecular methods employed in this study, namely PCR and LAMP, performed equally well in detecting the presence of *Vibrio cholerae* in samples collected. Figure 2 shows amplification using multiplex

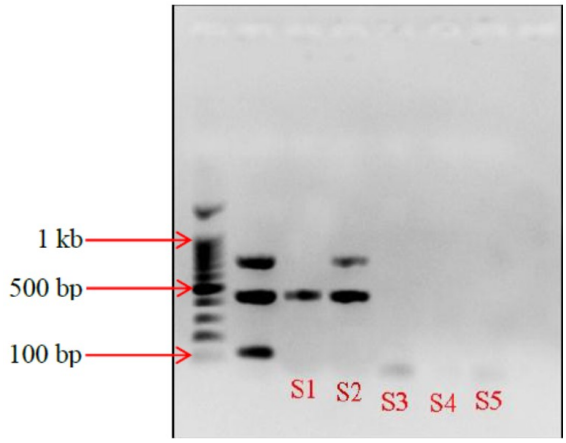

Figure 2. Gel electrophoresis of PCR amplification for *ctx*B (128 bp), *tcp*I (444 bp) and *hly*A (738/727 bp). Lane 1: 100 bp molecular ladder; Lane 2: positive control using DNA from *V. cholerae* clinical; Lane 3: DNA extracted from S1 BR (1); Lane 4: DNA extracted from S2 BR (1); Lane 5: DNA extracted from S3 PB (1); Lane 6: DNA extracted from S4 PB (1); Lane 7: DNA extracted from S5 PB (1); Lane 8: negative control.

Figure 3. A: LAMP amplification for DNA extracted from S6 BR (1) which showed positive LAMP reaction; B: LAMP amplification for DNA extracted from O4 PB (1) which showed negative LAMP reaction.

PCR assay detecting *ctxB, tcpI* and *hlyA* genes of *V.cholerae.* There was no *V. cholerae* isolate obtained from conventional culture detection method using TCBS agar. This result was in agreement with previous findings that showed that the culture method lacked sensitivity for the detection of low cell numbers compared to molecular method (Harwood *et al.,* 2004). Loop-mediated isothermal amplification (LAMP) was introduced in the year 2000 (Notomi *et al.,* 2000) uses single temperature at 63^{o}C and requires an hour to complete the assay. Visual observation of positive and negative reactions was possible from the colour change in the LAMP assay without gel electrophoresis step. These advantages greatly reduce the analysis time of from 4 hours in conventional PCR to 1 hour in LAMP assay. Such method is important for a small and less equipped laboratory setting or during field work. Figure 3 shows the positive and negative reaction of LAMP method.

4. Conclusion

Both multiplex PCR and LAMP method performed equally in the detection of *V. cholerae* in *satar* and *otak-otak*. The prevalence of *V. cholerae* was found to be low in *satar* and *otak-otak* sampled in Terengganu. However, proper food handling should be practised at all times to prevent foodborne diseases resulted from undercooked and contaminated products.

Conflict of interest

The authors declare that there is no conflict of interest in the present study.

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References

Anon. (1991). Cholera outbreak - Peru, Ecuador, and Colombia. *Morbidity and Mortality Weekly Report*, 40(13), 225-227.

Baker-Austin, C. (2015). Chapter 6 – Antimicrobial Resistance in *Vibrio* Species. In Chen, C.Y., Yan, X., and Jackson, C.R. (Eds.). Antimicrobial Resistance and Food Safety. Methods and Techniques, p. 105-118. London, UK: Elsevier. https://doi.org/10.1016/B978-0-12-801214-7.00006-5

Baumann, P., Furniss, A.L. and Lee J.V. (1984). Genus 1, *Vibrio*. In Holt, J.G. and Krieg, N.R. (Eds.), *Bergey's Manual of Systematic Bacteriology* p. 518-538. Baltimore, M.D.: Williams and Wilkins.

Colwell, R.R. (1996). Global climate and infectious disease: The cholera paradigm. *Science*, 274, 2025-2031. https://doi.org/10.1126/science.274.5295.2025

Elhadi, N., Son, R., Chen, C.H. and Nishibuchi, M. (2004). Prevalence of potentially pathogenic *Vibrio* species in the seafood marketed in Malaysia. *Journal of Food Protocol*, 67(7), 1469-1475. https:// doi.org/10.4315/0362-028X-67.7.1469

Etinosa, O.I. and Anthony, I.O. (2008). Emerging Vibrio species: An unending treat to public health in developing countries. *Research in Microbiology*, 159, 495-506. https://doi.org/10.1016/ j.resmic.2008.07.001

European Commission. (1998). Food Safety: From Farm to Fork. Retrieved on 24 May, 2018 from European Commission: http://ec.europa.eu/food/fs/sc/scf/ out12_en.html

Feldhusen, F. (2000). The role of seafood in bacterial foodborne diseases. *Microbes and Infection*, 2(13), 1651-1660. https://doi.org/10.1016/S1286-4579(00) 01321-6

Food and Environmental Hygiene Department (FEHD): Queensway, Hong Kong. (2005). *Vibrio* species in seafood. Retrieved on 25 May, 2018 from Food and Environmental Hygiene Department, Hong Kong: http://www.cfs.gov.hk/english/programme/ programme_rafs/files/vibrios_ra.pdf

Fraga, S.G., Pichel, M., Costagliola, M., Cecilia, M., Jurquiza, V., Peresuti, S., Caffer, M.I., Aulet, O., Hozbor, C., Tracanna, B.C., de Gamundi, A.V., Hernandez, D. and Ramirez, F.C. (2007). Environment and virulence factors of *Vibrio cholerae* strains isolated in Argentina. *Journal of Applied Microbiology*, 103(6), 2448-2456. https:// doi.org/10.1111/j.1365-2672.2007.03468.x

Harwood, V.J., Gandhi, J.P. and Wright, A.C. (2004). Methods for isolation and confirmation of *Vibrio vulnificus* from oysters and environmental sources: a review. *Journal of Microbiological Methods*, 59(3), 301-316. https://doi.org/10.1016/ j.mimet.2004.08.001

International Commission on Microbiological Specifications for Foods (ICMSF). (1996). *Vibrio cholerae*. In Microorganisms in Foods 5 Microbiological Specifications of Food Pathogens, p. 414-425. London: Blackie Academic and

Professional.

Kaper, J.B., Morris Jr, J.G. and Levine, M.M. (1995). Cholera. *Clinical Microbiology Reviews*, 8(1), 48-86.

Kolvin, J.L. and Roberts, D. (1982). Studies on the growth of *Vibrio cholerae* biotype classical in foods. *Journal of Hygiene*, 89, 243-252. https://doi.org/10.1017/S0022172400070777

Mrityunjoy, A., Kaniz, F., Fahmida, J., Shanzida, J.S., Md. Aftab, U. and Rashed, N. (2013). Prevalence of *Vibrio cholerae* in different food samples in the city of Dhaka, Bangladesh. *International Food Research Journal*, 20(2), 1017-1022.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, 28(12), E63. https://doi.org/10.1093/nar/28.12.e63

Oliver, J.D. (2003). Culture media for the isolation and enumeration of pathogenic *Vibrio* species in foods and environmental samples. In Corry, J.E.L., Curtis, G.D.W. and Baird R.M. (Eds.). Handbook of Culture Media for Food and Water, p. 249-269. London, UK: Elsevier. https://doi.org/10.1016/S0079-6352(03)80020-6

Ottaviani, D., Leoni, F., Rocchegiani, E., Santarelli, S., Masini, L., Trani, V.D., Canonico, C., Pianetti, A., Tega, L. and Carraturo, A. (2009). Prevalence and virulence properties of non-O1 non-O139 *Vibrio cholerae* strains from seafood and clinical samples collected in Italy. *International Journal of Food Microbiology*, 132(1), 47-53. https://doi.org/10.1016/j.ijfoodmicro.2009.03.014

Rabbani, G.H. and Greenough, W.B. (1999). Food as a vehicle for the transmission of cholera. *Journal of Diarrhoeal Disease Research*, 17(1), 1-9.

Shrestha, S.D., Malla, S., Adhikari, B.R., Shakya, G., Basnyat, S.R. and Sharma, S. (2010). Antibiotic susceptibility patterns of *Vibrio cholerae* isolates. *Journal Nepal Medicine Association*, 49(179), 232-236.

Supungul, P., Klinbunga, S., Pichyangkura, R., Hirono, I., Aoki, T. and Tassanakajon, A. (2004). Antimicrobial peptides discovered in the black tiger shrimp *Penaeus monodon* using the EST approach. *Diseases of Aquatic Organisms*, 61(1-2), 123-135. https://doi.org/10.3354/dao061123

Sutherland, J. and Varnam, A. (2000). Enterotoxin-producing *Staphylococcus, Shigella, Yersinia, Vibrio, Aeromonas* and *Plesiomonas*. In Blackburn, C.W. and McClure, P.J. (Eds.). Foodborne Pathogens, Hazards, Risk Analysis and Control, p. 358-415. Cambridge, UK: Woodhead Publishing Limited

Suzita, R., Abdulamir, A.S., Fatimah, A.B. and Son, R. (2009). A mini review: cholera outbreak via shellfish. *American Journal of Infectious Diseases*, 5 (1), 40-47. https://doi.org/10.3844/ajidsp.2009.40.47

Tang, J.Y.H., Mat-Sa'ad, S.H., Banerjee, S.K., Ho, L.H. and Son, R. (2017). Survivability of *Vibrio parahaemolyticus* in satar and otak-otak, Malaysian fish-based street food. *International Food Research Journal,* 24(3), 1000-1005.

Tang, J.Y.H., Wan-Rosli, W.F., Abdul-Razak, N.H., Yeo, C.C., Abu Bakar, C.A. and Son, R. (2014). Incidence and antibiogram of *Vibrio parahaemolyticus* in processed and frozen bivalve mollusks in Kuala Terengganu, Malaysia. *International Food Research Journal,* 21(3), 1949-1953.

Weinke, T. Liebold I., Burchard, G.D., Frühwein, N., Grobusch, M.P., Hatz, C., Kollaritsch, H., Nothdurft, H.D., Reisinger, E., Rieke, B., Schönfeld, Ch., Steffen R. and Stich, A. (2008). Prophylactic immunization against traveller's diarrhoea caused by enterotoxin forming strains of *Escherichia coli* and against cholera, travel medicine and infectious disease. *Travel Medical Infectious Disease*, 6(6), 362-336. https://doi.org/10.3844/ajidsp.2009.40.47

Wong, F.Y.K. and Desmarchelier, P.M. (1999). *Vibrio cholera.* In Robinson, R.K., Batt, C.A. and Patel, P.D. (Eds.). Encyclopedia of Food Microbiology, p. 2242-2257. Australia: Academic Press,. https://doi.org/10.1006/rwfm.1999.1655

