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Risk of transmission of *Vibrio parahaemolyticus* in foods

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Abstract

Vibrio parahaemolyticus is well known to be abundantly distributed in marine, coastal and estuarine environments. Since 1951, *V. parahaemolyticus* had been the source of numerous outbreaks related to contaminated or mishandled seafood. However, *V. parahaemolyticus* had been detected on other types of food. This issue has prompted this study to investigate on the prevalence of *V. parahaemolyticus* in various food samples and determine the risk associated with it. The results of the MPN-plating technique of the study indicated that *V. parahaemolyticus* was detected in seafood (33.3%, 95% Confidence Interval [CI] 31.9 – 34.8 , 94 – 290 MPN/g) and vegetables (10.0%, 95% CI 9.7 – 10.3 , 9.2 – 23 MPN/g) while negative *V. parahaemolyticus* was detected in fruits (0.0%, 95% CI 0 – 1, <3 MPN/g) and chicken (0.0%, 95% CI 0 – 1.5, <3 MPN/g). Microbial risk assessment using @risk was conducted based on the data collected on separate food classes. The probability of illness of consuming contaminated seafood and vegetables with *V. parahaemolyticus* was 1.86 x 10^{-8} with a rate of 0.505 per 100,000 Malaysians and 4.83 x 10^{-9} with a rate of 0.002 per 100,000 Malaysians respectively. The risk of consuming both contaminated seafood and vegetables was simulated and the probability of illness was 2.34 x 10^{-8} with 249 expected cases to occur per year and a rate of 0.831 per 100,000 Malaysians. The underlying factors that contribute to the transmission of *V. parahaemolyticus* can cause an increased risk of infection and should be prevented to conserve the public health.

Introduction

Vibrio parahaemolyticus is a Gram-negative, enteric, and non-spore forming marine bacterium. The halophilic curved rod-shaped bacterium is abundantly distributed in coastal and estuarine environments rather than the open sea (Adam and Moss, 2008). It forms part of the indigenous microflora of aquatic habitats of various salinities (Colwell *et al*., 1984). The distribution of *V. parahaemolyticus* is affected by various factors such as temperature, salinity and geographical locations (Kaneko and Colwell, 1973; DePaola *et al.*, 1990).

Being infected with *V. parahaemolyticus*, one may suffer from gastrointestinal illness characterized by explosive diarrhoea, headache, vomiting, nausea, abdominal cramps and low-grade fever within 24 hours of ingestion. The symptoms may last for three days, but it is self-limiting. However, severe infection may cause septicaemia which is life threatening to immunocompromised individuals and severe dehydration leading to death (Su and Liu, 2007). *V. parahaemolyticus* also causes traveller's diarrhoea, wound infection, and ear infection (Pavia *et al.*, 1989). Pathogenic strains are responsible for the outbreaks of acute gastroenteritis and it is only a sub-population (1 – 10%) of the environmental strains (Iida *et al*., 1998; DePaola *et al*., 2000; Institute of Food Technologists (IFT), 2004; Paranjype *et al*., 2012). The pathogenicity of *V. parahaemolyticus* has been correlated with the production of thermostable direct haemolysin (TDH) encoded with the tdh gene, which is responsible for the haemolytic activity named Kanagawa phenomenon (Honda and Iida, 1993). Nishibuchi *et al.* (1992) concluded that the tdh gene is able to induce intestinal chloride secretion. The TDH-related haemolysin (TRH) is also a putative virulence factor of *V. parahaemolyticus* after its discovery from Kanagawa phenomenon negative strains by Honda *et al.* (1987, 1988). Sixty-seven percent of the amino acid sequences of the TRH gene were similar to TDH including the biological activities (Honda and Iida, 1993).

The first outbreak reported was related to the consumption of dried sardines in Osaka, Japan dated back in 1951 when *V. parahaemolyticus* was first discovered as a foodborne pathogen. Over six decades, *V. parahaemolyticus* has made itself well-known with numerous outbreaks due to contaminated or mishandled seafood, particularly in Asia, due to

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the higher consumption of seafood. The number of outbreaks is still on the rise. The Asian climate was known to be a contributing factor to high growth and contamination of *V. parahaemolyticus* as mentioned earlier. A correlation between the isolation of *V. parahaemolyticus* and water temperature also exists (IFT, 2004) and was studied extensively by Kaneko and Colwell (1973), DePaola *et al*. (1990) and Duan and Su (2005). On the Western side, outbreaks were mostly reported attributable to consumption of contaminated raw oysters – the food which they are most associated. According to Scallan *et al*. (2011), *V. parahaemolyticus* causes an estimated 35,000 domestically acquired foodborne infections annually in the United States.

In the previous years, foodborne outbreaks apart from seafood due to various *Vibrio* spp. were reported through ready to eat lunch box (38.5%), meat and meat products (12.8%), cakes and confectioneries (7.8%), vegetables (17.3%), cereals (7.3%) and egg products (1.7%) in which *V. parahaemolyticus* accounted 52.3% as the causative agent (Anonymous, 1998). Tunung *et al.* (2011) highlighted again the prevalence of *V. parahaemolyticus* in raw vegetables from retail outlets in Malaysia. Based on the data reported by other researchers, there is a possibility that *V. parahaemolyticus* can be transmitted secondarily to other foods. Pathogen secondary transmission can amplify the consequences of the pathogen as some microbes can remain viable for days, weeks, and months which increase the potential of transmission leading to new point-source outbreaks (United States Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) and United States of Environmental Protection (EPA), 2012). The spread of secondary transmission of V. parahaemolyticus is at a question as minimal data was collected regarding on this matter. Hence, this study would like to investigate on the prevalence of *V. parahaemolyticus* in various food samples and to evaluate the risk associated with it.

Materials and Method

Sampling

A total of 50 food samples (seafood, n = 15; vegetables, n = 30; chicken, n=3; fruits, n=2) were randomly purchased from local supermarkets and wet markets in Serdang, Selangor, Malaysia. They were transported immediately to the laboratory and analysed upon arrival.

Most probable number (MPN) procedure

The MPN procedure described by Bacteriological Analytical Manual was employed with modifications (Kaysner and DePaola, 2004). Briefly, 10 g portion of the sample was weighed approximately into a sterile stomacher bag and plunged with 90 ml of Alkaline Peptone Water (APW) [1% peptone; 1% sodium chloride, NaCl (Merck, Germany)] for 60 seconds. The stomached mixture was diluted tenfold for three successive times, and pre-enriched at 37°C for 18 to 24 hours prior to MPN analysis. For MPN analysis, 1 ml of the each dilution tube was transferred into three tubes set containing 9 ml of APW and further incubated at 37°C for 18 to 24 hours. After the incubation period, turbid MPN tubes were then streaked onto Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar (Merck, Germany). Presumptive green centre colonies on TCBS agar plates were picked and confirmed on CHROMagar™Vibrio. Mauve colonies were identified as positive *V. parahaemolyticus*. The positive *V. parahaemolyticus* isolates were subjected to Polymerase Chain Reaction (PCR) for confirmation based on New *et al*. (2014).

Microbial Risk Assessment

The @risk software package (Palisade Corporation, USA) in combination with Microsoft Excel was used to run the simulations. The Monte Carlo simulation was adopted in performing the calculations and a total of 30,000 iterations were simulated. The prevalence of total *V. parahaemolyticus* were used as the input data in this risk assessment which was to calculate the risk estimate along with other required information on the latter.

Exposure Assessment

Contamination level of *V. parahaemolyticus* at retail for each class of food sample was assessed separately. The following explains the methodology of the model used for the assessment and, the parameters used were described in Table 1. The initial contamination rate (Pp) of *V. parahaemolyticus* was estimated using a Beta (s+1, n-s+1) function in @risk. The contamination level of positive *V. parahaemolyticus* (LP) was modelled using a lognormal distribution with mean and standard deviation values of the log MPN/g data.

This study adopted 'fail-safe' condition in which it is inferred as non-detectable level of *V. parahaemolyticus* in negative samples may indicate the true absence of the bacteria or the level of detection is below the detection limit (Jarvis, 2000). The equation developed by Jarvis (2000) was adopted to calculate the non-detectable level of *V. parahaemolyticus* (Ln) using the Uniform distribution.

$$M = -\left(\frac{2.303}{V}\right)\log\frac{Z}{N} \quad (1)$$

Table 1. Description of parameters and model for exposure assessment of *V. parahaemolyticus*

Parameters	Description of Parameters	Input Model
P_p	Original *V. parahaemolyticus* contamination rate	Beta (s+1, n-s+1)[a]
$1-P_p$	Non-detectable *V. parahaemolyticus* contamination rate	
L_p	Contamination level of positive *V. parahaemolyticus*	Normal (μ, σ)[b]
L_n	Non-detectable level of *V. parahaemolyticus*	Equation (1)
		Uniform (min, max)[c]
N_0	Contamination level of *V. parahaemolyticus* at retail	Discrete (L_p:L_n,P_p:1-P_p)
C_e	Customers Exposure	Discrete (L_p:L_n,P_p:1-P_p)

[a] s = number of positive samples, n = total number of sample
[b] μ = mean value in $\log_{10}$, σ = standard deviation value in $\log_{10}$
[c] The min and max are the respective values calculated from equation 1 in $\log_{10}$

Where M is the true density in the batch; V is the quantity of the material examined; Z is the number of negative samples detected while N is the total number of samples examined. Using the equation, the calculated M value will be used as an average non-detectable level of *V. parahaemolyticus* in negative samples, the minimum and maximum values were calculated as the input values of the uncertainty. The maximum value was taken from the lowest detection level of *V. parahaemolyticus* from the samples. The contamination level of *V. parahaemolyticus* (N_0) at retail will be calculated by using the discrete distribution.

The model assumes that the concentration of *V. parahaemolyticus* remained constant when purchased at retail until the food is being consumed with minimal processing. The process of the model was as shown in Figure 1. The model also adopted the assumption that consumers are exposed to *V. parahaemolyticus* infection by single exposure and have the possibility to suffer from adverse health effects with the minimal consumption of number of bacteria cells.

Hazard characterization

The probability of illness (P_{ill}) per meal was estimated using the Beta-Poisson model proposed in the United States Food and Drug Administration (US FDA) as shown in the equation below.

$$P_{ill} = 1 - (1 + D/\beta)^{-\alpha}$$

Where D is the dose and α and β are parameters corresponding to the beta distribution parameters for a specific range. Infection by *V. parahaemolyticus* is characterized by an acute gastroenteritis with symptoms such as abdominal cramps, explosive watery diarrhoea, nausea, vomiting and sometimes fever. Symptoms have often ranged from mild to severe, but self-limiting (Lake *et al*., 2003).

Risk characterization

Risk characterization is the integration of the exposure and dose-response assessments to provide an overall evaluation of the likelihood that the population will suffer adverse effects as a result of the hazard (Buchanan *et al.*, 2000). The probability of illness yearly was calculated based on the equation below as described by Robertson *et al.* (2005).

$$\text{Pill/year} = 1 - (1 - \text{Pill})^{\text{no. of serving per year}}$$

In this model, it was assumed that the Malaysian population, 29,947,600 people (Department of Statistics Malaysia, 2013) consumed 365 servings per year. The number of expected cases to occur was calculated by multiplying the exposed population obtained from the Ministry of Health Malaysia, (2003) with the probability of illness yearly.

Results and Discussion

Prevalence

Food risk is the opposite of food safety. The presence of marine bacteria, *Vibrio parahaemolyticus,* in various food samples poses food risk to consumers of suffering from foodborne illness when one

Table 2. Prevalence of *V. parahaemolyticus* in different food classes

Food Class	Sample Size	Positive Detection of *V. parahaemolyticus*	95% Confidence Interval	MPN range (MPN/g)
Vegetables	30 (60.0%)	3 (10.0%)	31.9 – 34.8	9.2 – 23
Seafood	15 (30.0%)	5 (33.33%)	9.7 – 10.3	94 – 290
Fruits	3 (6.0%)	0 (0.0%)	0 – 1.0	<3
Chicken	2 (4.0%)	0 (0.0%)	0 – 1.5	<3
Total	**50 (100.00%)**			

contracted with pathogenic *V. parahaemolyticus*. Some researchers such as Kothary *et al.* (2000) and Lee *et al*. (2002) discovered other potential virulence factor of *V. parahaemolyticus*. This notes that virulence factors other than TDH and TRH may yet be identified (Su and Liu, 2007) and the presence of *V. parahaemolyticus* on foods should be deemed a risk.

Table 2 summarized the results of the types of food that were sampled. Based on Table 2, vegetables were sampled more frequently (n = 30, 60.0%) as we would like to investigate the range of detectable *V. parahaemolyticus* present in vegetables. Tunung *et al.* (2011) studied on the rapid detection of pathogenic *V. parahaemolyticus* in raw vegetables from retail outlets and concluded that 12% of the 276 samples analysed were contaminated with *V. parahaemolyticus*. In our study, we managed to detect a total of 3 (10.0%, 95% CI 9.7 – 10.3) vegetable samples contaminated with *V. parahaemolyticus* with a range of 9.2 – 23 MPN/g which supported Tunung *et al*. (2011) research.

Bacteria can come from human, workspace or environment and even from other foods. Bacteria transfer or cross contamination can happen directly or indirectly (Pérez-Rodríguez *et al.*, 2008). Direct cross contamination occurs when the bacteria is transferred from one food to another food. On the other hand, indirect cross contamination occurs when the bacteria is transferred through a series of sources such as work surfaces, air, hands, before being transferred to the food.

From the results, it can be inferred that *V. parahaemolyticus* was most likely being indirectly transferred to vegetables through food handlers practicing poor hygiene and sanitation as stated by Tan *et al.* (2008). Hands are the most obvious culprit for bacteria transfer when there is minimal or no hand washing in between by the food handlers. Not only that, the surface-to-food contact can occur in numerous ways and situations. It can happen at different stages in the food chain through contact with raw food, in this case vegetables contact with seafood with a high microbial load of *V. parahaemolyticus*, as well as dishcloths, apart from food handlers. According to Pérez-Rodríguez *et al.* (2008), bacteria with hydrophilic characteristics, such as *V. parahaemolyticus*, attach better to hydrophilic surfaces. Foods with high water activity will be a potential attachment for *V. parahaemolyticus* if there is contact. More moisture yields a looser attachment of the bacteria on surfaces and eventually contributes to higher bacterial transfer. Merry *et al*. (2001) had proven that additional moisture facilitates the transfer from contaminated hands and, from contaminated fabrics by Marples and Towers (1979) and Sattar *et al.* (2001).

The irrigation system used in agriculture, if it is not being well-treated or polluted, may contain vibrios. It is also possible that the salt content in the water was elevated, allowing vibrios to grow in this condition (IFT, 2004) which resulted in contamination of the fresh produce. Although *V. parahaemolyticus* is widely disseminated in estuarine, marine and coastal surroundings (Su and Liu, 2007; Nelapti *et al*., 2012; Ceccarelli *et al*., 2013; Zhang and Orth, 2013), *V. parahaemolyticus* have been isolated from fresh-water and non-marine fish (IFT, 2004). Whitaker and Boyd (2012) reported that *V. parahaemolyticus*

has higher osmotic tolerance compared with other vibrios and is able to grow in conditions that contains more than 0.05% salt concentration. Adaptation to stress environments can result in a pathogen becoming better suited to survival and growth, or to becoming more virulent (Beuchat, 2002). This is supported by Whitaker *et al.* (2010) that conducted a study on the growth of *V. parahaemolyticus* at different salt concentration and, concluded that *V. parahaemolyticus* grown in 1% salt condition was more virulent compared to *V. parahaemolyticus* that was grown in 3% salt condition. Besides that, if the contaminated water temperature increases due to increasing global temperature, this might induce the higher growth level of *V. parahaemolyticus*. According to Devendra (2012), the Earth's temperature increases every year, causing all regions to become warmer and subsequently the heat will dissipate into the water and the temperature will rise. Eventually, the temperature condition becomes favourable to the bacteria, allowing it increase in number rapidly. The spread of *V. parahaemolyticus* and other vibrios through the irrigation system will then be uncontrollable and widely disseminated.

Despite that, the water system that was used for cleaning, if it is improperly treated will contain *V. parahaemolyticus* which attach itself onto surfaces forming biofilms. *V. parahaemolyticus* was reported to be one of the bacteria producing biofilm, exopolysaccharide (EPS) as a protective matrix (Leigh and Coplin, 1992). Furthermore, the cohabitation of *V. parahaemolyticus* with other microorganisms tends to induce the production of biofilm. A slight contact of the biofilm, which is adherent to an inert or a living surface, will initiate the secondary transmission of *V. parahaemolyticus* from seafood to other foods or even to other seafood. In this case, the improper cleaning of the stalls or the detergents used that was ineffective to eliminate the biofilm. This raise the concern of food safety as other foods may possibly be the next *V. parahaemolyticus* vehicle of contamination.

A total of 15 (30.0%) seafood samples was analysed from various wet market and hypermarkets as shown in Table 2. From the 15 seafood samples, 5 (33.3%, 95% CI 31.9-34.8) samples were contaminated with *V. parahaemolyticus* with a range of 94 – 290 MPN/g which indicated a higher detection level of *V. parahaemolyticus* in seafood rather than vegetables. Being a halophilic organism, it is inevitable to be able to detect *V. parahaemolyticus* from seafood. It will be a concern if the detectable level is more than 10,000 viable *V. parahaemolyticus* cells/g. However, the consumption of raw oysters causing a *V. parahaemolyticus* outbreak were detected as few as 100 cells/g (Food and Drugs Administration/ Center of Food Safety and Applied Nutrition (FDA/CFSAN), 2001). This initiated the range of detectable *V. parahaemolyticus* in seafood for safe and quality checking to be lowered in some countries such as Australia and New Zealand. Based on the data collected, it can be said that the contaminated seafood is likely to cause foodborne illness if they are mishandled or consumed raw. As five samples were only contaminated with *V. parahaemolyticus*, it is likely that *V. parahaemolyticus* present in other seafood was undetectable due to method limitation and its ability to fall under viable but non-culturable (VBNC) state. However, pathogens falling under VBNC state are still alive, potentially pathogenic as well as metabolically active which put consumers at risk (Colwell, 1993; Colwell and Huq, 1994; Oliver *et al.*, 1995; Oliver, 2005).

Other food samples as shown in Table 2, which were fruits (0.0%, 95% CI 0 – 1) and chicken (0.0%, 95% CI 0 – 1.5), were reported negative presence of *V. parahaemolyticus*. The MPN/g for both food classes were <3 MPN/g. The prevalence results could be due to data constraint and was suggested for further studies on these food classes. As of now, there were no reports on the contamination of fruits and chicken with *V. parahaemolyticus*.

Outputs and risk estimation

The outputs of the @risk simulation carried out separately according the food categories showed that detectable initial contamination of *V. parahaemolyticus* was higher for seafood compared to vegetables with a mean of -0.1230 log MPN/g and -1.5894 log MPN/g respectively. The obtained values reflect the detectable *V. parahaemolyticus* in MPN/g in food which have higher detectable *V. parahaemolyticus* will simulate a higher initial contamination of *V. parahaemolyticus*. As *V. parahaemolyticus* is a natural habitant in the sea, it is obvious seafood will attain a higher detection of *V. parahaemolyticus*. Another point to add on is most shellfish are filter feeders which they feed by filtering the sea water. This will enable them to accumulate pathogenic bacteria within themselves.

The simulation was further carried out according to Figure 1 until risk characterization. The outputs of the simulation for seafood indicated that a probability of 1.86×10^{-8} of Malaysian will contract foodborne illness and a probability of 6.78×10^{-6} yearly. The expected number of cases to occur is about 151 cases when it was estimated that 74.47% of the Malaysian population (22,302,976 people) consumed seafood

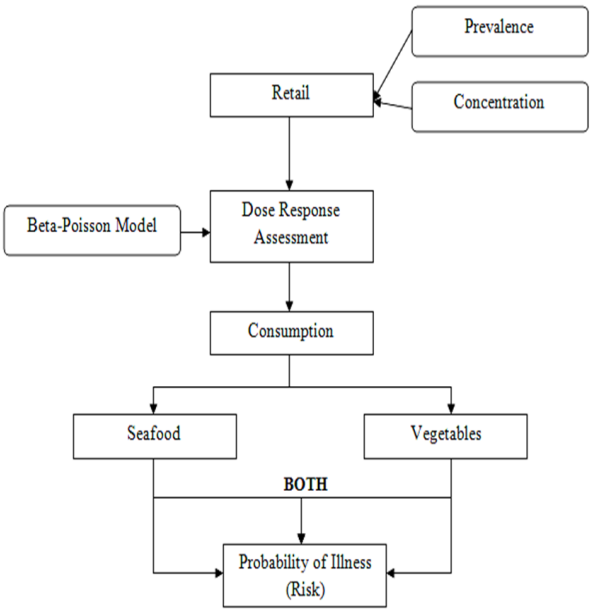

Figure 1. Model structure of microbial risk assessment of *V. parahaemolyticus* on the consumption of various contaminated food with *V. parahaemolyticus*

with a rate of 0.505 per 100,000 people. In contrast, only a rate of 0.002 per 100,000 people will contract foodborne illness caused by *V. parahaemolyticus* from vegetables. It was estimated there is a probability of 4.83 x 10^{-9} and a probability of 1.76 x 10^{-6} yearly. Nevertheless, there will not be likely any expected cases to occur per year from 89.34% of the Malaysian population (26,754,437 people) that consumed vegetables.

In addition, the risk of consuming both seafood and vegetables that are contaminated with *V. parahaemolyticus* at the same time was studied as shown in Figure 1. The model assumed that there is a possibility that consumers may cross contaminate while minimally preparing their meals and caused a combination of infection risks when both food classes are consumed. From the outputs of the simulation, it was estimated that there was a probability of illness of 2.34 x 10^{-8} and the probability of illness annually was 8.54 x 10^{-6}. With a combination of the seafood and vegetable consuming population, which is a total of 97.28% of the Malaysian population (29,132,491 people), it was expected that 249 cases are expected to occur per year with a rate of 0.831 per 100 000 people. The expected number of cases to occur was relatively higher compared to single food class consumption in a meal. The results suggested that the food classes are independent sets mathematically. The union of both sets will present an increase dosage of *V. parahaemolyticus* infection. When translated, it is to say that the possibility of one getting infected by *V. parahaemolyticus* will be higher if one consumes both *V. parahaemolyticus* contaminated seafood and vegetables with more cases expected to occur.

It should be noted that the dose-response assessment adopted from United States Food and Drug Analysis (US FDA) was based on the assessment on healthy individuals. Thus, the risk of *V. parahaemolyticus* infection for this study was likely underestimated due to the information on the susceptibility of higher risk subpopulations or potential gender effects is generally not available (Buchanan *et al.*, 2000). Nevertheless, a scenario assessment can be conducted to assume the percentage of infected individuals that will become symptomatic, either the individual is immunocompetent or immunoimpaired. This certainly requires more data collection and the assumptions made should be clear, kept simple, and valid with the purpose and scope of the risk assessment. Most often the available data will not be exactly representative for the required assessment (Lammerding and Fazil, 2000). It is recommended for more collection or adaptation of data to roughly visualize the realistic scenario and perform a quantitative microbial risk assessment. This enables the researcher to be able to estimate the potential magnitude of risk which becomes the baseline to determine the need of a more detailed analysis (Lammerding, 2007). According to Lammerding and Fazil (2000), assessors should consider the sensitivity, specificity and overall validity of the sampling and testing procedures prior to adaptation of data and the data should be clearly acknowledged.

Microbial risk assessment is often used as a tool to scientifically investigate the significance of microbial hazard in the food of concern (Lammerding and Fazil, 2000). The final outcome of it, as what is described as risk characterization, should be made transparent in order to ensure proper measurement and evaluations are made. The presence of *V. parahaemolyticus* in foods posed a critical risk at consumer level and if it is not being well-controlled, the infection rate will increase among the Malaysian population. The transfer of the bacteria should be studied in depth and by identifying the decisive factors, risk can be located and reduced (Pérez-Rodríguez *et al*., 2008).

Thus, it is suggested that the Malaysian Government and responsible authorities to put in strict enforcement in food safety regulatory and constant checking to ensure the appropriate level of protection of the public health. Proper, clear and easy to understand information should be channelled and educated to the industries and the public. The public should be constantly reminded to practice proper hygiene and sanitation while handling food to prevent further cross contamination and eventually, reduce the possibility of secondary transmission of

the pathogen. In addition, they should be informed of the probable risks that will be occurred if they do not practice proper hygiene and sanitation. Such practices are relatively simple, but yet less practiced in reality which induced the contamination of the pathogens to other foods. It could be highly due to the communication of the details in which caused the dejection. Above all, it is critical that the authorities provide technical transparency and interpret it in a simple manner which is well understood by the public to ensure the conservation of public health.

Conclusion

The presence of *V. parahaemolyticus* on various types of food significantly indicates its wide transmission from one food source to another and the risk that it will impose. 33.3% of the seafood samples were contaminated with *V. parahaemolyticus* while 10.0% of the vegetable samples were contaminated with *V. parahaemolyticus*. Fruits and chicken recorded 0.0% of detected *V. parahaemolyticus.* The combined risk of consuming both contaminated seafood and vegetables estimated was a rate of 0.831 per 100,000 people with 249 cases expected to occur. The bacteria transfer route should be studied in depth to identify the risk located due to the complexity of numerous pathways of transmission. Above all, the risk of transmission of *V. parahaemolyticus* can be greatly reduced when proper food safety measures are taken especially in practicing good hygiene and sanitation.

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