Bacterial diversity and potential risk factors associated with Salmonella contamination of seafood products sold in retail markets in Bangkok, Thailand

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Abstract

The objectives of this study are to determine the levels of indicator bacteria, *Salmonella* and *Vibrio* in these seafood commodities and to identify risk factors associated with *Salmonella* contamination of these samples. A total of 335 samples were collected comprised of Pacific white shrimp (n = 85), oysters (n = 82), blood cockles (n = 84), and Asian seabass (n = 84) during October 2018 to July 2019 in Bangkok Thailand. The prevalence and concentrations (sd) of fecal coliforms were 100% and ~~8.709~~2×10^4 (4~~.09~~×10^4) MPN/g; regarding *E. coli*, the prevalence was 85% and concentration (sd) was ~~1.85~~2×10^4 (~~3.684~~×10^4) MPN/g. The overall prevalence of *V. parahaemolyticus* (59%), *V. cholerae* (49%), *V. alginolyticus* (19%), *V. vulnificus* (18%), and *Salmonella* (36%) were reported. ~~The h~~Highest concentrations of fecal coliforms and *E. coli* were found in oysters, and *Salmonella* was reported in shrimp. Matopeni (31%) was a predominant serotype. The association between the presence of *Salmonella* and type of sample, sampling location, selling condition, and the presence of *E. coli*, *V. alginolyticus* and *V. vulnificus* in the samples was observed under logistic regression ($P < 0.05$). The optimal cutoff value of concentration of *E. coli* to predict the contamination of *Salmonella* was 1.33×10^4 MPN/g with sensitivity 84~~.43~~ % and specificity 61~~.03~~ %. Display products on ice, presence of *E. coli* and *Vibrio*, seafood derived from Eastern Thailand were associated with an increased risk of *Salmonella* contamination. Continuous monitoring of pathogenic bacteria through food chain under the "One Health" concept is needed to enhance seafood safety.

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Introduction

The total fisheries and aquaculture both inland and marine ~~are increasing with an average~~ reached 171~~0.9~~ million tonnes in 2016~~, and the major exporters are China, Norway, Viet Nam, and Thailand~~ (*Food and Agriculture Organization of the United Nations, 2018*). ~~The~~ ~~c~~Consumption ~~rate~~ of fish, and fishery products, ~~per capita per year continuingly increases. Over the past decades, the consumption of seafood products~~ per capita ~~per year has increasingly than~~ double from ~~nearly~~ 10 kg in 1960 to greater than 20 kg in 2016 (*Food and Agriculture Organization of the United Nations, 2018*). ~~The consumption of fish and fishery products has been demanded in many countries due to essential nutritional component of protein source.~~ In Southeast Asia, the consumption of fish and fishery products varies from 6~~.1~~ to 64~~3.5~~ kg per capita per year ~~depending on accessibility to the products, geographical location, and the environment~~ (*Food and Agriculture Organization of the United Nations, 2015*). In Thailand, the consumption of fish and fishery products ~~was approximately~~ is 31~~.4~~ kg per capita per year, which was accounted for 12~~1.7~~% of total protein consumption ~~in the country~~ (*Food and Agriculture Organization of the United Nations, 2015*).

Due to the rapid growth of global consumption of fish and fishery products, seafood safety ~~and seafood-associated diseases~~ are ~~increasingly~~ concerning. Foodborne diseases each year afflict ~~One~~ a third of the world population ~~is affected by foodborne diseases~~ each year (*World Health Organization, 2004*). Most of seafood outbreaks have been examined in ~~In~~ the United States, where approximately 9.4 million illnesses, almost 56,000 hospitalizations, and 1,351 deaths, ~~were affected by~~are associated with foodborne contamination per year (*Scallan et al., 2011*). Almost half (45%) of foodborne outbreaks reported in the U.S. ~~were~~ are from pathogenic bacteria, and fish are ~~is considered as one of the seafood commodities~~frequently implicated with ~~the~~ bacterial contamination (*Gould et al., 2013*). ~~Most of seafood outbreaks have mainly examined in the U.S. than Europe, Australia, and Asia. Therefore,~~ limited data of the number of illnesses from seafood-borne outbreaks has observed in many parts of the world.

~~Seafood is considered as an important vector in human pathogens. The increase of environmental~~ Pollution, animal ~~densities~~density, and ~~world~~ global trading ~~are considered as important contributing factors leading a concern of~~ contribute to pathogen contamination of seafood products (*Papadopoulou et al., 2007*). ~~According to the epidemiology of seafood infection in the U.S., t~~The most common bacterial pathogens associated with seafood-borne diseases are *Vibrio*, *Salmonella*, *Shigella*, and *Clostridium botulinum* (*Iwamoto et al., 2010*). Seafood-borne outbreaks caused by *V. parahaemolyticus*, *V. cholera* serogroup O139, *V. vulnificus*, *Salmonella* serotype Weltevreden, and *Escherichia coli* have been reported (Bonnin-Jusserand et al., 2019; Heinitz et al., 2020; Martinez-Urtaza et al., 2016; Raymond & Ramachandran, 2019).

In Thailand, *V. parahaemolyticus* and *Salmonella* sp. are the leading causes ~~pathogenic bacteria causing human~~ of foodborne diarrhea. ~~Taken together, most of studies relevant to seafood-borne outbreaks and bacterial contamination in fish and fishery products varies by~~

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~~specific pathogens and geographical location. Hence, sample at selling points that close to human consumption is required to reduce the seafood-borne contamination.~~

Even though Thailand is one of the major exporters, monitoring and surveillance of distribution of bacterial pathogens in seafood products ~~that potentially risk to human health~~ is still limited. Therefore, the objectives of this study are to 1) evaluate the levels of fecal coliforms and *E. coli* contaminated in Pacific white shrimp, oyster, blood cockle, and Asian seabass samples sold in fresh markets in Bangkok, Thailand; 2) examine the prevalence of *V. parahaemolyticus*, *V. cholerae*, *V. vulnificus*, *V. alginolyticus*, and *Salmonella*; 3) identify serotypes of *Salmonella* among various seafood samples; and 4) determine risk factors for *Salmonella* contamination and a potential cutoff value of the levels of *E. coli* affecting the presence of *Salmonella* in the samples.

Materials & Methods

Sample collection

A total of fresh fish and fishery samples (n = 335) was collected from Pacific white shrimp (*Litopenaeus vannamei*) (n = 85), oyster (*Saccostrea cucullata*) (n = 82), blood cockle (*Tegillarca granosa*) (n = 84), and Asian seabass (*Lates calcarifer*) (n = 84) between October 2018 and July 2019. ~~The s~~Samples were ~~sold~~ collected in open-air fresh markets in ~~Bangkok, Thailand. The sampling location~~ select from four districts ~~was selected based on a high human population density~~ in Bangkok. These districts vary in population ~~, which were Din Daeng, Huay Kwang, Samphanthawong, and Dusit~~density (Table 1).

Individual seafood samples ~~was~~ were purchased in the early morning (5 to 7 a.m.) and comprised at least 200 g where were placed into a double sterile plastic bag. The samples were kept on ice during transportation and submitted to the laboratory within 3 h. Microbiological determination was performed within 6 h after receiving samples in the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University.

The samples were analyzed for coliforms, *E. coli*, *Salmonella*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* and *V. cholerae*. Average and standard deviation (sd) of minimum and maximum ambient air temperature (°C), wind speed (km/h), precipitation (mm), and relative humidity (%) in Bangkok, Thailand, were retrieved from the Thai Meteorological Department (www.tmd.go.th)

Predictor variables

~~Putative r~~Risk factors for *Salmonella* contamination tested included type of seafood (Pacific white shrimp, oyster, blood cockle, or Asian seabass), sampling district (Din Daeng, Huay Kwang, Samphanthawong, or Dusit), regional source of seafood (central, eastern, southern Thailand, or unidentified source), retail storage of fish and shellfish samples (pooling and combining different seafood products for retail display versus keeping each seafood type separate when on display), and retail display condition (on ice or without ice). The concentrations of fecal coliform (MPN/g) and *E. coli* (MPN/g), and the prevalence of *V.*

parahaemolyticus, *V. vulnificus*, *V. alginolyticus* and *V. cholerae* were evaluated as putative risk factors for *Salmonella* contamination.

Determination of fecal coliform and *E. coli* concentrations

The concentrations of fecal coliform and *E. coli* were enumerated according to the U.S. Food and Drug Administration (U.S. FDA) Bacteriological Analytical Manual (BAM) with slight modification (*Feng et al., 2002*). Briefly, a 25 g of individual sample (shrimp and Asian seabass) was weighed, aseptically cut into small pieces, and placed into 225 mL of Buffered Peptone Water (BPW) (Difco, MD, USA). Then, the samples were homogenized for 1 to 2 min. A 10-fold serial dilution was performed using three-tube most probable number (MPN) at different dilutions from 10^{-1} to 10^{-4}. One mL of each solution was diluted in Lactose Broth (LB) (Difco), and then incubated at 35 °C for 24 h. A loopful of the mixture solution was transferred to Brilliant Green Lactose Bile (BGLB) (Difco) and EC broth (Difco), respectively. After overnight incubation, positive tubes were recorded and calculated as concentration of fecal coliforms (MPN/g). One loopful of EC broth was streaked on Eosin Methylene Blue (EMB; Difco) agar plates and reported as *E. coli* concentration (MPN/g).

For enumeration of indicator bacteria in oysters and blood cockle, a 100 g of oyster meat sample was weighed and added into 100 mL of Phosphate Buffered Saline (PBS) (Difco), which was then blended aseptically for 1 to 2 min. The oyster suspension was diluted in LB to different concentrations of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. The diluted suspensions were transferred to BGLB and EC broth. Biochemical tests including indole test and Triple Sugar Iron (TSI; Difco) were performed on suspect colonies for all samples. The lower and upper limits of the detection of fecal coliforms and *E. coli* were 1.0 and 1.11×10^{5} MPN/g, respectively.

Isolation and confirmation of *Salmonella*

The detection of *Salmonella* followed ISO 6579-1:2017 (*International Organization for Standardization, 2017*). Briefly, a total of 25 g of each seafood sample was weighed, cut, and placed into 225 mL of BPW. The sample was then homogenized for 2 min and incubated at 37 °C for 18 h. After incubation, 0.1 mL of the suspension was inoculated into Modified Semi-solid Rappaport-Vassiliadis (MSRV) (Difco) agar plate and incubated at 42 °C overnight. A loopful of incubated sample was streaked onto Xylose Lysine Deoxycholate (XLD) (Difco) agar. Presumptive colonies of *Salmonella* were pink to red colonies with black center. Biochemical tests (citrate utilization, TSI reaction, indole test) were used to confirm presumptive *Salmonella* colonies according to a standard protocol from the U.S. FDA BAM (*Andrews et al., 2007*).

Three typical colonies of *Salmonella* were selected for serotyping. Slide agglutination test was performed to determine serotype of *Salmonella* followed by Kauffmann-White Scheme, Pasture Institute (*Grimont and Weill, 2007*) using commercially available antiserum (S&A Reagents Lab Ltd., Lat Phrao, Bangkok, Thailand).

Isolation and confirmation of *Vibrio* spp.

The isolation of *Vibrio* was followed the U.S. FDA BAM (*Kaysner et al., 2004*). Briefly, 50 g of each sample was added to 450 mL of PBS, and homogenized for 1 to 2 min. One mL of resulting solution was added to 10 mL of Alkaline Peptone Water (APW) (Difco) and incubated at 37 °C

overnight. After incubation, one loopful of solution was streaked on Thiosulfate-Citrate-Bile Salts-sucrose (TCBS) (Difco) agar plate containing 2% of NaCl.

The presumptive colonies of *Vibrio* were confirmed using CHROMagar™ *Vibrio* (HiMedia Laboratories, Mumbai, India) agar. The TCBS and CHROMagar™ *Vibrio* plates were incubated at 37 °C for 24 h. Morphology of *V. parahaemolyticus* and *V. vulnificus* was colorless colonies with green center in TCBS agar. In CHROMagar™ *Vibrio* agar plate, colonies of *V. parahaemolyticus* was mauve, and of *V. vulnificus* was green blue to turquoise blue, whereas *V. alginolyticus* showed colorless.

For isolation of *V. cholerae*, 25 g of seafood sample was added to 225 mL of APW, homogenized for 1 to 2 min, and incubated at 35±2 °C for 8 h. A loopful of solution was streaked to TCBS agar plates. After incubation at 37 °C for 24 h, presumptive colonies of *V. cholerae* were confirmed on CHROMagar™ *Vibrio*. Typical colonies of *V. cholerae* on TCBS agar plate are 2 to 3 mm diameter, yellow, and flat colonies with opaque center, whereas the presumptive colonies of *V. cholerae* in CHROMagar™ *Vibrio* agar were green blue to turquoise blue. Biochemical tests including TSI, oxidase test, and growth in sodium chloride were conducted to confirmation.

Statistical analyses

~~The concentrations and sd of fecal coliforms (MPN/g) and *E. coli* (MPN/g) and prevalence of *Salmonella* and *Vibrio* in the samples were calculated.~~ Logistic regression was used to determine the association between *Salmonella* contamination and ~~various~~ risk factors~~, including type of seafood (Pacific white shrimp, oyster, blood cockle, or Asian seabass), sampling district (Din Daeng, Huay Kwang, Samphanthawong, or Dusit), regional source of seafood (central, eastern, southern Thailand, or unidentified source), retail storage of seafood (pooling and combining different seafood products for retail display versus keeping each seafood type separate when on display), and retail display condition (on ice or without ice), presence of *Vibrio* spp., and the concentrations of fecal coliforms and *E. coli*.~~ Univariate associations were first evaluated for all risk factors for *Salmonella* and an initial multivariable model constructed from only significant univariate risk factors ($P \leq 0.2$); a backward stepping algorithm was then used to eliminate non-significant ($P > 0.05$) risk factors based on a likelihood ratio test resulting in a final multivariable logistic regression model with only significant ($P \leq 0.05$) risk factors.

~~The r~~Receiver operating characteristic (ROC) analysis was performed to predict contamination of *Salmonella* using estimation of the concentration of *E. coli*. Based on ROC analysis, the optimal cutoff value was determined. All statistical analyses were performed using Stata version 14.0 (StataCorp, College Station, TX, USA). A *P*-value < 0.05 was considered as statistically difference under the two-sided hypothesis test.

Results

Distribution of fish and fishery products

~~Pacific white shrimp ($n = 85$), oyster ($n = 82$), blood cockle ($n = 84$), and Asian seabass ($n = 84$) were collected from four districts, which were composed of Din Daeng, Huay Kwang,~~

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~~Samphanthawong, and Dusit. Distribution of type of seafood, sampling district, regional source of seafood, retail storage and retail display condition are presented in Table 1. The regional sources of seafood samples were from various geographical regions, mainly Central (*n* = 257), Eastern (*n* = 28), and Southern (*n* = 25) Thailand, with the Central region further subdivided as Samut Sakhon (47.9%) and Samut Prakan (32.7%) provinces. The common regional sources of Pacific white shrimp, blood cockle, and Asian seabass were from Samut Sakhon, Samut Prakan, and Bangkok, whereas the main sources of oysters were from Samut Sakhon and Chon Buri provinces.~~

~~In fresh markets, most of the samples was stored in a separate container (75.5%, *n* = 253), while almost a quarter of the samples (24.5%, *n* = 82) were kept in the same container with other seafood products. All oyster and blood cockle samples were stored separately from other seafood products, whereas Asian seabass were kept as pooled samples 92.86% (*n* = 78/84). The majority of the samples (62.7%, *n* = 210) were displayed on ice.~~

~~The meteorological data was recorded from October 2018 to July 2019 according to the Thai Meteorological Department. The average (± sd) daily minimum and maximum ambient air temperature was 26.80 (± 1.75) °C and 34.08 (± 2.64) °C; average (± sd) wind speed was 12.95 (± 2.04) km/h, average 24-hour precipitation 1.39 (± 3.96) mm, and average relative humidity was 75.08 % (± 7.40).~~

Occurrence of indicator bacteria in seafood samples

~~The prevalence and concentrations of fecal coliforms and *E. coli* are shown in Table 2.~~ In general, seafood products were 100% positive for fecal coliforms with an average concentration (± sd) at 8.70×10^4 (± 4.09×10^4) MPN/g. The prevalence of *E. coli* was 85.1%, with an average concentration (± sd) of 1.85×10^4 (± 3.68×10^4) MPN/g. Oyster samples had the highest concentrations (± sd) of fecal coliforms at 1.10×10^5 (± 7.13×10^3) and *E. coli* at 5.13×10^4 (± 4.49×10^3), while blood cockle and seabass had the lowest concentrations of these indicator bacteria (Table 2).

Occurrence of *Vibrio* and *Salmonella* in seafood samples

~~The prevalence of *Vibrio* and *Salmonella* are shown in Figure 1.~~ Overall, the highest prevalence of *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. cholerae*, and *Salmonella* were observed in blood cockle (93~~2.9~~%), Pacific white shrimp (39~~8.8~~%), oyster (35~~.4~~%), Asian seabass (91~~0.5~~%), and Pacific white shrimp (47~~.1~~%), respectively. The lowest prevalence (< 10%) of *V. vulnificus* was observed in blood cockle and for *V. alginolyticus* in Asian seabass.

~~Regarding highest bacterial species in each seafood product, the~~ Pacific white shrimp exhibited a high prevalence of *V. parahaemolyticus* (59~~8.8~~%), *V. cholerae* (53~~2.9~~%) and *Salmonella* (47~~.1~~%), whereas oysters were mainly contaminated with *V. parahaemolyticus* (45~~.1~~%) and *Salmonella* (38~~7.8~~%). In blood cockles, a high prevalence of *V. parahaemolyticus* (93~~2.9~~%) were observed, although they had low prevalence of *V. cholerae*, *V. vulnificus*, and *V. alginolyticus*. Asian seabass was frequently contaminated with *V. cholerae* (90.5%) and *Salmonella* (46.4%).

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Matopeni (30.6%), Corvallis (4.7%), Give (4.7%), and Rissen (4.7%) were the most common serotypes of *Salmonella* isolated from seafood products (Table 3). Matopeni was the predominant serotype (52/56) observed from Asian seabass samples (n = 56 isolates), whereas Itami and Leith were common serovars isolated from the shrimp samples (n = 47 isolates). For oysters (n = 43 isolates) and blood cockles (n = 24 isolates), the major serotypes were Give (18.6%) and Rissen (33.3%), respectively.

The distribution of *Salmonella*, *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*, and *V. alginolyticus* among seafood products

~~The unilateral associations between *Salmonella* or *Vibrio* for the different seafood commodities are shown in Table 4.~~ Odds of *V. vulnificus* contamination in shrimp was 7.0 (1/0.143) times higher than that for blood cockle (P = 0.002). Odds of *V. cholerae* contamination in shrimp were 7.5 (1/0.134) and 23.6 (1/0.043) times higher than for oyster (P < 0.0001) and blood cockle (P = 0.002), respectively. The presence of *V. parahaemolyticus* in the blood cockle was higher than in shrimp (OR = 9.10, P < 0.0001). The odds of *V. alginolyticus* contamination in shrimp was 13.33 (1/0.075) and 20.0 (1/0.050) times higher than in blood cockles and seabass, respectively.

Risk factors associated with *Salmonella* contamination

Salmonella contamination of seafood sold in Bangkok was associated with type of seafood, sampling district, retail display condition, regional source of seafood products, and the presence of *E. coli*, *V. alginolyticus*, and *V. vulnificus* (Table 5). The odds of *Salmonella* contamination was not different for Asian seabass compared to Pacific white shrimp; in contrast, both oysters and blood cockles had significantly lower odds of *Salmonella* compared to shrimp. Seafood from markets in Huay Kwang (OR = 1.7) and Dusit (OR = 1.4) had a higher odds of *Salmonella* contamination compared to seafood from Din Daeng and Samphanthawong. Seafood displayed on ice (OR = 1.71, P < 0.0001) had a higher odds of *Salmonella* contamination compared to retail seafood products not displayed on ice. Seafood products sourced from Eastern Thailand had significantly higher odds of *Salmonella* contamination compared to seafood sourced from other regions (OR = 3.46, P < 0.0001). Lastly, the odds of *Salmonella* contamination were positively associated with the presence of *E. coli* and *V. alginolyticus*, but negatively associated with *V. vulnificus*.

ROC and area under the ROC curve

The area under the ROC curve (AUC) at 63.77% with standard error = 0.30 (C.I. = 57.8% - 69.7%) (Figure 2). The ROC AUC was statistically significance (P < 0.0001) compared to the null value of AUC = 0.5. The presence of *Salmonella* in seafood products was optimally predicted at a concentration of 1.33×10^4 *E. coli* MPN/g, with a sensitivity of 84.43% and specificity of 61.03% for this value.

Discussion

According to the Bureau of Quality and Safety of Food (BQSF), Department of Medical Science, Ministry of Public Health, Thailand, the concentration of *E. coli* should not excess 10 MPN/g of fresh or frozen seafood and less than 3 MPN/g of seafood consumed raw; in addition, all

products must not contain *Salmonella*, *V. cholerae*, *V. parahaemolyticus* in 25 g of sample (*Bureau of Quality and Safety of Food, 2020*). In this study, the average concentration of *E. coli* was 1.85×10^4 MPN/g for all the seafood samples, which is considerably higher than the standard BQSF guidelines in Thailand. In fact, only 17.9% (n = 60/335) of all seafood samples had concentrations of *E. coli* < 10 MPN/g and only 7.3% (n = 6/82) of oyster samples (often eaten raw) had < 3 MPN/g. Furthermore, the prevalence of *Salmonella* (36.4%), *V. cholerae* (49.0%), *V. parahaemolyticus* (58.8%) indicated widespread bacterial contamination of these seafood products, which also violates the Thailand standard requirements. Therefore, implementation of basic sanitation and evaluation of microbiological contamination of seafood products sold in Bangkok is needed to strengthen seafood safety in this area.

Salmonella is an important pathogen that is responsible for seafood-borne illness worldwide (*Barrett et al., 2017; European Food Safety Authority, 2014*). However, *Salmonella* is not a normal flora in finfish and shellfish products. The major sources of *Salmonella* contamination in seafood may originate from multitude of sources, including the natural aquatic environment, during aquaculture or seafood processing, insufficient hygiene practices during transport and storage, and improper food handling (*Amagliani et al., 2012; Fernandes et al., 2018*). In this study, the prevalence of *Salmonella* ranged from 14.3% to 47.1%. This finding was similar to a study of cultured shrimp in the Mekong Delta, Vietnam, which documented a prevalence of 24.5% *Salmonella* contamination (*Phan et al., 2005*), but substantially less to the 90 to 100% prevalence of *Salmonella* contamination in fish (93.1%) and shrimp (100.0%) collected from the Surabaya local market in Indonesia (*Pramono et al., 2019*).

Type of seafood, sampling retail location, use of ice during retail display, regional source of seafood, and presence of *E. coli* and *Vibrio* were all significantly associated with the presence of *Salmonella* (Table 5). The presence of *E. coli* in a seafood sample was associated with a 4-fold increase in the odds of *Salmonella* contamination (OR = 4.0, P < 0.0001); similarly, the presence of *V. alginolyticus* in a seafood sample was associated with a 1.4-fold increase in the odds of *Salmonella* contamination (OR = 1.4, P < 0.04). Seafood displayed on ice during retail had almost twice the odds *Salmonella* contamination (OR = 1.71, P < 0.0001) than seafood not displayed on ice. This curious finding may seem counterintuitive, but prior work has shown that the ice used to chill seafood can be contaminated with pathogenic microorganisms and become a risk of human infection (*Falcão et al., 2009*). Ice can be a vehicle for various pathogenic organisms, including diarrheagenic *E. coli*, *Aeromonas*, *S. enteritidis* and fecal coliforms (*Falcão et al., 2002*; *Falcão et al., 2004*; *Kirov, 1993*). In this study, most of the ice used to store seafood was at risk of rapidly melting due to high ambient temperatures in open air conditions. The melting ice can ~~be a mode of pathogenic bacteria dissemination that functions to~~ spread bacteria from one seafood item to nearby retail items, readily contaminating other seafood left standing in contaminated melt water. In addition, the physical placement of seafood for display in retail markets can ~~function to~~ spread bacterial contamination between seafood items if seafood handlers do not practice proper sanitation during handling (i.e., bare hands touching multiple seafood items; not replacing latex or plastic gloves at high enough frequency during retail

display placement of seafood items). Therefore, maintaining sanitary conditions during the production, storage, and use of ice to prevent microbial contaminates should be closely observed. Lastly, seafood that was sourced from Eastern Thailand had a 3.5-higher odds of *Salmonella* contamination compared to seafood from other regions (OR = 3.46, $P < 0.0001$). The coastal area of Eastern Thailand has concentrated areas of industrialization, agricultural development, and tourism-related urbanization, with major concerns of increased water pollution and resource depletion (*Nitivattananon and Srinonil, 2019*). Wastewater quality is a major concern for this area, especially in Chonburi and Rayong Provinces due to several industrial estates. Moreover, Chonburi, Chachoengsao, and Rayong Provinces have been designated for developing the Eastern Economic Corridor (EEC), so reduction of waste and wastewater is of increasing concern.

In this study, the diversity of *Salmonella* serovars varied between the different seafood products. Pacific white shrimp had the greatest diversity, 21 different serovars with prevalence per serovar ranging from 1-3%. Eleven serovars from isolated from oysters, with prevalence per serovar ranging from 1-5% similar to Pacific white shrimp. In contrast, only 5 serovars were isolated from blood cockles, with a similar range of prevalence per serovar of 2-5%. Least diverse were isolates from Asian seabass, where only two serovars were recovered with 92.9% (52/56) of these *Salmonella* isolates being Matopeni and the remainder being Paratyphi B. Serotype Matopeni has been reported in aquatic pet shops (*Gaulin et al., 2005*) and in food supplements from Germany (*European Commission, 2018*). Outbreak strains of *Salmonella* Paratyphi B have been associated with raw tuna sushi imported from Indonesia in 2015, and this outbreak caused 65 foodborne cases from 11 states in the U.S. (*Centers for Disease Control and Prevention, 2018*). *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi, and *S.* Paratyphi B were also detected in fresh fish in Iran (*Rahimi et al., 2013*).

The common serovars in Pacific white shrimp were Itami (10.6%, $n = 5/47$) and Leith (8.5%, $n = 4/47$). Itami was first documented as a new serovar from a traveler to Thailand suffering from gastroenteritis (*Sakazaki et al., 1981*). Itami has also been reported from infected humans in Taiwan (*Kuo et al., 2014*). In contrast to the serovars isolated during this study, serovars *S.* Weltevreden, *S.* Tennessee, and *S.* Dessau were isolated from shrimp from the Mekong Delta, Vietnam (*Phan et al., 2005*). The most common *S. enterica* serovar isolated from oysters was Give (18.6%, $n = 8/43$ isolates), which is different from oysters in the U.S. where Newport was the most common serotype (*Brands et al., 2005*). A previous study in Western Thailand found that the most common serovar in cultured oysters (*C. lugubris* and *C. belcheri*) from Phang Nga Province was Paratyphi B (*Jeamsripong et al., 2018*). This suggests that the distribution of *Salmonella* serovars within Thailand depends on geographical location and type of seafood. Serovar Give is an enteric serotype usually isolated from swine and ruminant, but rarely found in humans (*Higgins et al., 1997*). It is possible that the contamination of Give may be result of livestock or agricultural production near the oyster growing site. *S. enterica* ser. Give has been frequently reported in European national laboratories (*Jansen et al., 2005*). The higher virulence of the Give serovar compared to other non-typhoidal *Salmonella* may explain the

higher hospital rate associated with human Give infections (*Girardin et al., 2006*). Therefore, surveillance and monitoring of oysters due to this ~20% prevalence of *Salmonella* contamination, and fully cooking oysters prior to consumption are needed to reduce the risk of food-borne *Salmonella* infection from Thai cultured oysters.

In this study, the most common *Salmonella* serovar found in blood cockles was Rissen (33.3%, n = 8/24 isolates), similar to a study in India (*Kumar et al., 2009*), but it should be noted that none of the five different serovars isolated from cockles had a prevalence above 5%. Seafood such as cockles can acquire *Salmonella* from contaminated water or other environmental matrices during aquaculture, processing, shipping and retail display. Good hygiene and basic sanitation together with proper seafood handling and storage should be performed throughout the food chain (farm to fork) to reduce the risk of seafood-related *Salmonella*.

Prevalence of *V. parahaemolyticus* (58.8%), *V. cholerae* (49.0%), *V. alginolyticus* (18.5%), and *V. vulnificus* (17.9%) were observed in this study. According to BQSF, Thailand, seafood for human consumption should have no detectable *V. parahaemolyticus* and *V. cholerae* in 25 g of sample; however, 50-60% of samples contained these bacterial adulterants. This high prevalence is consistent with previous work demonstrating that between 2003 and 2015 the prevalence of *V. parahaemolyticus* was 63.4% in oysters, followed by clams (52.9%), fish (51.0%), and shrimp (48.3%) (*Odeyemi, 2016*). *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus* are considered important seafood-borne pathogens that cause gastroenteritis in humans due to consumption of raw and partly cooked seafood, while *V. alginolyticus* cause ear infection and intestinal disease in humans. In this study, the main source of *V. parahaemolyticus* was blood cockles (OR = 9.1, $P < 0.05$), while *V. cholerae* was commonly found in Asian seabass (OR = 4.0, $P > 0.05$). *V. parahaemolyticus* and *V. vulnificus* have been reported in bivalves in many countries such as Thailand, China, and Korea (*Changchai & Saunjit, 2014*; *Jiang et al., 2019*; *Ryu et al., 2019*). In this study, shrimp and oysters were predominantly contaminated with *V. vulnificus* and *V. alginolyticus*, respectively.

Regarding the use of *E. coli* concentrations to predict the presence of *Salmonella* contamination in seafood sold in Bangkok, the area under the curve (AUC) from an ROC analysis was 63.8%. Selection of the optimal cutoff value for *E. coli* levels was based on the Youden index that uses the maximal difference between sensitivity and 1- specificity (*Ruopp et al., 2008*). Based on this index, the optimal cutoff value for *E. coli* was 1.33×10^4 MPN/g, which can be implemented for both monitoring seafood for *Salmonella* contamination and to establish threshold control measures at processing or during retail storage. However, this cutoff for *E. coli* concentration in seafood is much higher than the microbiological criteria according to Commission Regulation (EC) No 2073/2005 (*European Commission, 2005*), and the BQSF, Thailand (*Bureau of Quality and Safety of Food, 2020*). This may be because high concentrations of *E. coli* in this sample collection generated a high cutoff value to discriminate the presence or absence of *Salmonella* in the samples. Lastly, given that the detection of *Salmonella* and *Vibrio* spp. is of similar expense and similar technical difficulty as quantifying *E. coli* concentrations in seafood matrices, it may be more expeditious and more accurate to focus seafood safety

monitoring protocols on *Salmonella* and *Vibrio* spp. detection rather than rely on indicator bacteria like *E. coli* that invariably suffer from false positive and false negative signals for the presence of common seafood-borne pathogens.

Conclusions

Finfish and shellfish products sold in Bangkok were found to be contaminated with a diversity of Salmonella serovars and species of Vibrio, with substantial differences between seafood commodities (Asian sea bass, oysters, blood cockle, Pacific white shrimp) ~~regarding bacterial pathogen prevalence, pathogen species, and bacterial diversity~~. Although the concentration of *E. coli* ~~was~~ predicted~~ive of~~ Salmonella contamination for these seafood samples, the high cutoff value (1.33×10^4 MPN/g) for maximal test accuracy will likely prevent this method from being adopted as a food hygiene surveillance tool. ~~because c~~Current Thai BQSF regulations require no more than 10 E. coli MPN/g for fresh or frozen seafood. ~~Given that this project focused on sampling seafood at retail markets, we could not discern where the point of contamination occurred, but it is not surprising that b~~Bacterial contamination varied by seafood commodity, which may reflect ~~given the significantly~~ different culturing, harvesting, processing, and retail display practices ~~for each of these commodities in Bangkok. Consumers should be made aware that proper handling and cooking of these seafood commodities is needed to minimize the risk of seafood-borne illness, and future work should ascertain where along the farm to retail continuum these bacterial contaminants are entering the food chain.~~

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Acknowledgements

The authors thank Chailai Chareamchainukul, Mullika Kuldee, Varangkana Thaotumpitak, and Saweeyah Toodbat for their technical assistance.

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