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

Consumption of seafood contaminated with bacteria causes ## of cases of food poising, including ## of fatal infections every year. Thailand is a leading producer and consumer of seafood but little is known about ..... In particular, public health officers need to know the relationship between ...., as assessed with readily available culture-dependent and bacterial phenotyping methods. To address this, ~~The objectives of this study were to determine the~~ levels of indicator bacteria, *Salmonella* and *Vibrio* in various seafood commodities were determined and to identify risk factors associated with *Salmonella* contamination ~~of these samples~~. A total of 335 samples were collected from October 2018 to July 2019 throughout Bangkok, Thailand; overall sample composition was Pacific white shrimp (n = 85), oysters (n = 82), blood cockles (n = 84), and Asian seabass (n = 84). The prevalence ~~and concentrations (sd) of fecal coliforms were~~ was 100% for fecal coliforms and ~~9×10^4 (4×10^4) MPN/g; regarding *E. coli*, the prevalence was~~ 85% ~~and concentration (sd) was 2×10^4 (4×10^4) MPN/g~~ for *E. coli*. In contrast, ~~the overall~~ prevalence ~~for~~was 59% for *V. parahaemolyticus* was ~~59%~~, 49% for *V. cholerae*, 19% for *V. alginolyticus*, 18% for *V. vulnificus*, and 36% for *Salmonella*. Highest concentrations of fecal coliforms and *E. coli* were ~~found~~ in oysters~~;~~. ~~shrimp had the h~~ Highest concentrations of *Salmonella* ~~#~~with Matopeni (31%) being the predominant serotype~~-~~ were in shrimp. ~~The presence of~~ *Salmonella* contamination ~~in these seafood commodities~~ was significantly associated with type of seafood ~~sample~~, sampling location, retail condition~~s of seafood~~, and the presence of *E. coli*, *V. alginolyticus* and *V. vulnificus* ~~in the samples~~. ~~The optimal cutoff value for the~~An *E. coli* concentration of *E. coli* 1.33×10^4 MPN/g~~to~~ predicted contamination of *Salmonella* ~~was 1.33×10^4 MPN/g~~, with a sensitivity of 84% and specificity of 61%. Displaying seafood products on ice,

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presence of *E. coli* and *Vibrio*, and 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 global fisheries and aquaculture both inland and marine reached 171 million tonnes in 2016 (*Food and Agriculture Organization of the United Nations, 2018*) and consumption of fish, and fishery products, per capita double from 10 kg in 1960 to greater than 20 kg in 2016 (*Food and Agriculture Organization of the United Nations, 2018*). In Southeast Asia, ~~the~~ consumption of fish and fishery products varies from 6 to 64 kg per capita per year (*Food and Agriculture Organization of the United Nations, 2015*). In Thailand, ~~the~~ consumption of fish and fishery products is about 31 kg per capita per year, which accounts for 12% of total protein consumption per person (*Food and Agriculture Organization of the United Nations, 2015*). Thailand is one of the top ten exporters of fish and fishery products, which accounted for 4% of global exports in 2016 (*Food and Agriculture Organization of the United Nations, 2018*).

Due to the rapid growth of global consumption of fish and fishery products, seafood safety is concerning. Foodborne diseases each year afflict a third of the world population each year (*World Health Organization, 2004*).but~~. Limited~~ data ~~of~~ about the number of illnesses from seafood-borne outbreaks ~~has observed~~is limited ~~in~~for many parts of the world. Most examinations of seafood outbreaks have been ~~examined~~ done in the United States, where approximately 9.4 million illnesses, almost 56,000 hospitalizations, and 1,351 deaths, are associated with foodborne contamination per year (*Scallan et al., 2011*). Almost half (45%) of foodborne outbreaks reported in the U.S. are from ~~pathogenic~~ bacteria, and fish are frequently implicated ~~with bacterial contamination~~ (*Gould et al., 2013*). In Europe, 5,175 foodborne outbreaks were reported in 2019~~,~~. ~~with~~ *Salmonella* spp. ~~the leading causative~~ cause agent ~~for~~most of these outbreaks. There were 87,923 and 7,775 confirmed cases of salmonellosis and infections from Shiga-toxin-producing *Escherichia coli*, respectively (*European Food Safety Authority; European Centre for Disease Prevention and Control, 2021*).~~Limited data of the number of illnesses from seafood-borne outbreaks has observed in many parts of the world.~~

Pollution, animal density, and global trading contribute to ~~pathogen~~ bacterial contamination of seafood products (*Papadopoulou et al., 2007*). 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 *E. 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* spp. are the leading causes of foodborne diarrhea. Even though Thailand is one of the major exporters of seafood products but~~,~~ monitoring ~~and surveillance of distribution~~ of bacterial pathogens ~~in seafood products~~of these exports is ~~still~~ limited. Therefore, the objectives of this study were 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

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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

Samples of fresh fish and shellfish)*n* = 335(were collected from open-air retail fresh markets between October 2018 and July 2019 from four districts in Bangkok, Thailand, resulting in a sample composition of 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( )Table 1(. Due to varying availability of these four different seafood commodities at each market, there were slightly different total sample sizes for some seafood commodities ranging from n=82 to n=85 (Table 1). ~~Production of~~ Pacific white shrimp, oysters, and blood cockles are raised in ~~from aquaculture (~~saltwater ponds~~)~~; the majority of Asian seabass ~~were~~ are raised in ~~cages in~~ estuaries, but ~~a minority were~~some are raised in saltwater ponds.

Individual seafood samples were purchased in the early morning (5 to 7 a.m.). At least 200 g of the samples were placed into a double sterile plastic bag. The samples were kept on ice (< 10 °C) during transportation and kept in the cooler. All samples were 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 in triplicate 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). The average (± sd) daily minimum and maximum ambient air temperature was 26.8 (± 1.8) °C and 34.1 (± 2.6) °C; average (± sd) wind speed was 13.0 (± 2.0) km/h, average 24-hour precipitation 1.4 (± 4.0) mm, and average relative humidity was 75.1 (± 7.4) %.

Predictor variables

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.

Bacterial concentration and phenotyping

~~The~~Seafood samples were analyzed in triplicate for coliforms, *E. coli*, *Salmonella*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* and *V. cholerae*.

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~~Determination of fecal coliform and *E. coli* concentrations~~

~~The oncentrations of fecal~~ 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~~ Pieces were then homogenized for 1 to 2 min. The resulting suspension was ~~A 10-fold~~ serially dilut~~ed~~~~ion, 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(.

Fecal coliform and *E. coli* were enumerated~~For enumeration of indicator bacteria~~ in oysters and blood cockles following (ref). Briefly,~~,~~ ~~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~~ resulting suspension was serially diluted in LB to ~~different concentrations of 10^{-1}, 10^{-2}, 10^{-3}, and~~ 10^{-4}. These ~~diluted~~ dilutions ~~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 1.1~~1~~ × 10^5 MPN/g, respectively.

~~Isolation and confirmation of *Salmonella*~~

~~The detection of~~ *Salmonella* detection 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 in~~added to 225 mL of BPW. The ~~sample was~~pieces were 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 plates and incubated at 42 °C overnight. A loopful of ~~incubated sample~~from the MSRV plates was restreaked 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, Pasteur Institute (*Grimont and Weill, 2007*). Commercial antiserums (S&A Reagents Lab Ltd., Lat Phrao, Bangkok, Thailand) used to determine the serotype of *Salmonella*.

Isolation and confirmation of *Vibrio* spp.

~~The i~~Isolation of *Vibrio* spp. ~~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~~ suspension was added to 10 mL of Alkaline Peptone Water)APW) (Difco(and

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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 p~~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. ~~and was colorless colonies~~ Colonies with green center ~~in~~ on TCBS agar were presumed to be *V. parahaemolyticus*. Colorless colonies were presumed to be *V. vulnificus*. ~~In~~ On CHROMagar™ *Vibrio* agar plate, mauve colonies were presumed to be *V. parahaemolyticus* and green blue to turquoise blue were presumed to be *V. Vulnificus*-, Colorless colonies were presumed to be *V. alginolyticus*.

~~For i~~Isolation of *V. cholerae* followed (ref). Briefly, 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 confirm Vibrio idenifications.

Statistical analyses

Chi-square test and odds ratios were used to examine the association between different species of bacterial contamination and different types of seafood. For the odds ratio calculations of the association between bacteria contamination among seafood samples, shrimp was set as the referent category based on its popularity in Thai cuisine and largest sample size (n = 85) of the four seafood commodities. In addition, logistic regression was used to determine the association between *Salmonella* contamination and various risk factors To construct the final logistic regression model, 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. 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 for the concentration of *E. coli*. 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

Occurrence of indicator bacteria in seafood samples

~~In general,~~All seafood products sampled in Bangkok were ~~100%~~ positive for fecal coliforms with total average concentration (± sd) at 9×10^4 ($\pm 4\times10^4$) MPN/g (Table 2). The prevalence of *E. coli* was 85%, with total average concentration (± sd) of 2×10^4 ($\pm 4\times10^4$) MPN/g. Oyster samples had

the highest concentrations (± sd) of fecal coliforms at 1×10^5 (± 7×10^3) and *E. coli* at 5×10^4 (± 5×10^3), while blood cockle and seabass had the lowest concentrations of these indicator bacteria (Table 2).

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

The overall prevalence for the various bacterial pathogens observed in all 335 seafood samples was 59% for *V. parahaemolyticus*, 18% for *V. vulnificus*, 19% for *V. alginolyticus*, 49% for *V. cholerae* and 36% for *Salmonella*. The highest prevalence of *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. cholerae*, and *Salmonella* were observed in blood cockle (78%), Pacific white shrimp (33%), oyster (29%), Asian seabass (76%), and Pacific white shrimp (40%), respectively (Figure 1). The lowest prevalence (< 10%) of *V. vulnificus* was observed in blood cockle and for *V. alginolyticus* in Asian seabass. Moreover, shrimp were most likely to have any of the four pathogens, followed by oysters (Figure 1). ~~However, b~~Blood cockles exhibited very high contamination of *V. parahaemolyticus*, while Asian seabass tended to harbor *V. cholerae* (Figure 1). Based on Chi-square tests, the was a significant association between different types of samples and the occurrence of *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. cholerae*, and *Salmonella* contamination ($P < 0.0001$).

Pacific white shrimp exhibited a high prevalence of *V. parahaemolyticus* (59%), *V. cholerae* (53%) and *Salmonella* (47%), whereas oysters were mainly contaminated with *V. parahaemolyticus* (45%) and *Salmonella* (38%). In blood cockles, a high prevalence of *V. parahaemolyticus* (93%) were observed, although they had low prevalence of *V. cholerae*, *V. vulnificus*, and *V. alginolyticus*. Asian seabass ~~was frequently contaminated with~~ exhibited a high prevalence of *V. cholerae* (91%) and *Salmonella* (46%).

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Matopeni (31%), Corvallis (5%), Give (5%), and Rissen (5%) 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 (19%) and Rissen (33%), respectively.

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

Odds of *V. vulnificus* contamination in shrimp was 7.0 (1/0.143) times higher than that for blood cockle ($P = 0.002$) (Table 4). 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 = 9.1, $P < 0.0001$). The odds of *V. alginolyticus* contamination in shrimp was 13.3 (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 throughout Bangkok was significantly associated with type of seafood, sampling district, retail display condition, regional source of seafood, and the presence of *E. coli*, *V. alginolyticus*, and *V. vulnificus* (Table 5). *Salmonella* contamination in

Pacific white shrimp was not different form Asian seabass; in contrast, both oysters and blood cockles had significantly lower odds of *Salmonella* contamination 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 = 1.7, $P < 0.0001$) 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 = 3.5, $P < 0.0001$). Lastly, the odds of *Salmonella* contamination were positively associated with the presence of *E. coli* and *V. alginolyticus*, but negatively associated with *V. vulnificus*. ~~These risk factors should be interpreted with caution given the observational nature of this study and the possibility of undetected confounding in the statistical analysis.~~

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ROC and area under the ROC curve

The area under the ROC curve (AUC) at ~~63.77~~64% with standard error = 0.30 (C.I. = ~~57.8~~58% - ~~69.7~~70%) (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~~by a concentration of 1.3~~3~~ × 10^4 *E. coli* MPN/g, with a sensitivity of 84~~.43~~% and specificity of 61~~.03~~% ~~for this value~~.

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Discussion

According to the ~~Bureau of Quality and Safety of Food (BQSF), Department of Medical Science,~~ Ministry of Public Health~~,~~ for Thailand, the concentration of *E. coli* should not exceed 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 detectable *Salmonella*, *V. cholerae*, *V. parahaemolyticus* in a 25 g ~~of~~ sample (*Bureau of Quality and Safety of Food, 2020*). In this study, the ~~total average~~ concentration of *E. coli* ~~was~~ averaged 2×10^4 MPN/g for all the seafood samples~~, which is considerably higher than the standard BQSF guidelines in Thailand~~. In fact, only 18% (n = 60/335) of all seafood samples had concentrations of *E. coli* < 10 MPN/g and only 7% (n = 6/82) of oyster samples (often eaten raw) had < 3 MPN/g. Furthermore, the prevalence of *Salmonella* (36%), *V. cholerae* (49%), *V. parahaemolyticus* (59%) indicated widespread bacterial contamination of these seafood products, which also violates food safety ~~the Thailand~~ standards ~~requirements~~. Therefore, implementation of basic sanitation and evaluation of microbiological contamination of seafood products sold in Bangkok are needed ~~to strengthen seafood safety for this region~~.

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~~ include ~~the natural~~ aquatic ~~environment, during~~and aquaculture systems, ~~or~~ seafood processing facilities, ~~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% to 47%. This ~~finding~~ prevalence was similar to ~~a study of cultured shrimp in the Mekong Delta, Vietnam, which documented a~~the prevalence of (25%) *Salmonella* contamination of shrimp cultured in Vietnam (*Phan et al., 2005*), but substantially less than the 90 to 100% prevalence of *Salmonella* contamination in fish (93%) and shrimp (100%) collected from ~~the Surabaya local~~ a 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 of *Salmonella* contamination (OR = 1.7, $P < 0.0001$) than seafood not displayed on ice. This ~~curious finding~~ may seem counterintuitive, but ~~prior work has shown that~~ 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 m~~Melting ice can 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 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 contamination should be closely observed. ~~The i~~Implementation of programs for food safety and also for prevention and control of diarrheal diseases have reduced mortality and morbidity rates of diarrheal diseases and strengthened food safety in Thailand (*Food Control Division, Food and Drug Administration, Thailand. 2004*).

~~Lastly, s~~Seafood ~~that was~~ sourced from Eastern Thailand had a 3.5-higher odds of *Salmonella* contamination ~~compared to~~than seafood from other regions (OR = 3.5, $P < 0.0001$). 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~~/~~ of 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*). The infection of *S.* Matopeni has been reported in Malaysian children (*Lee et al., 2003*). ~~Outbreak strains of~~ *Salmonella* Paratyphi B ~~have been associated with~~ in 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*). *S.* Paratyphi B can be classified as d-tartrate fermenting (dT+) and d-tartrate non-fermenting (dT-) strains. The dT+ strain is less virulent and commonly reported with gastroenteritis, while the dT- strain is associated with paratyphoid fever. The dT+ strain is associated with a significant emerging disease worldwide and of public health concern (*Denny et al., 2007*, *Hassan et al., 2018*). Hence, classification of *S.* Paratyphi B biotype should be further investigated.

The common serovars in Pacific white shrimp were Itami (11%, n = 5/47) and Leith (9%, 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 (19%, 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 ruminants, but rarely found in humans (*Higgins et al., 1997*). It is possible that the contamination of Give may be the result of livestock or agricultural production near the oyster growing site. *S. enterica* 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*).

Even though typhoid and paratyphoid salmonellosis are endemic diseases in Thailand, ~~a decline in trend of~~ typhoid fever rates declined and ~~a stable rate of~~ paratyphoid ~~have been observed~~stabilized from 2003 to 2014 in this nation (*Techasaensiri et al., 2018*). In Thailand, *S. enterica* Weltevreden ~~was~~ is commonly reported in human, frozen seafood, frozen ducks, and

polluted water (*Bangtrakulnonth et al., 2004*). *S.* Weltevreden, *S.* Stanley, *S.* Anatum, and *S.* Rissen ~~have been~~are frequently reported in human from northern and central Thailand (*Prasertsee et al., 2019*; *Sirichote et al., 2010*). Therefore, surveillance and monitoring of oysters due to this ~20% prevalence of *Salmonella* contamination, and fully cooking oysters prior to consumption are both 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%, 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* (59%), *V. cholerae* (49%), *V. alginolyticus* (19%), and *V. vulnificus* (18%) were observed in this study.~~ According to BQSF ~~,~~for 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 bacteria~~l adulterants~~. This high prevalence is consistent with previous work demonstrating that between 2003 and 2015 the prevalence of *V. parahaemolyticus* was 64% in oysters, followed by clams (53%), fish (51%), and shrimp (48%) (*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* can 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~~.

~~According to the annual epidemiological surveillance report from the Department of Disease Control, Ministry of Public Health in Thailand, the infection of~~ *Clostridium perfringens*, *Staphylococcus aureus*, *V. parahaemolyticus,* and *Salmonella* spp. Are the leading ~~pathogens of~~ causes of foodborne illnesses in Thailand (*Bureau of Epidemiology, 2019*). In Thailand, human salmonellosis caused 167 illnesses per 100,000 persons in 2019~~,~~. ~~and c~~Contaminated produce and water have been indicated as important sources of *Salmonella* infection (*Bureau of Epidemiology, 2019*). The trend of *V. cholerae* infection ~~has~~ decreased from 2.51 to 0.02 cases per 100,000 persons during 2010-2019, and contaminated water and seafood, poor sanitation, and dense housing have been blamed as sources of contamination (*Bureau of Epidemiology, 2019*).

In this study, the determination of bacterial prevalence and abundance ~~species~~ was made using ~~.~~culture -dependent methods. ~~for~~ For *Salmonella* spp. and *Vibrio* spp. detection this

approach has high accuracy and sensitivity compared with certain molecular techniques (*Almeida et al., 2013; Eriksson & Aspan 2007; Hara-Kudo et al., 2001; Mainar-Jaime et al., 2013; Yeung &Thorsen, 2016*). However, ~~molecular assays commonly used to improve specificity and are often less time-consuming compared to culture-based methods. Especially,~~ these methods can fail to detect viable but nonculturable state (VBNC) strains. VBNC bacteria can preserve metabolic activity and generate virulent proteins~~, which can be detected by molecular techniques~~ (*Alleron et al., 2013; Morishige et al., 2015*). Hence, molecular techniques are recommended ~~when feasible~~ to determine bacterial contamination. However, equipment, supplies and training required to implement these molecular techniques are not readily available to food safety officers, even in the developed world.....

In this study, the determination of bacteria species was made using bacterial phenotyping methods. This approach is justifiable because..... Additionally, identification of specific serogroups of *Vibrio* spp., virulence factors, and bacterial toxins should be further examined.

~~Regarding the use of~~ *E. coli* concentrations appeared well suited to predicting ~~the presence of~~ *Salmonella* contamination ~~in~~ of seafood ~~sold in Bangkok~~, ~~the~~ The ~~area under the curve (AUC) from an ROC analysis was 64%. 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~~ a cutoff value for *E. coli* was 1.3~~3~~ $\times 10^4$ 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, t~~This cutoff ~~for *E. coli* concentration in seafood~~ is much higher than the microbiological criteria set forth in the 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~~ false-positive and ~~false~~ false-negative signals ~~for the presence of common seafood-borne pathogens~~.

Conclusions

Finfish and shellfish products sold in Bangkok ~~were found to be~~ are contaminated with ~~a diversity~~ diverse ~~of~~ *Salmonella* serovars and species of *Vibrio*~~, with substantial differences between seafood commodities (Asian sea bass, oysters, blood cockle, Pacific white shrimp)~~. Although the concentration of *E. coli* predicted *Salmonella* contamination for these seafood samples, the high cutoff value (1.3~~3~~$\times 10^4$ MPN/g) for maximal test accuracy will likely prevent this method from being adopted as a food hygiene surveillance tool. Current Thai BQSF regulations require no more than 10 *E. coli* MPN/g for fresh or frozen seafood. Bacterial contamination varied by seafood commodity, with substantial differences between Asian sea

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bass, oysters, blood cockle, and Pacific white shrimp. ~~which~~ This may reflect different aquaculturing, harvesting, processing, and retail display practices.

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