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Step-wise risk assessment of *Vibrio vulnificus* infection associated with the consumption of cockles

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Abstract

In this study, a simple point estimate approach was used to evaluate the risk of *Vibro vulnificus* infection from the consumption of contaminated cockles. Comparisons between ethnic groups, gender and susceptible populations in Malaysia scenario were carried out utilizing data from the previous prevalence study and related sources to make an attempt to evaluate the health risk. The results obtained showed that the estimated annual number of septicaemia cases associated with cockle consumption was 8.028 cases per 100,000 Malaysian population. Malays were found exposed to the greater risk due to the higher frequency of cockle consumption, in comparison to Chinese (Malay, 5.965 cases; Chinese, 0.733 cases). Among susceptible populations, the elderly population was found exposed to the greatest risk followed by diabetes, alcoholism and AIDS populations (elderly, 0.395; diabetes, 0.347 cases; alcoholism, 0.207 cases; AIDS, 0.019 cases). Likewise, the male population was found exposed to the greater risk of septicaemia infections, compared to the female population regardless of their binge drinking habit (male, 4.881 cases; female, 3.166; male binge drinker, 0.242 cases; female binge drinker, 0.040 cases).

1. Introduction

Foodborne diseases have caused major consequences which can have significant impacts on public human health and the economy in food industries. Advanced microbiological identification techniques, rapid outbreak recognition, and targeted surveillance have enhanced our ability to trace and identify foodborne microbes and the execution of effective controlling measures (El Sheikha *et al*., 2018; Hennekinne, 2018). However, these microbes are able of adapting and evolving in a new host, new niches and new vehicles of transmission, which end up acquired certain resistance traits and modifications in their virulence mechanisms during the course of survival (Newell *et al*., 2010). The understanding of food safety risks and its management are further complicated by modern technology shifts in population demographics, global food trade marketplace and consumer preferences (Lammerding, 2013).

V. vulnificus is generally part of the natural inhabitant of coastal and estuarine waters, plankton, sediment and shellfish around the world (Vezzulli *et al*., 2013). While human infections reported are relatively uncommon, the causing illness is the most severe among *Vibrio* species. This bacterium is associated with over 95% deaths through seafood consumption in the United States and reported to cause the highest mortality rate of any foodborne pathogen (Iwamoto *et al*., 2010). According to a review submitted to the Food and Drug Administration (FDA), more than 50% of patients infected by *V. vulnificus* reported in 459 cases died. Surprisingly, this case fatality rate is congruent with a range of viruses in Biosafety Level 3 and 4 categories such as anthrax, Ebola and Marburg fever (Baker-Austin and Oliver, 2018).

Cockles *(Anadara granosa),* is an ark clam species that is commonly found in the soft sediment intertidal zones of Malaysia coast, particularly Selangor and Perak (Thia-Eng *et al*., 2000). It has been traditionally harvested as a protein and mineral source for local

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people (Hazmi *et al*., 2007; Taniguchi *et al*., 2017). In Malaysia, they are commonly prepared in sambal belacan which served with nasi lemak and sometimes cooked as ingredients in curry laksa and char kuey teow in popular cuisine of different ethnic groups. In addition to being associated with popular gourmet seafood, cockles are commonly eaten raw or partially cooked by people in many other Asian countries such as Thailand, Vietnam and Indonesia (Watanabe, 2009; Tu *et al*., 2011; Yurimoto *et al*., 2014). However, there is a relatively high risk for consumers who eat undercooked cockles because of the foodborne pathogen that may harbor inside.

Several risk assessment studies on *Vibrio* species in a variety of seafood products have been reported. Yamamoto *et al*. (2008) conducted a health risk study associated with the consumption of bloody clams in Thailand. Meanwhile, Huang *et al*. (2018) investigate the risk assessment of *Vibrio* in raw oysters consumed in Taiwan. While in Malaysia, Malcolm *et al*. (2015) and Sani *et al*. (2013) conducted a risk assessment of *V. parahaemolyticus* in bloody clams and tiger shrimps, respectively. These studies demonstrated how international cooperation on health risk analysis in microbiological terms with available data successfully provided valuable insights on food safety.

In risk analysis, risk assessment should be cooperated effectively with management and communication organizations to be used as a valid methodology for policymakers and related parties to take the action to mitigate the situation (Lammerding, 2013). Risk management is the process of identification and evaluation of the estimated risks, in consultation among the related parties, concluding the factors, and deciding which action to be taken. While risk communication is the process of an exchange of opinions and information interactively among the stakeholders and plays an integral component of risk management, including the description on the risks associated with real or perceived hazards identified in risk assessment (Cope *et al*., 2010).

The current stepwise risk assessment using data from the previous microbiological studies of *cockles (A. granosa)*. The available data were used to estimate the risks of acquiring *V. vulnificus* infections following the consumption of cockles in Malaysia scenario. This study also shows how risk assessment works as a supportive tool to enhance the assessment of public health risks.

2. Materials and methods

2.1 Statement of purpose

The purpose of the present risk assessment work was to estimate the likelihood of consumers in Malaysia contracting *V. vulnificus* related diseases following the consumption of cockles *(Anadara granosa)*. The cockles risk assessment is based on limited data for the prevalence of total *V. vulnificus* in 199 samples collected for one-year period.

The present risk assessment is a scientifically based process consisting of the following steps (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization (Figure 1).

2.2 Hazard identification

This process includes the identification of potential health hazards (microorganism) and other risk factors that may be presented in a particular food product and able to cause harm in humans. The identified hazard in this study is *V. vulnificus* that might be presented in cockles. The negative health impacts caused by this bacterium will be described in detail.

V. vulnificus is a motile, non-spore forming gram negative curved rod-shaped halophile microorganism with a single polar flagellum. To date, three biotypes have been described; with biotype 1 strain exclusively associated with human diseases. Biotype 2 strain was first thought to be pathogenic only to eels but several cases in human infections have been reported (Amaro and Biosca, 1996; Koton *et al*., 2015). Biotype 3 strain has only been reported from wound infections in patients acquired by handling Tilapia in Israel (Baker-Austin and Oliver, 2018).

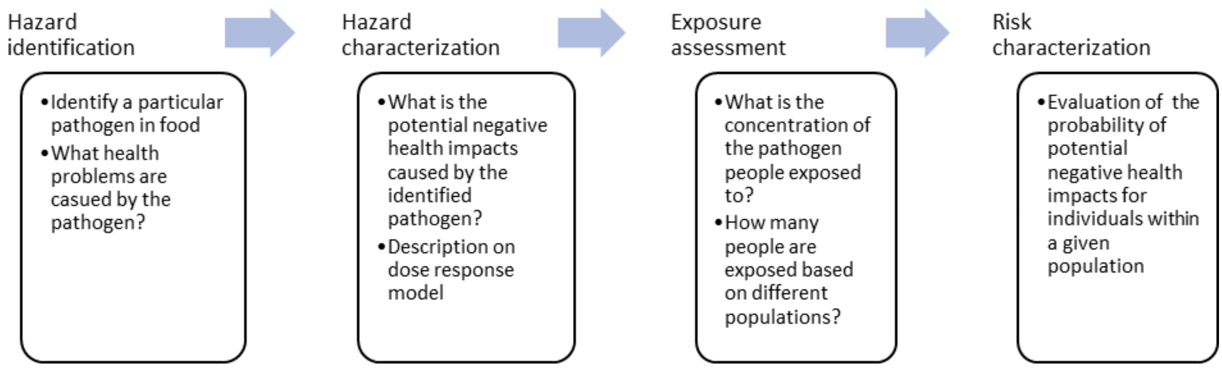

Figure 1. Conceptual model for risk assessment.

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Human infections with *V. vulnificus* can lead to primary septicaemia and severe wound infections. The symptoms and severity of disease in a human depends on the host susceptibility and mode of infection (Horseman and Surani, 2011; Heng *et al*., 2017). Infections usually reported associated with two recognizably sources; consumption of contaminated shellfish or physical exposure to the bacterium through seawater and seafood. Septicaemia and gastroenteritis are caused by ingestion of raw or undercook shellfish, particularly oysters. While wound infections with *V. vulnificus* are commonly occurring through exposure of a wound to seawater or seafood drippings during seaside activities and handling of seafood such as seafood with hard and pointed surfaces (Haq and Dayal, 2005; Wang *et al*., 2017).

In Malaysia, fatal septicaemia *V. vulnificus* associated case has been reported by Zetti *et al*. (2009). However, gastroenteritis caused *V. vulnificus* is characterized by mild vomiting, diarrhoea, and abdominal pain and these symptoms are rarely severe enough for the infected people looking for medical assistance. Thus, it is possible that shellfish related cases associated with *V. vulnificus* go undetected and underreported.

2.3 Hazard characterization

This process includes evaluation and characterization of the relationship between the level of revelation to a particular pathogen (dose) and the possibility of negative health impacts for contracted individuals within a population (response) (WHO/FAO, 2005). For this study, a quantitative relationship was built to evaluate the number of diseases resulting from the consumption of food contaminated with *V. vulnificus.*

V. vulnificus associated illnesses are identified by a short incubation period between the beginning of the symptoms and subsequent clinical outcomes, it is typically within 24 hrs of exposure. Patients can usually begin with an abrupt rise in temperature, often with abdominal pain, vomiting, and diarrhoea. The symptoms and severity of disease in humans are varied and depend on the host susceptibility and mode of infection.

Patients with primary septicaemia are often characterized by severe skin lesions; often leads to necrotic which required surgical intervention and amputation. The mortality rate is 60-75% in individuals contracted with primary septicaemia. Patients with severe wound infections are often characterized by soft tissue infection and necrotizing skin, including gangrene and fasciitis. The mortality rate is lower, estimated from 20-30% (Horseman and Surani, 2011; Baker-Austin *et al.*, 2017).

2.3.1 Normal population

The normal population may be susceptible to relatively mild gastroenteritis after the consumption of cockle harbouring *V. vulnificus,* but this rarely leads to primary septicaemia.

2.3.2 Susceptible population

People who are most susceptible to *V. vulnificus* infections usually are individuals suffer from hepatic or immune system dysfunction and other chronic diseases (Table 1). Liver disorders include hepatitis, alcoholic liver disease, metastatic cancer or liver transplantation. Immuno-compromised conditions that predispose people to *V. vulnificus* infections include AIDS and chemotherapy. Other underlying conditions include chronic intestinal and renal diseases, diabetes mellitus or steroid dependency.

2.3.3 Ethnic group

Malaysia is a multiracial country with food cross culturing gradually evolves through education, social interaction, and media as part of acculturation (Ishak *et al*., 2013). Food consumption patterns of each ethnic group are influenced by many factors such as lifestyle practices, socio-demographic status, dietary behaviors, and education levels. Foodborne infections associated with various ethnic cuisines have been reported around the globe (Lee *et al*., 2014). However, there is no epidemiological data associated with ethnic cuisines in Malaysia. Data for the serving size of cockles among the Indian population is not available. Thus, this study was focused on Malay and Chinese populations.

2.3.4 Gender

Interestingly, a clear gender disparity with regards to *V. vulnificus* infections was reported by several researchers. Jones and Oliver (2009) reported that up to 85.6% of the reported cases submitted to the FDA between 1992 and 2007 were in males. A similar observation was reported by Baker-Austin *et al*. (2017), which stated 86% of the cases reported to Cholera and other *Vibrio* Information Service (COVIS) from 1988 to 2010 were in males. The underlying factors behind are ambiguous, but a greater percentage of men carrying underlying health problems especially liver associated disease is thought to attribute it.

2.3.5 Dose-response model

To date, there are no human volunteer studies with *V. vulnificus* available for the construction of a dose-response relationship because of the severity of the illness. A beta-Poisson *V. vulnificus* dose-response model published by the World Health Organization and

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Table 1. *V. vulnificus* cases reported in different countries. Adapted from Heng *et al*. (2017)

Country	Year	Mortality rate (%)	Primary septicaemia (%)	Wound infections (%)	Association
Japan	1978-1987	68	60.5	7.9	Liver disease, leukopenia
	1999-2003	61.7	72.3	22.3	Alcoholic liver disease
	2001-2010	58.3	-	-	Underlying disease
Korea	2001-2010	48.5	-	-	Liver disease alcoholism
	2000-2011	-	63	-	-
Taiwan	1985-1990	42.3	64.3	28.6	-
	NA-1994	55.6	77.8	22.2	Liver disease
	1996-2011	18	58	78	
Israel	1996-1997	0	0	100	Immuno-compromised

Food and Agriculture Organization (WHO/FAO, 2005) was used in this study. This model was based on estimates for the susceptible population with the monthly epidemiological date on the number of cases associated to oyster consumption reported to the USA Centers for Disease Control and Prevention (CDC) from 1995 to 2001 (Figure 2).

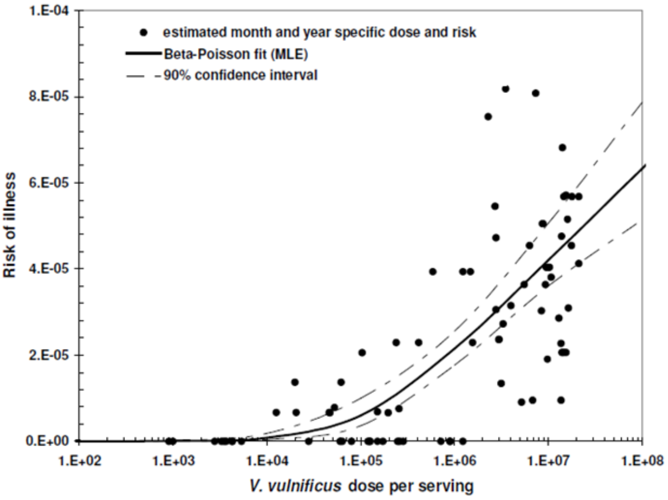

Figure 2. The Beta-Poisson dose-response curve for *V. vulnificus* associated septicaemia. The solid line is the best estimate of the Beta-Poisson Model and the parallel dashed lines shows the 90% confidence interval on the predicted risk. The dots represent the best estimates of the month and year specific exposure based on the observed epidemiology data from 1995–2001 (Adapted from WHO/FAO, 2005). The best estimates of the parameters for the Beta-Poisson model are $\alpha = 9.3 \times 10^{-6}$ and $\beta = 110\ 000$.

This model was used to estimate the *V. vulnificus* septicaemia risk probability ($P_{inf/pos}$) for a population ingesting a given pathogen dose (*D*) per positive serving. The value incorporates pathogenicity heterogeneity, not every ingested microorganism survives to cause an infection. The formulation of the proposed model is as follows:

$$P_{inf/pos} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}$$

Where α (9.3×10^{-6}) and β (1.1×10^{5}) are parameters of the beta distribution that describe the relationship between host and pathogen.

2.4 Exposure assessment

In this step, the frequency and likelihood of exposure to *V. vulnificus* when consuming contaminated cockles were defined (WHO/FAO, 2005).

2.4.1 Prevalence and concentration

The prevalence data on *V. vulnificus* in cockles was obtained from the previous detection study carried out in the year 2016 was used to assess the hazard in cockles. Assumptions were made for some not available data and information (Yamamoto *et al*., 2008). Thus, it is important to be informed that the results calculated were mainly based on deterministic approach (point estimates).

A total of 199 fresh cockles were purchased at wet markets in Kuala Selangor area, Malaysia. Of the 199 cockles purchased from wet markets, 10.55% were found to harbour *V. vulnificus*. The quantity of *V. vulnificus* detected in cockle samples (mean concentration) was approximately 300.87 MPN/g. Although *V. vulnificus* demonstrates variation in pathogenicity and virulence characteristics, all cells were assumed to be potentially pathogenic in the assessment ('worst case' scenario).

2.4.2 Consumption

Information on the serving size of cockles was obtained from the Food Consumption Statistics of Malaysia: The Food Consumption Statistics also provides comprehensive information on the food consumption pattern for Malaysian adults according to geographical zones, strata, ethnicity, and gender. The serving size for total populations, ethnicity, and gender was summarized in Table 2.

2.4.3 Population exposure

In this study, total population and selected ethnic groups (Malay and Chinese), susceptible populations (diabetes, AIDS, alcoholism and elderly), male and female populations, and binge drinkers population in Malaysia were selected. Information on the number of populations estimated in the different category of health and susceptibility was obtained from the latest online

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Table 2. Summary of estimated mean intake (g/day) in cockles for the total population, selected ethnicity and gender

	Estimated mean intake (g/day)	Standard error of mean	Estimated population
Total population	1.42	0.13	19439869
Ethnicity			
Malay	1.94	0.21	10417667
Chinese	0.66	0.09	3817115
Indian	-	-	-
Gender			
Male	1.66	0.20	10126853
Female	1.17	0.15	9313016

(Adapted from Ministry of Health, 2014)

sources of the Ministry of Health, Malaysian Adults Nutrition Survey and Department of Statistics Malaysia.

2.5 Risk characterization

In risk characterization, prediction of the probability of potential negative health impacts for individuals within a given population was carried out through the integration of the dose-response model and exposure assessment (WHO/FAO, 2005). For this study, the potential likelihood for causing severe diseases through the consumption of cockles contaminated with *V. vulnificus* was evaluated for the Malaysian population based on characteristics of the host such as the comparison between healthy and susceptible populations, gender and ethnic groups.

All analysis was carried out in simple Excel worksheet software. The results of the calculations for *V. vulnificus* infection leading to septicaemia through the consumption of contaminated cockles for the total population and various ethnic groups (Malay and Chinese) were shown in Table 3. Meanwhile, Table 4 showed the risk estimates for different susceptible populations in Malaysia. Table 5 summarized the risk estimates for male and female population and binge drinker population in Malaysia (male and female respectively).

3. Results

Table 3 summarizes the risk estimates for the total population and selected ethnic groups (Malay and Chinese). Overall, the estimated number of septicaemia attributed to contaminated cockle consumption was 8.028 cases per 100,000 Malaysian populations. Per 100,000 populations, the evaluated risk in Malay and Chinese ethnic group were 5.865 and 0.733 cases, respectively.

Table 4 demonstrates the risk estimates for susceptible populations (diabetes, AIDS, alcoholism and elderly) in Malaysia. Overall, the estimated risks for susceptible populations are relatively low. Per 100,000 population, the highest risk observed was in the elderly population (0.395 cases) followed by diabetes (0.347 cases), alcoholism (0.207 cases) and AIDS (0.019 cases).

Table 5 summarizes the risk estimates for male and female populations, and binge drinkers' population. The observed risk was higher for the male population (4.881 cases), compared to the female population (3.166 cases) as per 100,000 population. Similarly, the risk estimates for *V. vulnificus* in cockles for male binge drinkers (0.242 cases) was higher than female counterpart (0.040 cases) as per 100,000 population.

4. Discussion

Previous microbiological risk estimations in Malaysia scenarios had been mainly focused on the estimation of the *V. parahaemolyticus* in food (Tunung *et al*., 2010; Sani *et al*., 2013; Malcolm *et al*., 2015). The purpose of the present risk assessment work was to estimate the likelihood of consumers in Malaysia contracting septicaemia caused by *V. vulnificus* following the consumption of cockles. The step-wise risk estimation of *V. vulnificus* infections from the consumption of cockles conducted in this study has made use of the prevalence data and the various external associated sources. For a better understanding of the data and calculations, several assumptions were adopted to evaluate the potential health risk caused by *V. vulnificus* concerning cockle consumption in Malaysia scenario.

The results obtained showed that the estimated number of septicaemia attributed to contaminated cockle consumption was 8.028 cases per 100,000 Malaysian populations (Table 3). Per 100,000 population, higher risk was observed in the Malay ethnic group (5.865 cases), compared to Chinese (0.733 cases). The results of the calculations were found to be dependent on the frequency of cockle consumption among the various ethnic groups; it showed that the Malay ethnic group was subjected to a greater health risk due to the higher consumption frequency of cockles in their daily intake. Cockles are a favourite shellfish among the ethnic groups in Malaysia. One of the favourite methods of cockles' consumption is to pour boiling water over them until the shells are partially opened. The temperature attained by such method of preparation prior to consumption may be

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Table 3. Risk estimates for total population and selected ethnic groups

	Total population	Malay	Chinese
Prevalence			
No. of samples	199.00	199.00	199.00
No. positive	21.00	21.00	21.00
Prevalence (%)	10.60	10.60	10.60
Probability of positive sample	0.11	0.11	0.11
Concentration			
Mean concentration (per gram)	300.87	300.87	300.87
Serving size (g)	1.42	1.94	0.66
Dose (organism/serving)	427.24	583.69	198.57
Beta-Poisson dose response (α = 9.3x 10^{-6}; β = 1.1 x10^{5})			
Probability of infection per serving			
Probability of infection per positive serving	$3.61\text{x}10^{-8}$	$4.92\text{x}10^{-8}$	$1.68\text{x}10^{-8}$
Probability of infection per serving	$3.97\text{x}10^{-9}$	$5.41\text{x}10^{-9}$	$1.85\text{x}10^{-9}$
Probability of infection per year			
No. of servings per year	104	104	104
Probability of infection per year	$4.13\text{x}10^{-7}$	$5.63\text{x}10^{-7}$	$1.92\text{x}10^{-7}$
Estimated no. of cases per 100,000 population			
Population	32,049,700	22,050,193	7,435,530
Estimated population exposed	19,439,869	10,417,667	3,817,115
Expected no. of cases	8.028	5.865	0.733
Rate per 100,000 population	0.041	0.056	0.019
50% infection-to-illness ratio assumption			
Probability of infection to illness	0.5	0.5	0.5
Probability of illness per serving	$1.99\text{x}10^{-9}$	$2.71\text{x}10^{-9}$	$9.25\text{x}10^{-10}$
Probability of illness per year	$2.06\text{x}10^{-7}$	$2.81\text{x}10^{-7}$	$9.62\text{x}10^{-8}$
Expected no. of cases	4.012	2.930	0.367
Rate per 100,000 population	0.021	0.028	0.009

Table 4. Risk estimates for susceptible populations

	Diabetes	AIDS	Alcoholism	Elderly
Prevalence				
No. of samples	199.00	199.00	199.00	199.00
No. positive	21.00	21.00	21.00	21.00
Prevalence (%)	10.60	10.60	10.60	10.60
Probability of positive sample	0.11	0.11	0.11	0.11
Concentration				
Mean concentration (per gram)	300.87	300.87	300.87	300.87
Serving size (g)	1.42	1.42	1.42	1.42
Dose (organism/serving)	427.24	427.24	427.24	427.24
Beta-Poisson dose response (α = 9.3x 10^{-6}; β = 1.1 x10^{5})				
Probability of infection per serving				
Probability of infection per positive serving	$3.61\text{x}10^{-8}$	$3.61\text{x}10^{-8}$	$3.61\text{x}10^{-8}$	$3.61\text{x}10^{-8}$
Probability of infection per serving	$3.97\text{x}10^{-9}$	$3.97\text{x}10^{-9}$	$3.97\text{x}10^{-9}$	$3.97\text{x}10^{-9}$
Probability of infection per year				
No. of servings per year	104	104	104	104
Probability of infection per year	$4.13\text{x}10^{-7}$	$4.13\text{x}10^{-7}$	$4.13\text{x}10^{-7}$	$4.13\text{x}10^{-7}$
Estimated no. of cases per 100,000 population				
Population	1,678,680	93,089	1,003,582	1,914,100
Estimated population exposed (50% of target group)	839,340	46,545	501,791	957,050
Expected no. of cases	0.347	0.019	0.207	0.395
Rate per 100,000 population	0.041	0.041	0.041	0.041
50% infection-to-illness ratio assumption				
Probability of infection to illness	0.5	0.5	0.5	0.5
Probability of illness per serving	$1.99\text{x}10^{-9}$	$1.99\text{x}10^{-9}$	$1.99\text{x}10^{-9}$	$1.99\text{x}10^{-9}$
Probability of illness per year	$2.06\text{x}10^{-7}$	$2.06\text{x}10^{-7}$	$2.06\text{x}10^{-7}$	$2.06\text{x}10^{-7}$
Expected no. of cases	0.173	0.009	0.103	0.197
Rate per 100,000 population	0.021	0.019	0.021	0.021

*These estimates are based on the assumption that all individuals of the susceptible populations are equally susceptible to the *V. vulnificus* infections.

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insufficient to kill the *Vibrio* species, harbouring in the shellfish. Hence, sufficient cooking is necessary to reduce the risk of infection.

Table 4 summarizes the risk estimate for *V. vulnificus* in cockles for susceptible populations. People who are most susceptible to *V. vulnificus* infections are individuals suffer from hepatic or immune system dysfunction and other chronic diseases. In this study, patients with diabetes, AIDS, alcoholism and elderly were selected (with an assumption of all individuals of the at-risk population is equally susceptible to the *V. vulnificus* infections). From the obtained results, the overall estimated risks for susceptible populations in Malaysia are relatively low. The highest risk was observed for elderly (0.395 cases per 100,000 population), followed by diabetes (0.347 cases per 100,000 population), alcoholism (0.207 cases per 100,000 population), and AIDS (0.019 cases per 100,000 population).

The risk estimates for *V. vulnificus* in cockles for male and female populations were shown in Table 5. The risk of infection was higher for males (4.881 cases per 100,000 population), compared to females (3.166 cases per 100,000 population). Interestingly, a clear gender disparity with regards to *V. vulnificus* infections was reported by several researchers (Lee et al., 2013; Baker-Austin and Oliver, 2018). The underlying factors behind this are undefined, but it is suspected that men with underlying health risks particularly liver diseases are attributable to it. Excessive consumption of alcohol is considered the most significant cause of death from liver associated disease in the United States (Younossi *et al*., 2011). According to several epidemiological studies, consumption of a threshold dose of alcohol is responsible for serious liver diseases to become apparent (Mezey *et al*. 1988). The development of liver disease in man through long-term excessive alcohol consumption had been reported (Moriya *et al*., 2015; Tajima *et al*., 2017).

Though the prevalence of alcohol consumption in Malaysia was considerably low compared with other Western countries, the reported proportion engaging in binge drinking was relatively high. According to the National Health and Morbidity Survey in 2015, the prevalence of current drinker in Malaysia was 8.4% while the proportion of binge drinker for 18 years old and above was as high as 59.4%. The estimated male binge drinker population was higher than the female counterpart (male, 64%; female, 45.7%) (MOH, 2015). The risk estimates for *V. vulnificus* in cockles for male and female binge drinker populations were shown in Table 5. Similarly, the male population was found exposed to the greater risk (0.242 cases per 100,000 population), compared to female (0.040 cases per 100,000 population).

While comparing with the risk estimates of oysters reported by the WHO/FAO (2005), the estimated risk in this study was lower than their predicted number of cases in summer was 12.2 per 100,000 people. The risk per serving for susceptible populations reported was 4.28 $\times 10^{-5}$, while the risk per serving for susceptible populations in this study was 3.97×10^{-9}, a lower value. Similarly, Yamamoto *et al*. (2008) reported a mean number of times an individual would acquire *V. parahaemolyticus* illness from the consumption of bloody clams is 5.6 x 10^{-4}/person/year. Although these studies may provide an informative perspective, they do not correspond to the real scenario in Malaysia due to the variation in consumption patterns and cultural practices in different countries.

The estimated risk in the current study was much lower in comparison with the risk evaluated by Malcolm *et al*. (2015) concerning *V. parahaemolyticus* infections through the consumption of contaminated cockles which estimated at 250 cases per 100,000 people annually. In addition, the risk estimate reported by Sani *et al*. (2013), stated that 123 cases of infection per 100,000 people annually through consumption of contaminated tiger shrimps. The prevalence of different *Vibrio* species may vary by food or geographical area. However, the lack of supportive data and information for quantitative evaluation of risk including information on the consumption pattern, pathogenesis, and nature of *V. vulnificus* in foods may hinder more practical and accurate estimates.

In the current study, assumptions were made that 100% of infections can cause septicaemia in the population. However, a 100% infection-to-septicaemia rate is unrealistic. Also, information to translate the possibility of *V. vulnificus* infection into defined diseases is inadequate including information on the variance of pathogen's virulence by region and food source, the definition of at-risk population and host characteristics. Thus, more data will be needed to obtain a more conclusive result. Likewise, the pathology of different *V. vulnificus* strains to cause infections leading to septicaemia in humans is still unidentified; thus, this study also stipulated a 50% ratio of infection to illness in the risk analysis. An assumption was also made that for dose-response analysis, the use of mean concentration rather than median as a measure for exposure assessment is considered appropriate (for data within each group with varying individual values, it is considered more appropriate to relate the average dose to response).

This research demonstrated how deterministic step-wise risk analysis with limited narrow data availability

Table 5. Risk estimates for male and female populations

	Male	Female	(Binge drinker ≥ 18 years old) Male	Female
Prevalence				
No. of samples	199.00	199.00	199.00	199.00
No. positive	21.00	21.00	21.00	21.00
Prevalence (%)	10.60	10.60	10.60	10.60
Probability of positive sample	0.11	0.11	0.11	0.11
Concentration				
Mean concentration (per gram)	300.87	300.87	300.87	300.87
Serving size (g)	1.66	1.17	1.66	1.17
Dose (organism/serving)	499.44	352.01	499.44	352.01
Beta-Poisson dose response ($\alpha = 9.3\text{x } 10^{-6}$; $\beta = 1.1 \text{ x}10^{5}$)				
Probability of infection per serving				
Probability of infection per positive serving	$4.21\text{x}10^{-8}$	$2.97\text{x}10^{-8}$	$4.21\text{x}10^{-8}$	$2.97\text{x}10^{-8}$
Probability of infection per serving	$4.63\text{x}10^{-9}$	$3.27\text{x}10^{-9}$	$4.63\text{x}10^{-9}$	$3.27\text{x}10^{-9}$
Probability of infection per year				
No. of servings per year	104	104	104	104
Probability of infection per year	$4.82\text{x}10^{-7}$	$3.40\text{x}10^{-7}$	$4.82\text{x}10^{-7}$	$3.40\text{x}10^{-7}$
Estimated no. of cases per 100,000 population				
Population	16,362,500	15,298,200	809,835	193,746
Estimated population exposed	10,126,853	9,313,016	501,287	117,945
Expected no. of cases	4.881	3.166	0.242	0.040
Rate per 100,000 population	0.048	0.034	0.048	0.034
50% infection-to-illness ratio assumption				
Probability of infection to illness	0.5	0.5	0.5	0.5
Probability of illness per serving	$2.32\text{x}10^{-9}$	$1.64\text{x}10^{-9}$	$2.32\text{x}10^{-9}$	$1.64\text{x}10^{-9}$
Probability of illness per year	$2.41\text{x}10^{-7}$	$1.71\text{x}10^{-7}$	$2.41\text{x}10^{-7}$	$1.71\text{x}10^{-7}$
Expected no. of cases	2.441	1.593	0.121	0.020
Rate per 100,000 population	0.024	0.017	0.024	0.017

*These estimates are based on the assumption that the estimated population exposed for binge drinker ≥ 18 years old, male and female (%), are equally with general male and female populations.
*Binge drinking was defined as consumption of 6 and more of standard drink per-sitting.

can provide a valuable local insight scenario. The predicted risk for septicaemia among cockle's consumers could be improved in the future with better available data and knowledge collected from other ongoing scientific research. The prevalence of the bacterium in the cockles can be influenced by the annual temperature and salinity which can be another significant factor to influence the incidence of illness and the risk outcome. Thus, continual updates and modification to the current model are needed when new data become available through ongoing research to minimize the degree of uncertainty.

5. Conclusion

In recent years, cockles are no longer considered as cheap shellfish indulgence because of declining production. However, cockles are still commonly consumed shellfish by Malaysians. Overall, the estimated health risk of septicaemia cases attributed to the consumption of contaminated cockles was 8.028 cases per 100,000 Malaysian populations. The step-wise risk evaluation demonstrated that the levels of *V. vulnificus* present in cockles play an important role to cause disease in people. Thus, cockles' preparation in the kitchen level such as proper storage and sufficient cooking time is necessary to safeguard public health. With several key assumptions, this step-wise risk assessment was able to estimate the health risk of acquiring *V. vulnificus* infections following the consumption of cockles in Malaysia scenario.

Conflict of Interest

The authors declare that the research was conducted in the absence of any potential conflict of interest.

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