Incidence and prevalence of *Vibrio parahaemolyticus* in seafood: a systematic review and meta-analysis

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Abstract

*Vibrio parahaemolyticus* is an important seafood borne human pathogen worldwide due to it occurrence, prevalence and ability to cause gastrointestinal infections. This current study aim at investigating the incidence and prevalence of *V. parahaemolyticus* in seafood using systematic review-meta-analysis by exploring heterogeneity among primary studies. A comprehensive systematic review and meta-analysis of peer reviewed primary studies reported between 2003 and 2015 for the occurrence and prevalence of *V. parahaemolyticus* in seafood was conducted using “isolation”, “detection”, “prevalence”, “incidence”, “occurrence” or “enumeration” and *V. parahaemolyticus* as search algorithms in Web of Science (Science Direct) and ProQuest of electronic bibliographic databases. Data extracted from the primary studies were then analyzed with fixed effect meta-analysis model for effect rate to explore heterogeneity between the primary studies. Publication bias was evaluated using funnel plot. A total of 10,819 articles were retrieved from the data bases of which 48 studies met inclusion criteria. *V. parahaemolyticus* could only be isolated from 2761 (47.5%) samples of 5811 seafood investigated. The result of this study shows that incidence of *V. parahaemolyticus* was more prevalent in oysters with overall prevalence rate of 63.4% (95% CI 0.592–0.674) than other seafood. Overall prevalence rate of clams was 52.9% (95% CI 0.490–0.568); fish 51.0% (95% CI 0.476–0.544); shrimps 48.3% (95% CI 0.454–0.512) and mussels, scallop and periwinkle: 28.0% (95% CI 0.255–0.307). High heterogeneity (p value <0.001; $I^2 = 95.291$) was observed mussel compared to oysters ($I^2 = 91.024$). It could be observed from this study that oysters harbor *V. parahaemolyticus* based on the prevalence rate than other seafood investigated. The occurrence and prevalence of *V. parahaemolyticus* is of public health importance, hence, more studies involving seafood such as mussels need to be investigated.

Keywords:  
Seafood safety and quality, Prevalence, Reservoir, *V. parahaemolyticus*, Shellfish

Background

*Vibrio parahaemolyticus* is a non-sucrose fermenting halophilic bacterium that grows between 10 and 44 °C and optimum temperature of 35–37 °C (Zamora-Pantoja et al. 2013; Wagley et al. 2009). The first outbreak of seafood borne disease due to consumption of *V. parahaemolyticus* contaminated sardine was reported in Japan in 1950 (Levin 2006). In this outbreak, 20 people were reported dead while over 270 people were
likewise hospitalized. More outbreaks involving consumption of contaminated raw or undercooked seafood like oyster has been reported in United States (Iwamoto et al. 2010; McLaughlin et al. 2005; Drake et al. 2007), China (Liu et al. 2004), Taiwan (Chiou et al. 2000), Spain (Lozano-Leon et al. 2003), Italy (Ottaviani et al. 2008), Chile (Garcia et al. 2009), Peru (Gil et al. 2007) and (Leal et al. 2008) V. parahaemolyticus infection is characterized with vomiting, acute abdominal pain, abdominal pain, vomiting, watery or bloody diarrhea and gastroenteritis as result of production of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) toxins respectively (Jahangir Alam et al. 2002; Wagley et al. 2009) with an incubation period of 4–96 h (Levin 2006) however, non-pathogenic V. parahaemolyticus strains do not cause any infection. Several studies have been conducted globally regarding occurrence and prevalence of total or pathogenic V. parahaemolyticus in seafood yet there exist variability among the studies in terms of incidence and prevalence.

Meta-analysis is a quantitative statistical summarizing techniques aimed at extracting and combining scientific results from multiple primary studies that have investigated the same research question (Gonzales-Barron et al. 2013). Meta-analysis explains possible differences in outcomes of primary studies by extracting and encoding study characteristics such as research design features, data collection procedures, type of samples and year of study (DerSimonian and Laird 1986). This involves several steps like systematic review of literatures, data extraction of both qualitative and quantitative information from relevant primary studies, selection of effect size as described from each study, estimation of overall effect size of all the primary studies, assessment of heterogeneity of studies and presentation of meta-analysis using numerical (odd ratios, fixed effects size, p values, publication bias, meta regression, and random effect) and or graphical methods forest plot, funnel plot and others (Gonzales-Barron et al. 2013). Method of data generation differs from one study to another. Hence, researchers can either perform experiment to generate data or utilize available data from previous study (primary study) without experimental work (den Besten and Zwietering 2012). It was recently that food safety researchers stated conducting meta analytical studies as most meta-analytical study are conducted only in medical and social sciences (Gonzales Barron et al. 2008; Gonzales-Barron and Butler 2011; Patil et al. 2004). Meta-analytical studies could be carried out in food safety research in order to help answer various research questions involving prevalence pathogens in foods, treatment interventions, predictive modelling, microbial risk assessment, food safety knowledge, attitude and practices (Xavier et al. 2014).

Currently, no meta-analysis has been conducted on estimation of overall incidence, detection and prevalence of V. parahaemolyticus in seafood has been carried out in order to gain insight to source(s) of reservoir for these bacterial pathogens. This study therefore aim to systematically review and summarize primary studies describing incidence and prevalence of V. parahaemolyticus in seafood worldwide.

**Methods**

**Definition**

For the purpose of this study, incidence is defined as occurrence (presence) of V. parahaemolyticus in seafood samples analyzed in the primary studies while prevalence (p) is
the number (n) of seafood that was positive for the presence of *V. parahaemolyticus* from the total sample (N). Primary studies imply all the studies carried out by other researchers used in this study. Population of study is the type of seafood investigated in each study. Seafood considered in this study are mollusks (oysters, clams, and mussels), fin-fish (salmon and tuna) and crustaceans (shrimp, crab, and lobster) (Iwamoto et al. 2010). In order to achieve the aim of this study, modified methods of Preferred Reporting Items for Systematic Reviews and Meta-Analyses—PRIMA (Moher et al. 2009) and (Gonzales-Barron and Butler 2011) were used. The steps consist of systematic review of literatures, data extraction of both qualitative and quantitative information from relevant primary studies, selection of effect size as described from each study, estimation of overall effect size of all the primary studies, assessment of heterogeneity of studies and meta-analysis representation of obtained result using numerical (odd ratios, fixed effects size, p values, publication bias, meta regression, and random effect) and or graphical methods forest plot, funnel plot and others).

**Literature search, selection and relevance screening**

This review was guided by a research question and problem statement. The research question was how prevalent is *V. parahaemolyticus* in seafood? While a problem statement describing the incidence and prevalence of *V. parahaemolyticus* in different seafood samples was formulated. Presence or absent of *V. parahaemolyticus* was considered as possible outcome of each primary study. Thereafter, a comprehensive literature search of electronic databases (ISI Web of science and ProQuest) and systematic review of available primary studies aimed at producing summary of relevant, quality and initial findings from such studies was carried out. The following search algorithms: “isolation” and *V. parahaemolyticus*, “detection” and *V. parahaemolyticus*, “prevalence” and *V. parahaemolyticus*, “incidence” and *V. parahaemolyticus*, “occurrence” and *V. parahaemolyticus* and “enumeration” and *V. parahaemolyticus* were used. Preliminary screening (Abstract-based relevance screening) of titles and abstracts of retrieved primary studies was carried out for eligibility and relevance to this study. Relevance of each article was screened using both inclusion and exclusion criteria. The inclusion criteria are: description of isolation method of *V. parahaemolyticus* from seafood using both conventional method (use of Thiosulphate Citrate Bile Salt agar—TCBS) and or molecular methods (Polymerase chain reaction—PCR). Full text and peer reviewed articles in English. The total number (population) of samples studied and number of samples that are positive for presence of *V. parahaemolyticus* clearly stated in the study. The exclusion criteria are: review articles, detection of *V. parahaemolyticus* in artificially contaminated samples, non-peer reviewed articles such as thesis, opinion articles, non-food related sources of *V. parahaemolyticus* such as clinical samples and conference abstract due to lack of access to full articles. Thereafter, full text screening of eligible primary studies were obtained from the databases. Articles that are not freely available were obtained via the service of the University of Tasmania’s library. Citations identified were retrieved and further checked for duplication using Endnote x7.1 software.
Data extraction and assessment of quality
Based on the inclusion and exclusion criteria, first author, year of publication or study, location, type of seafood studied, microbiological methods, number of sample positive for presence of *V. parahaemolyticus* were extracted.

Statistical analysis of extracted data
The pooled estimates of prevalence of *V. parahaemolyticus* in seafood were obtained by fixed effect meta-analysis model. The model was used to analyze combined extracted data while variation of incidence and prevalence of *V. parahaemolyticus* between the primary studies was evaluated using heterogeneity ($I^2$). Heterogeneity of prevalence estimates between the studies was investigated using Q statistic (Bangar et al. 2014) and quantified by $I^2$ Index (Higgins et al. 2003) as shown in below equations.

$$Q = \sum \{ w_i (\beta_i - \beta_w)^2 \}$$  \hfill (1)

$$I^2 = \{ (Q - df) / Q \} \%$$  \hfill (2)

where df is the degree of freedom ($N - 1$), $\beta_w$ is the pooled estimate, $\beta_i$ is the estimate of individual primary study. Presence of bias in the publications was determined using funnel plots (odd of presence of *V. parahaemolyticus* in the samples) of standard error. Forest plots were however used to estimate the event rate at 95 % confidence intervals. Prevalence (p) and standard error (s.e.) were calculated by the following formulae: $p = n/N$ and $s.e. = \sqrt{p (1 - p) / N}$: where $n =$ number of positive samples and $N =$ number of samples (Tadesse and Tessema 2014). Modified method of (Greig et al. 2012) was used for the assessment of risk bias. Statistical analyses was carried out using Comprehensive Meta-Analysis (CMA) software. Statistical p values ($p < 0.05$) were considered as statistically significant.

Results and discussion
Literature search
The numbers of studies on *V. parahaemolyticus* has increased over the years. This current study is the first meta-analytical study to be carried out on incidence and prevalence of *V. parahaemolyticus* in seafood. Figure 1 shows results obtained from literature search. Literature search yielded 10,819 primary studies. However, when the source of articles was limited to peer review journals, 6876 articles were obtained. Further limiting of the subject to full text academic journals, *V. parahaemolyticus*, seafood and or shellfish, 149 articles were obtained. Abstract relevance screening of published articles reduced the study to 86 while only 63 articles remained after de-duplication. Hence, only few primary studies met the inclusion requirement of this meta-analysis. The primary studies considered in this meta-analysis described standard method for isolation and detection of *V. parahaemolyticus* from seafood samples. First author, year of publication or study, location of study, type of seafood studied, microbiological methods and number of sample positive for presence of *V. parahaemolyticus* were extracted from the following 48 primary studies: (Abd-Elghany and Sallam 2013; Amin and Salem 2012; Anjay et al. 2014; Bilung et al. 2005; Blanco-Abad et al. 2009; Chakraborty and Surendran 2008;
10819 articles were identified from databases: Web of Science (Science Direct), ProQuest and other sources like Google scholar and University of Tasmania Library MegaSearch database.

6876 articles were obtained after limiting publications to peer review articles.

149 articles were obtained after limiting subject to full text academic journals, *Vibrio parahaemolyticus*, seafood and or shellfish.

86 eligible for quantitative review

63 excluded after de-duplication

48 studies included in meta-analysis.

Fig. 1 Flow diagram of selected studies included in fixed effect meta-analysis
et al. 2008a, b; Yano et al. 2014; Zarei et al. 2012; Zhao et al. 2011; Zulkifli 2009). The outcome of this study revealed that oysters are more contaminated with this pathogen than other samples. It could be observed from this study that more studies have carried out on oyster than other samples. Oysters are eaten either raw or undercooked. This practice tend to increase the prevalence of outbreak of *V. parahaemolyticus* in oysters especially in countries like United States, China and Japan. There are limitations in meta-analysis study. Only studies that are published in English are used in this study. There could be possibility that positive results involving incidence of *V. parahaemolyticus* from other seafood are reported. This correlates with the publication bias observed in the study which involve publication of study with significant results. Additionally, primary research studies involving clinical samples were not included in this study.

**Descriptive characteristics of eligible studies**

As seen in Table 1, the studies were conducted and published between 2003 and 2015 from the following 24 countries: Brazil (3 studies); India (6 studies); Iran (1 study); United Kingdom (1 study); China (5 studies); Thailand (4 studies); Vietnam (1 study); Malaysia (3 studies); Indonesia (3 studies); Italy (5 studies); Japan (1 study); Chile (1 study); Egypt (2 studies); United States (3 studies); Turkey (1 study); France (3 studies); Spain (1 study); Mexico (1 study); Korea (1 study); Sri Lanka (1 study); Nigeria (1 study); Tunisia (1 study); New Zealand (1 study) and Switzerland (1 study). *V. parahaemolyticus* was isolated from 2761 (47.5 %) of 5811 mussel, scallop and periwinkle (1670) in 15 studies, oyster (951) in 17 studies, clam and cockle (830) in 18 studies, shrimps, prawn and crab (1422) in 23 studies, fish, squid and cephalopod (998) in 20 studies of seafood investigated.

**Meta-analysis of prevalence of *V. parahaemolyticus* in mussel, scallop, and periwinkle**

Meta-analysis of incidence and prevalence of *V. parahaemolyticus* in mussel, scallop, and periwinkle was carried out using data of 1670 samples from 15 studies. The results of estimates of prevalence are summarised in Table 2. The pooled prevalence estimate of *V. parahaemolyticus* was found to be 28.0 % (95 % CI 0.255–0.307) as shown in Table 2. The studies included in this meta-analysis were found to be of significant heterogeneity (\(Q = 297.293, \text{df} = 14, p < 0.001\)) between 15 studies. Heterogeneity quantified by \(I^2\) index was observed as 95.291 % as shown in the forest plot in Fig. 2. Squares represent effect estimates of individual studies with their 95 % confidence intervals of prevalence with size of squares proportional to the weight assigned to the study in the meta-analysis (Fig. 3).

**Meta-analysis of prevalence of *V. parahaemolyticus* in shrimp, prawn and crab**

Meta-analysis of incidence and prevalence of *V. parahaemolyticus* in shrimp, prawn and crab was carried out using data of 1422 samples from 24 studies. The pooled prevalence estimate of *V. parahaemolyticus* was found to be 48.3 % (95 % CI 0.454–0.512). The primary studies included in this meta-analysis were found to be of significant heterogeneity (\(Q = 232.099, \text{df} = 22, p > 0.001\)) between 24 studies. Heterogeneity quantified by \(I^2\) index was observed as 90.521 % as shown in the forest plot in Fig. 4. Squares represent effect estimates of individual studies with their 95 % confidence intervals of prevalence
| Sn | Sr                        | Country   | Year | Species | Detection Method | N   | n   | P (%) |
|----|---------------------------|-----------|------|---------|------------------|-----|-----|-------|
| 1  | Sobrinho Pde et al. (2011)| Brazil    | 2011 | Oyster  | TCBS/PCR         | 74  | 74  | 100   |
| 2  | Sudha et al. (2012)       | India     | 2012 | Finfish | TCBS/PCR         | 182 | 82  | 45.1  |
| 3  | Zarei et al. (2012)       | Iran      | 2012 | Shrimps | TCBS/PCR         | 300 | 146 | 43.9  |
| 4  | Wagley et al. (2009)      | England   | 2009 | Crabs   | TCBS/PCR         | 22  | 22  | 100   |
| 5  | Zhao et al. (2011)        | China     | 2011 | Oyster  | TCBS/PCR         | 80  | 39  | 48.8  |
|    |                           |           |      | Clam    | TCBS/PCR         | 72  | 46  | 63.8  |
|    |                           |           |      | Scallop | TCBS/PCR         | 70  | 42  | 60.0  |
|    |                           |           |      | Mussel  | TCBS/PCR         | 76  | 45  | 59.2  |
| 6  | Nakaguchi (2013)          | Thailand  | 2013 | Cockle  | TCBS/PCR         | 109 | 76  | 69.4  |
|    |                           |           |      | Mussel  | TCBS/PCR         | 106 | 73  | 70.1  |
|    |                           |           |      | Oyster  | TCBS/PCR         | 32  | 27  | 84.4  |
|    |                           |           |      | Clam    | TCBS/PCR         | 86  | 52  | 60.0  |
| 7  | Di Pinto et al. (2008)    | Italy     | 2008 | Mussel  | TCBS/PCR         | 144 | 47  | 32.6  |
| 8  | Yamamoto et al. (2008)    | Thailand  | 2008 | Clams   | MPN/PCR          | 32  | 32  | 100   |
| 9  | Miwa et al. (2006)        | Japan     | 2006 | Fish    | MPN/PCR          | 30  | 11  | 36.7  |
|    |                           |           |      | Shrimp  | MPN/PCR          | 20  | 11  | 55.0  |
|    |                           |           |      | Cockle  | MPN/PCR          | 10  | 9   | 90    |
| 10 | Fuenzalida et al. (2006)  | Chile     | 2006 | Mussel  | TCBS/PCR         | 35  | 9   | 25.7  |
|    |                           |           |      | Clam    | TCBS/PCR         | 8   | 2   | 25    |
|    |                           |           |      | Oyster  | TCBS/PCR         | 5   | 1   | 20    |
| 11 | Anjay et al. (2014)       | India     | 2014 | Fish    | TCBS/PCR         | 182 | 140 | 76.9  |
|    |                           |           |      | Prawn   | TCBS/PCR         | 42  | 31  | 73.8  |
| 12 | Abd-Elghany and Sallam (2013) | Egypt | 2013 | Shrimp  | TCBS/PCR         | 40  | 9   | 22.5  |
|    |                           |           |      | Crab    | TCBS/PCR         | 40  | 8   | 20    |
|    |                           |           |      | Cockle  | TCBS/PCR         | 40  | 3   | 7.5   |
| 13 | Changchai and Saunjit (2014) | Thailand | 2014 | Raw oysters | MPN/PCR       | 240 | 219 | 91    |
| 14 | Ramos et al. (2014)       | Brazil    | 2014 | Oyster  | MPN/PCR          | 60  | 29  | 48.3  |
| 15 | Chakraborty and Surendran (2008) | India | 2008 | Finfish | TCBS/MPN        | 12  | 8   | 66.6  |
|    |                           |           |      | Shelffish | TCBS/MPN       | 25  | 21  | 84.0  |
|    |                           |           |      | Cephalopods | TCBS/MPN    | 5   | 4   | 80    |
| 16 | Bilung et al. (2005)      | Malaysia  | 2005 | Cockle  | MPN/PCR         | 100 | 62  | 62    |
| 17 | Rosec et al. (2012)       | France    | 2012 | Oyster  | TCBS/C/PCR      | 60  | 19  | 31.6  |
|    |                           |           |      | Clams/mussel | TCBS/C/PCR  | 9   | 1   | 11.1  |
| 18 | Terzi et al. (2009)       | Turkey    | 2009 | Fish    | TCBS/PCR         | 30  | 9   | 30    |
|    |                           |           |      | Mussel  | TCBS/PCR         | 60  | 35  | 58.3  |
| 19 | Suffredini et al. (2014)  | Italy     | 2014 | Mussel  | TCBS/PCR         | 75  | 31  | 41.3  |
|    |                           |           |      | Clams   | TCBS/PCR         | 51  | 22  | 43.1  |
| 20 | Sun et al. (2012)         | China     | 2012 | Oyster  | TCBS/LAMP        | 10  | 2   | 20    |
|    |                           |           |      | Clam    | TCBS/LAMP        | 16  | 2   | 12.5  |
| 21 | Parveen et al. (2008)     | US        | 2008 | Oyster  | TCBS/DCH/PCR     | 33  | 22  | 67    |
| 22 | Di Pinto et al. (2012)    | Italy     | 2012 | Mussel  | PCR/EUSA         | 195 | 26  | 13.3  |
### Table 1 continued

| Sn | Sr                     | Ls     | Yp     | Ts     | M         | N   | n  | P (%)  |
|----|------------------------|--------|--------|--------|-----------|-----|-----|--------|
| 23 | Rizvi and Bej (2010)   | Mexico | 2010   | Oyster | SYBR/PCR  | 24  | 14  | 58.3   |
| 24 | Blanco-Abad et al. (2009) | Spain  | 2009   | Mussel | TCBS/PCR  | 48  | 5   | 10.4   |
| 25 | Marlina et al. (2007)  | Indonesia | 2007 | Clam   | RAPD/PCR  | 35  | 13  | 37.1   |
| 26 | Luan et al. (2008)     | China  | 2008   | Shrimp | MPN/PCR   | 80  | 66  | 82.5   |
|    |                        |        |        |        | Crab      | 15  | 14  | 93.3   |
|    |                        |        |        |        | Clam      | 100 | 64  | 64     |
|    |                        |        |        |        | Fish      | 10  | 10  | 100    |
|    |                        |        |        |        | Scallop   | 20  | 11  | 55     |
| 27 | Lu et al. (2006)       | US     | 2006   | Oyster | RAPD/PCR  | 13  | 9   | 69     |
|    |                        |        |        |        | Mussel    | 22  | 7   | 32     |
|    |                        |        |        |        | Clam      | 48  | 13  | 27     |
| 28 | Robert-Pillot et al. (2014) | France | 2014   | Fish   | RT/PCR    | 27  | 5   | 18.5   |
|    |                        |        |        |        | Mussel/Scallop | 10  | 1   | 10     |
| 29 | Zulkifi (2009)         | Indonesia | 2009 | Cockle | C/PCR     | 50  | 25  | 50     |
| 30 | Nelapati and Krishnaiah (2010) | India  | 2010   | Fish   | TCBS/PCR  | 105 | 69  | 65.7   |
| 31 | Yano et al. (2014)     | Thailand | 2014  | Shrimp | MPN/PCR   | 16  | 6   | 37.5   |
| 32 | Duan and Su (2005a)    | US     | 2005   | Oyster | TCBS/PCR  | 74  | 31  | 41.9   |
| 33 | Copin et al. (2012)    | France | 2012   | Shrimp | MPN/PCR   | 36  | 28  | 77.8   |
| 34 | Yang et al. (2008a)    | China  | 2008   | Fish   | RAPD/PCR  | 197 | 58  | 29.7   |
|    |                        |        |        |        | Crab      | 49  | 22  | 44.9   |
|    |                        |        |        |        | Shrimp    | 71  | 28  | 39.4   |
| 35 | Ottaviani et al. (2005) | Italy  | 2005   | Mussel | TCBS/PCR  | 144 | 35  | 24.3   |
| 36 | Sobrinho et al. (2010) | Brazil | 2010   | Oyster | MPN/PCR   | 123 | 122 | 99.2   |
| 37 | Xu et al. (2014)       | China  | 2014   | Shrimp | TCBS/PCR  | 273 | 103 | 37.7   |
| 38 | Lee et al. (2008)      | Korea  | 2008   | Oyster | TCBS/PCR  | 72  | 48  | 66.7   |
| 39 | Amin and Salem (2012)  | Egypt  | 2012   | Shrimp | TCBS/PCR  | 20  | 4   | 20     |
|    |                        |        |        |        | Crab      | 20  | 6   | 30     |
| 40 | Koralage et al. (2012) | Sri Lanka | 2012 | Shrimp | TCBS/PCR  | 170 | 155 | 91.2   |
| 41 | Schärer et al. (2011)  | Switzerland | 2011 | Squid  | TCBS/PCR  | 2   | 2   | 100    |
| 42 | Paydar et al. (2013)   | Malaysia | 2013  | Fish   | TCBS/mPCR | 27  | 21  | 77.8   |
|    |                        |        |        |        | Squid     | 7   | 4   | 57.1   |
|    |                        |        |        |        | Cockle    | 5   | 3   | 60     |
|    |                        |        |        |        | Shrimp    | 11  | 9   | 81.8   |
|    |                        |        |        |        | Clam      | 3   | 2   | 66.7   |
|    |                        |        |        |        | Prawn     | 7   | 5   | 71.4   |
|    |                        |        |        |        | Oyster    | 9   | 6   | 66.7   |
| 43 | Dileep et al. (2003)   | India  | 2003   | Finnfish | TCBS/PCR  | 18  | 4   | 22.2   |
|    |                        |        |        |        | Shrimp    | 10  | 3   | 30     |
| 44 | Eja et al. (2008)      | Nigeria | 2008  | Shrimp | TCBS/Biotyping | 120 | 26  | 21.7   |
|    |                        |        |        |        | Clam      | 90  | 7   | 7.7    |
|    |                        |        |        |        | Periwinkle | 98  | 9   | 9.2    |
| 45 | Khouadja et al. (2013) | Tunisia | 2013  | Oyster | TCBS/PCR  | 20  | 2   | 10.0   |
|    |                        |        |        |        | Mussel    | 20  | 1   | 5.0    |
| 46 | Kirs et al. (2011)     | New Zealand | 2011 | Oyster | TCBS/RT/PCR | 58  | 55  | 94.8   |
| 47 | Normanno et al. (2006) | Italy  | 2006   | Mussel | TCBS/API  | 600 | 47  | 7.83   |
| 48 | Pal and Das (2010)     | India  | 2010   | Fish   | TCBS/PCR  | 90  | 60  | 66.7   |

i, shucked oyster; tb, Tillamook Bay; yb, Yaquina Bay; s, Selangor; pj, Padang and Jakarta; m, use of any molecular method like specie specific genes etc; k, mpn chrom agar; a, coastal province Jiangsu; China b, eastern coast of China. Sn = study number; Sr = study reference; Ls = location of study; Yp = year of publication; Ts = type of seafood; M = microbiological method(s); N = total sample; n = number of positive samples
Meta-analysis of prevalence of \( V. \) \textit{parahaemolyticus} in fish, squid and cephalopod

Meta-analysis of incidence and prevalence of \( V. \) \textit{parahaemolyticus} in fish, squid and cephalopod was carried out using data of 998 samples from 20 studies. The pooled prevalence estimate of \( V. \) \textit{parahaemolyticus} was found to be 51.0 % (95 % CI 0.476–0.544). The studies included in this meta-analysis were found to be significant heterogeneity (\( Q = 159.368, \text{df} = 19, p > 0.001 \)) between 20 studies. Heterogeneity quantified by \( I^2 \) index was observed as 88.078 % as shown in the forest plot in Fig. 6. Squares represent effect estimates of individual studies with their 95 % confidence intervals of prevalence with size of squares proportional to the weight assigned to the study in the meta-analysis.
with size of squares proportional to the weight assigned to the study in the meta-analysis (Fig. 7).

**Meta-analysis of prevalence of V. parahaemolyticus in clam and cockle**

Meta-analysis of incidence and prevalence of *V. parahaemolyticus* in clam and cockle was carried out using data of 830 samples from 18 studies. The pooled prevalence estimate of *V. parahaemolyticus* was found to be 52.9 % (95 % CI 0.490–0.568). The studies included in this meta-analysis were found to be significant heterogeneity (Q = 132.490, df = 17, p > 0.001) between 18 studies. Heterogeneity quantified by $I^2$ index was observed as 87.169 % as shown in the forest plot in Fig. 8. Squares represent
Effect estimates of individual studies with their 95% confidence intervals of prevalence with size of squares proportional to the weight assigned to the study in the meta-analysis (Fig. 9).

Meta-analysis of prevalence of *V. parahaemolyticus* in oyster

Meta-analysis of incidence and prevalence of *V. parahaemolyticus* in oyster was carried out using data of 951 samples from 17 studies. The pooled prevalence estimate of *V. parahaemolyticus* was found to be 63.40% (95% CI 0.592–0.674). The studies included in this meta-analysis were found to be significant heterogeneity ($Q = 178.260, df = 16, p < 0.001$) between 17 studies. Heterogeneity quantified by $I^2$ index was observed as
91.024 % as shown in the forest plot in Fig. 10. Squares represent effect estimates of individual studies with their 95 % confidence intervals of prevalence with size of squares proportional to the weight assigned to the study in the meta-analysis (Fig. 11).

Publication bias among the primary studies
Both publication bias and quality of primary studies are limiting factors in any meta-analytical study (Noble Jr. 2006). In meta-analysis, publication bias is usually graphically assessed using funnel plot (Soon et al. 2012; Gonzales-Barron and Butler 2011). This was obtained by plotting of study size (usually standard error or precision) on the vertical
axis as a function of effect size on the horizontal axis. In this current study, publication bias could be observed among the primary studies due to asymmetric nature of the plots. Solid vertical line in the funnel plots represents the summary of prevalence rate derived from fixed-effect meta-analysis while the diagonal lines represent 95% confidence interval. Studies with large samples appeared toward the top of the graph, and tend to cluster near the mean effect size while studies with smaller samples appeared toward the bottom of the graph. It should be noted that sampling variation in effect size estimates in the studies with smaller seafood samples affects the plots.
Conclusion

In conclusion, higher prevalence rate of *V. parahaemolyticus* was observed in oysters than other seafood investigated. The occurrence and prevalence of *V. parahaemolyticus* is of public health importance, hence, more studies involving seafood such as mussels need to be investigated. Additionally, the study is a trial to develop a new data analysis tool. There is need to investigate prevalence of this pathogen in other seafood and also intervention strategies to reduce *V. parahaemolyticus* in seafood.

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Competing interests

The author declares no competing interest.

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