Risk assessment of vibriosis by \textit{Vibrio cholerae} and \textit{Vibrio vulnificus} in whip-arm octopus consumption in South Korea

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Abstract

This study evaluated the risk of foodborne illness from highly pathogenic \textit{Vibrio} spp. (\textit{Vibrio vulnificus} and \textit{V. cholerae}) by raw whip-arm octopus (\textit{Octopus minor}) consumption. In total 180 samples of raw whip-arm octopus were collected from markets and examined for the prevalence of \textit{V. vulnificus} and \textit{V. cholerae}. Predictive models describing the kinetic behavior of \textit{Vibrio} spp. in raw whip-arm octopus were developed, and the data on amounts and frequency of raw whip-arm octopus consumption were collected. Using the collected data, a risk assessment simulation was conducted to estimate the probability of foodborne illness raw whip-arm octopus consumption using @RISK. Initial contamination levels of \textit{Vibrio} spp. in raw whip-arm octopus were \(-3.9\) Log colony-forming unit/g, as estimated by beta distribution fitting. The developed predictive models were appropriate to describe \textit{Vibrio} spp. in raw whip-arm octopus during distribution and storage with $R^2$ values of 0.946–0.964. The consumption frequency and daily consumption amounts of raw whip-arm octopus per person were 0.47% and 57.65 g, respectively. The probability of foodborne illness from raw whip-arm octopus consumption was estimated to be \(8.71 \times 10^{-15}\) for \textit{V. vulnificus} and \(7.08 \times 10^{-13}\) for \textit{V. cholerae}. These results suggest that the risk of \textit{Vibrio} spp. infection from raw whip-arm octopus consumption is low in South Korea.

Keywords: Microbial risk assessment, Highly pathogenic \textit{Vibrio} spp., Raw whip-arm octopus, \textit{Vibrio vulnificus}, \textit{Vibrio cholerae}
Introduction

_Vibrio_ spp. are facultative, oxidase positive (except _Vibrio met-schnikovii_), non-spore-forming gram-negative bacteria that survive in marine environments (Bross et al., 2007; Tantillo et al., 2004; Tsai et al., 2009). Pathogenic species such as _Vibrio parahaemolyticus_, _Vibrio vulnificus_, and _Vibrio cholerae_ are causative agents of foodborne diseases upon consumption of raw or undercooked seafood (Gauthier, 2015; Yan et al., 2019). Among the pathogenic _Vibrio_ spp., _V. parahaemolyticus_ has been subjected to microbial risk assessment in various foods and _V. vulnificus_ and _V. cholerae_ are categorized as highly pathogenic _Vibrio_ (Baker-Austin et al., 2018; Jones & Oliver, 2009). Especially in 2020, cases of sepsis caused by _V. vulnificus_ increased by two fold since 2019, and most infections were recorded between August and September (KCDC, 2020). This pathogen can cause death in approximately 50% of infected patients (KCDC, 2020).

While _V. cholerae_ originated abroad, domestic cases have been reported in Korea in 2016 (KCDC, 2017). _V. vulnificus_ and _V. cholerae_ infections may cause death in immunocompromised patients within few hours, owing to dehydration and systemic symptoms (KCDC, 2005).

The whip-arm octopus is a rich source of protein, taurine, various amino acids, iron, calcium, minerals, eicosapentaenoic acid, and docosahexaenoic acid, and its consumption decreases cholesterol levels (Lyu, 2003). Whip-arm octopus production ranks fourth among mollusks and it is the tenth most consumed seafood annually (KOSIS, 2018; KREI, 2014). In South Korea, the whip-arm octopus is occasionally consumed in the raw form. Hence, _Vibrio_ spp. infection can be caused by whip-arm octopus consumption, owing to incomplete washing of sediment from the foreshore and storage water contaminated with _Vibrio_ spp. _V. vulnificus_ has been detected in whip-arm octopus (MFDS, 2003).

Therefore, this study estimated the risk of infection with highly pathogenic _Vibrio_ spp. such as _V. vulnificus_ and _V. cholerae_ following whip-arm octopus consumption.

Materials and Methods

Investigation of the prevalence of highly pathogenic _Vibrio_ spp. and collection of transfer and storage temperature/time

To investigate the prevalence of highly pathogenic _Vibrio_ spp. in whip-arm octopus, 180 whip-arm octopus samples were collected from supermarkets, wet markets, and restaurants on the south shore (Tongyeong, Korea), east shore (Gangreung, Korea), west shore (Goonsan, Korea), and central area (Seoul, Korea) of Korea. The most probable number-polymerase chain reaction (MPN-PCR) method was used to detect highly pathogenic _Vibrio_ spp., as described by Jang et al. (2018), Kim et al. (2019), and Nandi et al. (2000). The samples (25 g) were aseptically transferred to filter bags (3M, St. Paul, MN, USA) containing 225 mL of 0.85% saline buffer, and the samples were pummelled using a pummeler (BagMixer®, Interscience, St. Nom, France) for 1 min. Homogenates (0.1, 1, and 10 mL) were inoculated into five test tubes containing 10 mL of alkaline peptone water (APW; Oxoid, Basingstoke, UK) to obtain a final concentration of 1 × APW. All tubes were incubated at 35°C for 14 h. For PCR analysis, the cultures were centrifuged at 13,475×g for 2 min, and the supernatants were discarded. The cell pellets were resuspended in 0.1 mL of distilled water and centrifuged at 13,475×g for 2 min. The suspension was heated at 100°C for 10 min to extract DNA, which was used as a DNA template for PCR (Ha et al., 2020). Primers for _V. vulnificus_ (F: CAG CCG GAC GTC GTC CAT TTT G, R: ATG AGT AAG CGT CCG ACG CGT) and _V. cholerae_ (F: CAC CAA GAA GGT GAC TTT ATT GTG; R: GGT TTG TCG AAT TAG CTT CAC C) were used, as suggested by Kim et al. (2015) and Nair et al. (1994), respectively. DNA amplification was performed using FastMix (Intron Bio, Seongnam, Korea), including dNTP, DNA polymerase, reaction buffer, and magnesium chloride (MgCl₂). The amplification of _V. vulnificus_ and _V. cholerae_ was performed as per the following procedures: initial denaturation at 94°C for 5 min; 25 cycles (_V. vulnificus_) or 30 cycles (_V. cholerae_) of denaturation (94°C for 30 s), elongation (60°C [ _V. vulnificus_] or 64°C [ _V. cholerae_] for 30 s), and extension (72°C for 30 s); and final extension at 72°C for 10 min. PCR products were electrophoresed on a 1.5% agarose gel for 20 min and visualized under UV light. The number of positive test tubes per five test tubes that were analyzed by PCR was counted for each dilution, and the MPN was determined using an MPN table (FDA, 2020). A loopful of the positive test tube from the PCR analysis was streaked on thiosulfate citrate bile salts sucrose agar (TCBS; Becton, Dickinson and Company, Sparks, MD, USA), and the plates were incubated at 35°C for 18–24 h. The typical colonies of _V. vulnificus_ and _V. cholerae_ on the plates were inoculated into 10 mL tryptic soy broth (TSB, Becton, Dickinson and Company; non-selective broth) containing 3% sodium chloride (NaCl) to isolate a single colony and incubated at 35°C for 18–24 h. Non-selective broth was used for the isolation of _Vibrio_ spp. to improve recovery of damaged cells.
or decrease stress on *Vibrio* spp. The DNA extraction method, primers, and PCR conditions used were the same as those described above. The sample showing positive results in both MPN-PCR and TCBS agar was determined as positive. In addition, temperature and time during transfer and storage were collected by interviewing people who work in supermarkets, wet markets, and restaurants.

**Development of predictive models**

**Preparation of inoculum**

In a previous study, the growth of *V. vulnificus* and *V. cholerae* was not different; thus, predictive models applicable to both species have been developed. Briefly, 100 μL aliquots of *V. vulnificus* strains ATCC27562, NCCP14549, and NCCP11887, and *V. cholerae* strains NCCP14552 (non-O1) and NCCP12842 (O23) in cryovials stored at −80°C were inoculated into 10 mL of Luria-Bertani (LB) broth (Becton, Dickinson and Company) with 2% NaCl and incubated at 37°C for 28 h. The cultures (0.1 mL) were then inoculated into 10 mL of LB broth plus 2% NaCl and incubated at 37°C for 12 h. The subcultures of *V. vulnificus* and *V. cholerae* strains were mixed and then centrifuged at 1,912×g and 4°C for 15 min. The cell pellet was washed twice with phosphate-buffered saline (PBS; 0.2 g potassium chloride [KCl], 0.2 g monopotassium phosphate [KH₂PO₄], 8.0 g NaCl, and 1.5 g disodium hydrogen phosphate [Na₂HPO₄·7H₂O] in 1 L distilled water pH 7.4) and resuspended in 50 mL of APW to obtain the inoculum at 6 Log CFU/mL.

**Infected whip-arm octopus with artificial seawater contaminated with highly pathogenic Vibrio spp.**

If sea water is infected with *V. vulnificus* and *V. cholerae*, the pathogens can contaminate the surface and accumulate in the intestine of the octopus during breathing. Thus, a mixture of 1,750 g sun-dried salt and 1,750 g artificial sea salt (Reef Salt, Ross, 1996) as follows:

$$N_i = N_0 + \mu_{max} \times \ln \left( 1 + \frac{\exp(\mu_{max} \times A_t) - 1}{\exp(N_{max} - N_i)} \right)$$

$$A_t = t + \frac{1}{\mu_{max}} \ln \left( \frac{\exp(-\mu_{max}) + q_h}{1 + q_h} \right)$$

$$q_h = \frac{1}{\exp(h_0) - 1}$$

The kinetic parameters were fitted into a secondary polynomial model as a function of storage temperature as follows:

$$LPD \text{ or } \mu_{max} = a_0 + a_1 T + a_2 T^2$$

The performance of the developed models was evaluated by comparing the derived predicted values and objective values collected at 13°C and 17°C. The differences between the predicted and observed values were indicated by the root mean square error (RMSE), bias factor (Bf), and accuracy factor (Af) (Ross, 1996) as follows:

$$\text{RMSE} = \sqrt{\frac{\sum\text{(observed value } - \text{ predicted value)²}}{n}}$$

$$B_f = 10^{\frac{\Sigma\text{log(predicted/observed)}/n}{n}}$$

$$A_f = 10^{\frac{\Sigma\text{log(predicted/observed)}/n}{n}}$$

**Collection time and temperature for transportation and storage**

The time and temperature data for transportation and storage of the whip-arm octopus were collected via personal communication with people who were in the business. Using these data, the appropriate probability distribution for time and temperature at
each step was determined using the @RISK program (Version 7.6, Palisade, Ithaca, NY, USA).

**Investigation of consumption amount and frequency**
Data on the daily consumption amount and frequency of whip-arm octopuses in South Korea were obtained from the Korea National Health and Nutrition Examination Survey (2017) (KCDC, 2019). Using these data, the appropriate probability distribution for the daily consumption amount was determined using the @RISK program.

**Determination of the dose-response model**
To evaluate the doses (CFU) of *V. vulnificus* and *V. cholerae* that cause foodborne illness, the most appropriate dose-response models were searched and selected in the literature.

**Simulation to estimate risk**
A simulation model was prepared in the @RISK program with data of highly pathogenic *Vibrio* spp. prevalence and initial concentration, the predictive models applied to distribution time and temperature, consumption amount and frequency, and dose-response models. The risk of foodborne illnesses from highly pathogenic *Vibrio* spp. upon raw whip-arm octopus consumption was estimated using a simulation model with 10,000 iterations.

**Statistical analysis**
Kinetic parameters (LPD and $\mu_{\text{max}}$) from the predictive (primary) models were analyzed using a general linear model (GLM) procedure in SAS® (Version 9.3, SAS Institute, Cary, NC, USA). The mean comparison of the kinetic parameters among storage temperatures was conducted using a pairwise *t*-test at $\alpha = 0.05$.

**Results and Discussion**

**Investigation of initial *V. vulnificus* and *V. cholerae* contamination in whip-arm octopus**
To detect pathogens, we collected and examined 180 whip-arm octopus samples from supermarkets, wet markets, and restaurants of four sections, including the south shore, east shore, west shore, and central area. MPN-PCR analysis showed that *V. vulnificus* count was below the detection limit (30 MPN/100 g) and *V. cholerae* count was 36 MPN/100 g in one sample from the east shore, one sample from the west shore, and three samples from the central area. However, the cultures from contaminated samples were streaked on TCBS plates and the colonies obtained on the plates were subjected to PCR analysis, which deemed all samples negative for *V. vulnificus* and *V. cholerae*. Thus, all whip-arm octopus samples were negative for the highly pathogenic *Vibrio* spp., and the beta distribution fitting, as suggested by Vose (1996), estimated an initial contamination level of $-3.9 \log \text{CFU/g}$ (Fig. 1).

**Probability distribution of temperature and time for transport and display**
Times for transport and display of whip-arm octopus were between 0 and 72 h, and the mode was 4 h. These values were fitted with the Pert distribution, and the result was as follows: Pert $[0, 4, 72]$. The collected temperatures were fitted with the minimum extreme value distribution, and the result was expressed as ExtValueMin $[15.1959, 2.4783]$.

**Predictive models**
The cell counts of highly pathogenic *Vibrio* spp. in live whip-arm octopus gradually decreased at 7°C and 10°C but increased at 15°C and 20°C (Fig. 2). FAO & WHO (2020) also suggested that *V. vulnificus* failed to grow below 10°C but could grow gradually above 15°C. According to the previous study by Kaspar & Tamplin (1993), the pathogenic *Vibrio* spp. can grow better as the temperature increases, and not survive well at temperatures below 8.5°C, which is similar to these results. The results of our study show that *Vibrio* spp. can grow in live seafood during storage at temperatures above 15°C. The fitting results from the Baranyi model with the cell counts produced kinetic parameters such as LPD and $\mu_{\text{max}}$, as shown in Table 1. Since the Baranyi model provides biological interpretation for the duration of the lag phase, and both growth and death rates, the model was used for primary modeling. The $R^2$ values were 0.694–0.930. The LPD decreased but increased as the temperature increased (Table 1). The LPD and $\mu_{\text{max}}$ values were fitted with a polynomial model as a function of temperature, and the secondary models are presented in Table 2. The $R^2$ values of the secondary models were 0.946 and 0.964 for LPD and $\mu_{\text{max}}$ respectively (Table 2). Thus, the developed secondary models were appropriate to describe the effect of temperature on the kinetic parameters (Fig. 3). The validation results showed that RMSEs were 0.307 and 0.263 at 13°C and 17°C, respectively. As RMSE approaches 0, the prediction of the secondary predictive model is accurate. Furthermore, $B_j$ values were 1.02 and 0.96 at 13°C and 17°C, respectively, indicating that the predictive models...
over-predict by 2% at 13°C and under-predict by 4% at 17°C. 
$A_f$ values were 1.06 and 1.05 at 13°C and 17°C, respectively. 
As $A_f$ value approaches 1, the prediction of the predictive model is accurate. 
Although $R^2$ values were not very high in the primary models, the validation results indicate that the developed predictive models were appropriate to describe cell count changes of highly pathogenic *Vibrio* spp. under the temperature and time during transport and display of live whip-arm octopus.

Fig. 1. Probable distributions for initial contamination levels of *Vibrio vulnificus* (A) and *Vibrio cholerae* (B) in raw whip-arm octopus.
Table 1. Kinetic parameters calculated by primary model for pathogenic *Vibrio* spp. in raw whip-arm octopus

| Kinetic parameters | Temperature (°C) | 4   | 10  | 15  | 20  |
|--------------------|------------------|-----|-----|-----|-----|
| $\mu_{\text{max}}$ | $-0.03 \pm 0.01$ | $-0.02 \pm 0.00$ | $0.02 \pm 0.00$ | $0.05 \pm 0.01$ |
| LPD                | $22.93 \pm 2.35$ | $8.69 \pm 1.81$ | $3.35 \pm 0.23$ | $0.98 \pm 0.08$ |
| $R^2$              | $0.718 \pm 0.136$ | $0.879 \pm 0.051$ | $0.694 \pm 0.080$ | $0.930 \pm 0.018$ |

$\mu_{\text{max}}$, maximum specific growth rate (Log CFU/g/h), indicating death and growth rates; LPD, lag phase duration (h), period of no cell count change in a growth/death curve.

Fig. 2. Results of primary modeling for highly pathogenic *Vibrio* spp. growth in raw whip-arm octopus during storage at 7°C (A), 10°C (B), 15°C (C), and 20°C (D) (•, observed data; —, fitted line).
Consumption amount and frequency

In Korea National Health and Nutrition Examination Survey (KCDC, 2019), 34 of 7,167 respondents had consumed whip-arm octopus, and the consumption frequency was 0.47% \((34/7,167) \times 100\). Hence, the discrete distribution was used to describe the probability of consuming a whip-arm octopus. The consumption amounts from the respondents with whip-arm octopus were fitted with @RISK. The appropriate probability distribution was the exponential distribution \((\text{Expon } [55.585, \text{Shift } 3.0651])\) that showed an average consumption of 58.65 g (Fig. 4).

Dose response models

To estimate the dose responses of \(V.\ vulnificus\) and \(V.\ cholerae\), the commonly used Beta-Poisson model \((\text{probability} = 1 - [1 + \text{dose} / \beta]^{-\alpha})\) was selected. For \(V.\ vulnificus\), the parameters were \(\alpha = 1.31 \times 10^{-1}\) and \(\beta = 1.49 \times 10^7\) (Cash et al., 1974; WHO & FAO, 2005b). For \(V.\ cholerae\), the parameters were \(\alpha = 1.31 \times 10^{-1}\) and \(\beta = 1.49 \times 10^7\) (Cash et al., 1974; WHO & FAO, 2005b).

Risk characterization

The simulation models were formulated with the estimated levels of initial contamination, predictive models, probability distributions for temperature and time, probability distribution of consumption amounts, consumption frequency, and dose response models, as presented in Table 3. The simulation showed that the contamination levels of \(V.\ vulnificus\) and \(V.\ cholerae\) in whip-arm octopus samples were \(-3.8 \text{ Log CFU/g}\) after transport and display (Fig. 5), indicating only \(0.1 \text{ Log CFU/g}\) increase from the initial contamination level \((-3.9 \text{ Log CFU/g})\). Thus, the highly pathogenic \(Vibrio\) spp. do not grow in live whip-arm octopus at the investigated temperature and time. Live whip-arm octopus was transported and displayed at \(0^\circ\text{C} - 10^\circ\text{C}\) and sold within 4 days. Therefore, the pathogen could not

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Table 2. Equations of secondary model for predicting \(\mu_{\text{max}}\) and \(LPD\) of pathogenic \(Vibrio\) spp. in raw whip-arm octopus

| Parameter | Equation | \(R^2\) |
|-----------|----------|--------|
| \(\mu_{\text{max}}\) | \(\mu_{\text{max}} = -0.0875 + 0.007 \times T\) | 0.964 |
| \(LPD\) | \(LPD = 60.0506 - 6.8472 \times T + 0.1961 \times T^2\) | 0.946 |

\(T\), temperature \(^\circ\text{C}\); \(\mu_{\text{max}}\), maximum specific growth rate \((\text{Log CFU/g/h})\); \(LPD\), lag phase duration \((\text{h})\).
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Fig. 4. Probabilistic distribution for raw whip-arm octopus consumption obtained from the Korea National Health and Nutrition Examination Survey (KNHNES) fitted by @RISK.

Table 3. Excel spreadsheet for calculating the risk of Vibrio spp. in raw whip-arm octopus with @RISK

| Input model                        | Unit | Variable | Formula | Reference                        |
|------------------------------------|------|----------|---------|----------------------------------|
| **DISTRIBUTION**                   |      |          |         |                                  |
| Pathogens contamination level      |      |          |         |                                  |
| V. vulnificus prevalence           |      | PR       | \(PR = \text{RiskBeta}(1,181)\) | This research; Vose (1996)       |
| V. cholerae prevalence             |      | PR       | \(PR = \text{RiskBeta}(1,181)\) |                                  |
| Initial contamination level        | CFU/g| C        | \(C = -\ln(1 - PR) / 25\)    | Sanaa et al. (2004)              |
|                                    | Log CFU/g | IC      | \(IC = \log(C)\)              |                                  |
| **MARKET**                         |      |          |         |                                  |
| Market display                     |      |          |         |                                  |
| Display time                       | h    | \(Time_{\text{mark-dis}}\) | \(\text{RiskPert}(0,4,72)\)    | This research; Personal communication |
| Display temperature                | ºC   | \(Temp_{\text{mark-dis}}\) | \(\text{RiskExtValueMin}(15.1959, 2.4783, \text{RiskTruncate}(0,18.8))\) | This research; Personal communication |
| Growth/death                       |      |          |         |                                  |
| Log CFU/g                          | \(h_0\) | \(\mu_{max}\) | \(\mu_{max} = -0.0875 + 0.007 \times Temp_{\text{mark-dis}}\) | This research; Baranyi & Roberts (1994) |
| Log CFU/g                          | \(\gamma_0\) | \(\gamma_{\text{mod}}\) | \(\gamma_{\text{mod}} = \text{Average}(\gamma_{\text{mod}}, \text{Fixed} 4.3)\) | This research; Baranyi & Roberts (1994) |
| Log CFU/g                          | \(\ln(q)\) |         | \(\ln(q) = \ln(1 / \exp(h_0) - 1)\) | This research; Baranyi & Roberts (1994) |
| \(h_{\text{max}}\)                | Log CFU/g/\(h_{\text{max}}\) | \(\gamma_{\text{mod}}\) | \(\gamma_{\text{mod}} = -0.0875 + 0.007 \times Temp_{\text{mark-dis}}\) | This research; Baranyi & Roberts (1994) |
| Pathogenic Vibrio growth/death     | Log CFU/g | \(C_1\) | \(C_1 = IC + 1 / (1 + \exp(-\ln(q))) \times (1 - 10^{\gamma_{\text{mod}}/\text{Temp}_{\text{mark-dis}}} / \ln(10)) \times \text{GR}_{\text{mark-dis}} \times Time_{\text{mark-dis}}\) | This research; Baranyi & Roberts (1994) |
| model                              |      |          |         |                                  |
| **CONSUMPTION**                    |      |          |         |                                  |
| Daily consumption frequency        | %    | ConFre   | Fixed 0.47 | KCDC (2019)                     |
|                                   | CF(0) | = 1 – 0.47/100 | | KCDC (2019)                     |
|                                   | CF(1) | = 0.47/100 | | KCDC (2019)                     |
|                                   | CF   | = \text{RiskDiscrete}(0,1, (CF(0), CF(1))) | | KCDC (2019)                     |
| Daily consumption amount           | g    | Consump  | \(\text{RiskExpon}(55.585, \text{RiskShift}(3.0651), \text{RiskTruncate}(4,190))\) | KCDC (2019)                      |
Table 3. Continued

| Input model          | Unit | Variable | Formula                          | Reference                              |
|----------------------|------|----------|----------------------------------|----------------------------------------|
| Final consumption amount | g    | Amount   | = IF(CF = 0, 0, Consump)         |                                        |
| DOSE-RESPONSE        |      |          |                                  |                                        |
| *V. vulnificus* amount | CFU  | D        | $= 10^{\alpha} \times Amount$   | WHO & FAO (2005a)                      |
|                      |      | $\alpha$ | Fixed $9.3 \times 10^{-6}$       | WHO & FAO (2005a)                      |
|                      |      | $\beta$  | Fixed $1.1 \times 10^{3}$        | WHO & FAO (2005a)                      |
| *V. cholerae* amount | CFU  | D        | $= 10^{\alpha} \times Amount$   | Cash et al. (1974); WHO & FAO (2005b) |
|                      |      | $\alpha$ | Fixed $1.31 \times 10^{-1}$      | Cash et al. (1974); WHO & FAO (2005b) |
|                      |      | $\beta$  | Fixed $1.49 \times 10^{7}$       | Cash et al. (1974); WHO & FAO (2005b) |
| RISK                 |      | Risk     | $= 1 - (1 + D/\beta)^{-\alpha}$ | Cash et al. (1974); WHO & FAO (2005a, 2005b) |

*Personal communication with experts.*

Fig. 5. Growth of *Vibrio* spp. in raw whip-arm octopus during domestic distribution (IC, initial concentration; C1, concentration after display at markets).
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In addition, the simulation showed that the probabilities of foodborne illness per person per day were $8.71 \times 10^{-15}$ for *V. vulnificus* and $7.08 \times 10^{-13}$ for *V. cholerae*.

In conclusion, the risk of infection by the highly pathogenic *Vibrio* spp. via raw whip-arm octopus consumption is low. This might be related to the low contamination level and low growth of pathogens under the conditions of temperature and time. In addition, a very low consumption frequency (0.47%) affected the risk, as indicated by the correlation coefficient (Fig. 6).

**Competing interests**

No potential conflict of interest relevant to this article was reported.

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Not applicable.

**Availability of data and materials**

Upon reasonable request, the datasets of this study can be available from the corresponding author.

**Ethics approval and consent to participate**

This article does not require IRB/IACUC approval because there are no human and animal participants.

![Fig. 6. Correlation coefficient values of risk factors, affecting the probability of foodborne illness caused by *Vibrio* spp. per person per day following raw whip-arm octopus consumption.](image)

(A)

(B)
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