Saudi Journal of Biological Sciences 27 (2020) 1602–1608

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Prevalence and antibiotic resistance patterns of *Vibrio parahaemolyticus* isolated from different types of seafood in Selangor, Malaysia

Chia Wanq Tan [a,*], Yaya Rukayadi [a], Hanan Hasan [a], Tze Young Thung [a], Epeng Lee [b], Wendy Dayang Rollon [b], Hirofumi Hara [c], Ahmad Yaman Kayali [d], Mitsuaki Nishibuchi [d], Son Radu [a,b]

[a] Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor Darul Ehsan, Malaysia
[b] Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia, UPM Serdang, 43400 Selangor Darul Ehsan, Malaysia
[c] Department of Environmental Engineering and Green Technology, Malaysia-Japan International Institute of Technology, Universiti Teknologi Malaysia, Kuala Lumpur 54100, Malaysia
[d] Center for Southeast Asian Studies, Kyoto University, Kyoto 606-8501, Japan

ARTICLE INFO

Article history:
Received 29 November 2019
Revised 5 January 2020
Accepted 6 January 2020
Available online 16 January 2020

Keywords:
Antibiogram
MAR
Multidrug-resistant
Seafood
Vibrio

ABSTRACT

Vibrio parahaemolyticus is a foodborne bacterial pathogen that may cause gastroenteritis in humans through the consumption of seafood contaminated with this microorganism. The emergence of antimicrobial and multidrug-resistant bacteria is another serious public health threat worldwide. In this study, the prevalence and antibiotic susceptibility test of *V. parahaemolyticus* in blood clams, shrimps, surf clams, and squids were determined. The overall prevalence of *V. parahaemolyticus* in seafood was 85.71% (120/140), consisting of 91.43% (32/35) in blood clam, 88.57% (31/35) in shrimps, 82.86% (29/35) in surf clams, and 80% (28/35) in squids. The majority of *V. parahaemolyticus* isolates from the seafood samples were found to be susceptible to most antibiotics except ampicillin, cefazolin, and penicillin. The MAR indices of *V. parahaemolyticus* isolates ranged from 0.04 to 0.71 and about 90.83% of isolates were found resistant to more than one antibiotic. The high prevalence of *V. parahaemolyticus* in seafood and multidrug-resistant isolates detected in this study could pose a potential risk to human health and hence appropriate control methods should be in place to minimize the potential contamination and prevent the emergence of antibiotic resistance.

1. Introduction

Vibrio parahaemolyticus is a facultative, anaerobic, Gram-negative, curved rod-shaped bacterium commonly found in marine and estuary environments. It is a moderate halophilic bacterium capable of survival and multiplication at a concentration of 1 to 9% sodium chloride (NaCl) while the optimal growth happens at 3% NaCl (Whitaker et al., 2010; Kalburge et al., 2014). The ecological habitat of *V. parahaemolyticus* can be free to live as bacterioplankton, associated with the seafood surface, and a parasite in the gastrointestinal tract of fish. Higher organisms such as crustacean and molluscan shellfish are frequently found to be associated with *V. parahaemolyticus* (Kirs et al., 2011; Rodgers et al., 2014; Malcolm et al., 2015; Mala et al., 2016; Yu et al., 2016). Shellfish and other aquatic organisms are therefore often used as a vehicle for the transmission of this microorganism. Although *V. parahaemolyticus* is a well-known halophile, some reports have shown that *V. parahaemolyticus* can also be found in freshwater organisms (Nair et al., 2007; Nelapati and Krishnaiah, 2010; Noorlis et al., 2011; Otomo et al., 2013).

Ingestion of food contaminated with *V. parahaemolyticus* can lead to gastrointestinal illness, including symptoms such as watery diarrhoea, abdominal cramps, nausea, vomiting, fever, headache and/or bloody diarrhoea (CDC, 2013). Open wounds in contact with *V. parahaemolyticus* may also result in wound infection and life-threatening septicemia. Based on the number of vibriosis infections reported to the Cholera and Other Vibrio Illness Surveillance (COVIS) system and Centers for Disease Control and Prevention (CDC) from 1996 to 2014, *V. parahaemolyticus* was identified as the most common foodborne pathogen which caused 39–51% of *Vibrio* infection compared to other *Vibrio* species such as

* Corresponding author.
E-mail address: chiawanq@gmail.com (C.W. Tan).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.01.002
1319-562X/

V. vulnificus, *V. cholerae* (non-O1 and non-O139), *V. alginolyticus*, *V. fluvialis*, *V. mimicus*, and *V. hollisae* (Newton et al., 2012; CDC, 2019). The high infection rate caused by *V. parahaemolyticus* results in high medical costs worldwide. For example, the annual health cost of ingestion of seafood contaminated with *V. parahaemolyticus* in the United States was estimated to be $21 million (Ralston et al., 2011).

Another global health concern in recent years has been the rising cost of the medical costs of antibiotic-resistant infections. Timely surveillance of antibiotic-resistant bacteria and dissemination of surveillance data are therefore essential to address these public health issues (Johnson, 2015). Antibiotic resistance profiles of bacteria are usually determined through phenotypic assays such as agar dilution, broth dilution and Kirby-Bauer disk diffusion. Agar and broth dilution methods are used to determine the minimal inhibitory concentration (MIC) of antimicrobial agents by inoculating the defined number of bacterial cells at different concentrations of the antimicrobial substance (Wiegand et al., 2008). Kirby-Bauer disk diffusion method involves different kinds of the antibiotic disc placed on a bacterium agar plate and the antimicrobial profile of the bacteria is interpreted as sensitive, intermediate and resistance based on the inhibition zone. This latter method is routinely used in many clinical microbiology laboratories to test common and fast-growing pathogens due to its simplicity, well standardized, and easily interpreted (Jorgensen and Ferraro, 2009; Syal et al., 2017).

The majority of *V. parahaemolyticus* strains isolated from clinical and environmental samples reported high resistance to multiple antibiotics such as amoxicillin, ampicillin, carbenicillin, cefazolin, ceftazidime, cephalothin, colistin, gentamicin, and tobramycin (Zanetti et al., 2001; de Melo et al., 2011; Al-Othrubi et al., 2014; Sudha et al., 2014; Yano et al., 2014). Extensive use and misuse of prophylactic antibiotics in aquaculture for the prevention of bacterial infection and rapid spread of disease is most likely the main cause of the emergence and widespread of multiple drug resistance (MDR) in *V. parahaemolyticus* isolates. In addition, the overuse of antibiotics in aquaculture not only increases the selection of antibiotic-resistant bacteria and the dissemination of the antibiotic-resistant genes but also results in the presence of antibiotic residues in aquatic organisms such as fish (Miranda et al., 2018). The presence of *V. parahaemolyticus* in seafood samples and the occurrence of *V. parahaemolyticus* antibiotic resistance should be evaluated frequently. The aim of this study was to determine the prevalence and antibiotic-resistant patterns of *V. parahaemolyticus* isolated from different types of seafood in Malaysia.

2. Materials and methods

2.1. Sample collection

A total of 140 seafood samples consists of 35 samples for each blood clam (*Anadara granosa*), shrimp (*Penaeus* spp.), surf clam (*Paphia undulata*), and squid (*Loligo* spp.) were purchased from different wet markets in Selangor, Malaysia, for a period of 6 months from January 2018 to June 2018. All samples were transported to the laboratory and analysed immediately on the same sampling date.

2.2. Enrichment and isolation

Samples were examined according to the US FDA Bacteriological Analytical Manual (BAM) for *Vibrio* species with some modifications (Kaysner and DePaola, 2004). Twenty-five grams of each sample was weighed and transferred to a sterile stomacher bag containing 225 mL of alkaline peptone water (APW; Merck, Germany). Sample in the stomacher bag was mixed with Stomacher Lab-Blender 400 (Seward Medical, UK) for 2 min. Serial 10-fold dilution was carried out up to 10^{-5} by transferring 1 mL of the mixture to 9 mL of APW. Each dilution tube in triplicate was incubated at 37 °C overnight. After incubation, one loopful of the sample in each tube was streaked onto the CHROMagar™ *Vibrio* (CV) plate and the plates were incubated at 37 °C for overnight. Bacterial colonies in mauve colour were considered to be presumptive *V. parahaemolyticus*. The mauve colony was picked, purified by streaking back onto the CV plate and incubated at 37 °C overnight. A single colony was transferred to tryptic soy broth (TSB; Merck, Germany) supplemented by 2.5% NaCl (Merck, Germany) and incubated at 37 °C overnight. Colonies growth in the TSB were subjected to DNA extraction and *V. parahaemolyticus* species-specific confirmation through PCR assay. Pure *V. parahaemolyticus* colonies were stored on tryptic soy agar (TSA; Merck, Germany) slanted and kept at room temperature until further analysis.

2.3. DNA extraction and PCR confirmation

Bacterial DNA extraction was done by boiling and freeze-thawing extraction procedures. Briefly, 1.5 mL of overnight culture growth in the TSB supplemented by 2.5% NaCl was centrifuged at 13,400 × *g* for 3 min. The supernatant was discarded and the pellet was suspended in 200 μL TE buffer (10 mM Tris-HCl and 1 mM EDTA•Na_2 at pH 8.0). The suspension was boiled at 100 °C for 15 min in a dry bath (Labnet, USA) and immediately kept in a −20 °C freezer for 15 min. The suspension was then again centrifuged at 13,400 × g for 1 min and the supernatant was used as a DNA template for the PCR assay.

Presumptive *V. parahaemolyticus* colony growth on the CV plate was confirmed by amplification of *V. parahaemolyticus* species-specific gene with the *toxR* primers (F: 5′-GTCTTCTGACG CAATCGTTG-3′ and R: 5′-ATACGAGTGGTTGCTGTCATG-3′) (Kim et al., 1999). The presence of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) genes was also detected by the use of the *tdh* primers (F: 5′-CCACTACCACTCTCATATGC-3′ and R: 5′-GGTACTAAATGGCTGACATC-3′) and *trh* primers (F: 5′-TT GGCTTCGATATTTTCAGTATCT-3′ and R: 5′-CATAACAAACATATGCC CATTTCCG-3′), respectively (Tada et al., 1992; Bej et al., 1999). The amplification of *toxR*, *tdh* and *trh* genes was performed in a single reaction according to the multiplex PCR procedure described by Malcolm et al. (2015).

A total of 25 μL of each reaction mixture consists of 7 μL of 1.4 × PCR buffer, 2.5 μL of 2.5 mM $MgCl_2$, 0.5 μL of 0.2 mM dNTPs, 0.5 μL of 0.2 μM primers mix, 0.4 μL of GoTaq® Taq polymerase (Promega, USA), 2 μL of DNA template, and 12.1 μL of sterilized distilled water. The amplification was performed in the Kyratec SuperCycler Trinity (Australia) and the following conditions were applied: initial denaturation at 95 °C for 5 min for 1 cycle, 30 cycles consisting of denaturation at 95 °C for 30 s, annealing at 60 °C for 45 s, extension at 68 °C for 1 min, and final extension cycle at 72 °C for 3 min.

2.4. Antibiotic susceptibility test (AST)

A single *V. parahaemolyticus* isolate from each positive sample was selected for the antibiotic susceptibility test. A total of 24 types of antibiotics including amikacin (30 μg), amoxicillin-clavulanic acid (20/10 μg), ampicillin (10 μg), ampicillin-sulbactam (10 μg), cefazolin (30 μg), cefepime (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), cefuroxime sodium (parenteral) (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), gentamicin (10 μg), imipenem (10 μg), levofloxacin (5 μg), meropenem (10 μg), ofloxacin (5 μg), penicillin G (10 unit), piperacillin (100 μg), piperacillin-tazobactam (100/10 μg), tetracycline (30 μg), and

trimethoprim-sulfamethoxazole (1.25/23.75 μg) was used in this study. All the antimicrobial susceptibility discs were purchased from Oxoid (England). Selection of antibiotics was based on their frequent usage in clinical practices and according to the Clinical and Laboratory Standards Institute (CLSI) M45 guideline for *Vibrio* spp. (not V. *cholerae*) (CLSI, 2010). Disk susceptibility testing was according to the Kirby-Bauer disk diffusion method and CLSI M45 guidelines (Bauer et al., 1966; CLSI, 2010).

Briefly, a direct colony suspension was prepared by suspending the bacterial colony in 0.85% NaCl solution and adjusting equivalent to 0.5 McFarland standard. The inoculum was swabbed uniformly on the Mueller-Hinton agar plate (MHA; Merck, Germany) using a sterile cotton swab and allowed to dry for 5–10 min before placing the antibiotic disks on the MHA plate by using Oxoid™ disk dispenser. The plates were then incubated at 35 °C for 16–20 h. *Escherichia coli* ATCC® 25922 was included and used as a quality control organism in this study to monitor the accuracy of disk diffusion tests.

2.5. AST interpretive criteria

The diameter of any inhibition zone around the antibiotic disk was measured in the nearest millimetre. The zone diameter value was used to categorize each isolate as susceptible, intermediate, and resistant according to the CLSI recommendation breakpoint (CLSI, 2010). The multiple antibiotic resistance (MAR) index was also determined by using the formula, a/b, where "a" is the number of antibiotics to which the particular isolate was resistant, and "b" is the total number of antibiotics tested (Krumperman, 1983).

3. Results

3.1. Prevalence of V. parahaemolyticus

A total of 120 (85.71%) samples were found to be positive for *V. parahaemolyticus*. *V. parahaemolyticus* was isolated from 91.43% (32/35) of blood clams, 88.57% (31/35) of shrimps, 82.86% (29/35) of surf clams, and 80% (28/35) of squids. The prevalence of *V. parahaemolyticus* in four different types of seafood samples is summarised in Table 1. A 368 bp DNA fragment was developed from the PCR assay detection of *V. parahaemolyticus* species-specific *toxR* gene (Fig. 1). None of the *V. parahaemolyticus* isolates isolated from seafood samples were positive for pathogenic *tdh* and *trh* genes.

3.2. Antibiotic susceptibility test (AST)

A total of 120 *V. parahaemolyticus* isolates collected from all positive samples were confirmed by a pre-tested PCR assay against 24 types of antibiotics. Antibiotic susceptibility profiles of 120 *V. parahaemolyticus* isolates are presented in Table 2. All the *V. parahaemolyticus* isolates are 100% penicillin G resistant. The majority of *V. parahaemolyticus* (84.17%) isolated from seafood samples were also found to be highly resistant to ampicillin and cefazolin. Antibiotic sensitivity profiles of *V. parahaemolyticus* have shown that chloramphenicol inhibits the growth of all isolates. Ampicillin-sulbactam, imipenem, meropenem, tetracycline, trimethoprim-sulfamethoxazole, and doxycycline were also found to be effective against more than 90% of *V. parahaemolyticus* isolates. The antibiotic resistance profile of each *V. parahaemolyticus* was found to differ with MAR indices ranging from 0.04 to 0.71 (Table 3). The BC4 isolate showed the highest MAR index of 0.71 that was resistant to 17 antibiotics. In this study, majority of *V. parahaemolyticus* isolates from seafood samples demonstrated resistance to at least 3 antibiotics.

Table 1
Prevalence of *Vibrio parahaemolyticus* in blood clam, shrimp, surf clam and squid.

Sample	No. of sample	Number of positive samples	(%) of positive samples
Blood clam	35	32	91.43
Shrimp	35	31	88.57
Surf clam	35	29	82.86
Squid	35	28	80.00
Total	140	120	85.71

4. Discussion

The prevalence of *V. parahaemolyticus* in different types of seafood samples ranged from 80 to 91.43% with an average of 85.71% in this study. Blood clam was detected at the highest prevalence rate (91.43%) followed by shrimp (88.57%), surf clam (82.86%), and squid (80.00%). The higher occurrence of *V. parahaemolyticus* in seafood was mainly due to the widely disseminated of *V. parahaemolyticus* in estuarine, marine and coastal environments (Su and Liu, 2007; Johnson et al., 2012; Givens et al., 2014; Wu et al., 2014). The warm and tropical climate of Malaysia is also likely to promote and favour the growth of *V. parahaemolyticus*. Schwab et al. (2014) reported that the warmer the weather, the higher the incidence density of gram-negative bacteria. Not only that, Sterk et al. (2015) reported that an average temperature increases by 3.7 °C could lead to the changes in the concentration of *V. parahaemolyticus* and increase the risk of illness by two to three times higher.

The results obtained from this study were found to be comparable to the findings of Tran et al. (2018). Tran et al. (2018) reported that 332 of 385 (86.2%) seafood samples, including molluscan shellfish and shrimp collected in Vietnam, had been contaminated with *V. parahaemolyticus*. On the other hand, Malcolm et al. (2015) reported a slightly higher prevalence of *V. parahaemolyticus* in seafood samples where all blood clams (84/84), 98.7% (75/76) surf clams, and 97.2% (70/72) shrimps were positive for *V. parahaemolyticus*. In contrast, Letchumanan et al. (2015) reported only 44% (200/450) shellfish samples, including mud crab, flower crab, carpet clam, hardshell clam, and mud creeper collected in Malaysia, were found to be positive for *V. parahaemolyticus* species-specific *toxR* gene. Similarly, Li et al. (2019) reported a low prevalence rate of *V. parahaemolyticus* in which 15.7% (365/2328) fish, 27.6% (164/594) crustaceans and 27.6% (84/304) molluscs collected in China were identified for *V. parahaemolyticus*.

Although a high prevalence of *V. parahaemolyticus* was detected in seafood samples, the majority of isolates were found to be non-pathogenic to humans due to lack of pathogenic *tdh* and *trh* genes. In this study, no seafood samples were detected with *tdh* and/or *trh* genes. Previous studies have also reported a low occurrence of *V. parahaemolyticus* isolates for *tdh* and *trh* genes, in accordance with the present results. Tran et al. (2018) reported that 25 out of 385 (6.5%) molluscan shellfish and shrimp samples were detected with pathogenic *tdh* and/or *trh* genes. Malcolm et al. (2015) revealed that 33.1% (77/232) and 6.9% (16/232) of seafood samples were detected with the presence of *tdh* and *trh* genes, respectively. Letchumanan et al. (2015) reported that only 6.5% (13/200) of the *V. parahaemolyticus* isolates collected from shellfish samples were *trh*-positive and none of the samples was *tdh*-positive. Consequently, majority of *V. parahaemolyticus* strains isolated from the seafood samples are found with the absence of *tdh* and *trh* genes. However, the pathogenicity of *V. parahaemolyticus* is complex and interactive (Sun et al., 2019). The significance of *V. parahaemolyticus* and its host-pathogen interactions for human infection is still questionable (Ghenem et al., 2017).

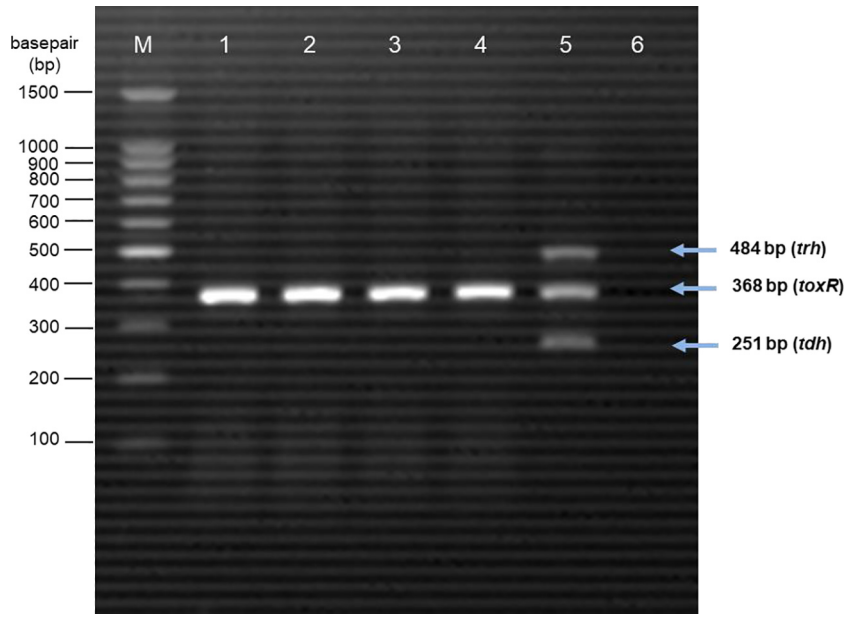

Fig. 1. Agarose gel electrophoresis of PCR products. M = 100 bp DNA marker; Lane 1: blood clam sample with *toxR* positive; Lane 2: shrimp sample with *toxR* positive; Lane 3: surf clam sample with *toxR* positive; Lane 4: squid sample with *toxR* positive; Lane 5: positive control; Lane 6: negative control.

Table 2
Antibiotic susceptibility profiles of *V. parahaemolyticus* isolated from seafood samples tested by disk diffusion method.

Antibiotics	Code	Interpretive criteria		
		Resistant (%)	Intermediate (%)	Sensitivity (%)
Amikacin (30ug)	Ak	45 (37.50)	40 (33.33)	35 (29.17)
Amoxicillin-clavulanic acid (20/10ug)	Amc	1 (0.83)	28 (23.33)	91 (75.83)
Ampicillin (10ug)	Amp	101 (84.17)	13 (10.83)	6 (5.00)
Ampicillin-sulbactam (10ug)	Sam	1 (0.83)	7 (5.83)	112 (93.33)
Cefazolin (30ug)	Cz	101 (84.17)	17 (14.17)	2 (1.67)
Cefepime (30ug)	Fep	4 (3.33)	30 (25.00)	86 (71.67)
Cefotaxime (30ug)	Ctx	6 (5.00)	72 (60.00)	42 (35.00)
Cefoxitin (30ug)	Fox	15 (12.50)	57 (47.50)	48 (40.00)
Ceftazidime (30ug)	Caz	6 (5.00)	29 (24.17)	85 (70.83)
Cefuroxime sodium (parental) (30ug)	Cxm	62 (51.67)	45 (37.50)	13 (10.83)
Cephalothin (30ug)	Kf	65 (54.17)	43 (35.83)	12 (10.00)
Chloramphenicol (30ug)	C	–	–	120 (100)
Ciprofloxacin (5ug)	Cip	16 (13.33)	80 (66.67)	24 (20.00)
Doxycycline (30ug)	Do	–	2 (1.67)	118 (98.33)
Gentamicin (10ug)	Cn	8 (6.67)	35 (29.17)	77 (64.17)
Imipenem (10ug)	Ipm	–	2 (1.67)	118 (98.33)
Levofloxacin (5ug)	Lev	2 (1.67)	30 (25.00)	88 (73.33)
Meropenem (10ug)	Mem	–	2 (1.67)	118 (98.33)
Ofloxacin (5ug)	Ofx	3 (2.50)	33 (27.50)	84 (70.00)
Penicillin G (10 unit)	P	120 (100)	–	–
Piperacillin (100ug)	Prl	43 (35.83)	28 (23.33)	49 (40.83)
Piperacillintazobactam (100/10ug)	Tzp	19 (15.83)	35 (29.17)	66 (55.00)
Tetracyline (30ug)	Te	–	7 (5.83)	113 (94.17)
Trimethoprimsulfamethoxazole (1.25/23.75ug)	Sxt	–	6 (5.00)	114 (95.00)

V. parahaemolyticus strain without the virulence *tdh* and *trh* genes have been also isolated from clinical specimens and reported in several studies (Bhoopong et al., 2007; Jones et al., 2012; Li et al., 2014; Pazhana et al., 2014). Besides the predominant *tdh* and *trh* hemolysin genes, the other factors contributing to human pathogenesis of *V. parahaemolyticus* infection have been addressed in some previous research studies. For instance, Makino et al. (2003) suggested that the presence of Type III Secretion Systems (T3SS) in *V. parahaemolyticus* strain is one of the important virulence factors closely related to *V. parahaemolyticus* pathogenicity. Urease present in many other foodborne pathogens, including *V. parahaemolyticus*, is defined as an enterovirulence factor that hydrolyses urea and increases pH in the immediate environment within the host (Okuda et al., 1997; Hongping et al., 2011; Berutti et al., 2014).

V. parahaemolyticus isolated from different types of seafood samples in this study were found to be highly resistant to the penicillin class of antibiotics, including penicillin G (10 unit) and ampicillin (10 μg). This finding is consistent with previous studies that the majority of *V. parahaemolyticus* isolates from seafood, such as grouper, shellfish, small mackerel and shrimp, were found to be highly resistant to penicillin (92.54–100%) and ampicillin (82.09–88%) (Srinivasan and Ramasamy, 2009; Letchumanan et al., 2015; Tan et al., 2017; Amalina et al., 2019). A total of 84.17% of *V. parahaemolyticus* isolates from this study were also found to be resistant to cefazolin (30 μg), which is a cephalosporin antibiotic.

Table 3
Antibiotics resistance profile and multiple antibiotic resistance (MAR) index of *V. parahaemolyticus* isolated from seafood samples.

MAR Index	Antibiotics Resistance Profile	Isolates[a]	Percentage of Isolate (%)
0.71	Amp, Amc, Sam, Prl, Tzp, Cz, Fep, Ctx, Fox, Cxm, Kf, Ak, Cn, Cip, Lev, Ofx, P	BC4	0.83
0.63	Amp, Prl, Tzp, Cz, Fep, Ctx, Fox, Caz, Cxm, Kf, Ak, Cip, Lev, Ofc, P	SC2	0.83
0.58	Amp, Prl, Tzp, Cz, Ctx, Fox, Caz, Cxm, Kf, Ak, Cn, Cip, Ofx, P	SQ3	0.83
0.54	Amp, Prl, Tzp, Cz, Fep, Ctx, Fox, Caz, Cxm, Kf, Ak, Cip, P	BC10	0.83
0.50	Amp, Prl, Tzp, Cz, Fep, Ctx, Fox, Cxm, Kf, Ak, Cip, P	SH3	0.83
0.46	Amp, Prl, Tzp, Cz, Fox, Cxm, Kf, Ak, Cn, Cip, P	BC16, SH19	1.67
0.42	Amp, Prl, Tzp, Cz, Cxm, Kf, Ak, Cn, Cip, P	SQ13, SH20	1.67
0.38	Amp, Prl, Tzp, Cz, Fox, Caz, Cxm, Kf, P	SH2	0.83
	Amp, Prl, Tzp, Cz, Cxm, Kf, Ak, Cip, P	BC1	0.83
	Amp, Prl, Tzp, Cz, Cxm, Kf, Ak, Cn, P	SQ4	0.83
	Amp, Prl, Cz, Ctx, Fox, Cxm, Kf, Ak, P	SC15	0.83
0.33	Amp, Cz, Fox, Cxm, Kf, Ak, Cip, P	SH1	0.83
	Amp, Prl, Tzp, Cz, Cxm, Kf, Cip, P	SC3	0.83
	Amp, Prl, Tzp, Cz, Cxm, Kf, Ak, P	SC11	0.83
	Amp, Prl, Tzp, Cz, Fox, Cxm, Kf, P	SC12	0.83
	Amp, Prl, Tzp, Cz, Caz, Cxm, Kf, P	BC13	0.83
	Amp, Prl, Cz, Cxm, Kf, Ak, Cn, P	SC19	0.83
0.29	Amp, Prl, Cz, Fox, Cxm, Kf, P	BC3	0.83
	Amp, Prl, Cz, Cxm, Kf, Cip, P	BC29	0.83
	Amp, Prl, Tzp, Cz, Cxm, Kf, P	SQ2, BC2, SQ7	2.50
	Amp, Cz, Cxm, Kf, Ak, Cip, P	SH9, SQ21, BC30	2.50
	Amp, Prl, Cz, Cxm, Kf, Ak, P	SH4, SH5, BC12, SQ4, SH18, SQ15, SQ23, BC23	6.67
0.25	Amp, Cz, Cxm, Kf, Cip, P	SQ1	0.83
	Amp, Prl, Cz, Cxm, Ak, P	SQ5	0.83
	Amp, Cz, Caz, Cxm, Kf, P	SC13	0.83
	Amp, Prl, Cz, Fox, Ak, P	SH13	0.83
	Amp, Cz, Fox, Cxm, Kf, P	SQ10, BC22	1.67
	Amp, Prl, Cz, Cxm, Kf, P	SC17, SQ22	1.67
	Amp, Prl, Cz, Cxm, Ak, P	SH16, SH17	1.67
	Amp, Cz, Cxm, Kf, Ak, P	SH11, SH14, SH15, SH25, SH28, SC24, SC26	5.83
0.21	Amp, Prl, Cz, Ak, P	SQ11, SQ24	1.67
	Amp, Cz, Cxm, Ak, P	BC17, BC32	1.67
	Amp, Cz, Kf, Ak, P	SH6, SQ6, SQ26	2.50
	Amp, Cz, Cxm, Kf, P	SC1, BC5, SQ8, SC10, SQ12, BC25, BC27, SC29	6.67
0.17	Amp, Cz, Ak, P	SQ17	0.83
	Amp, Cz, Kf, P	SC6, SH30, SH31	2.50
	Amp, Prl, Cz, P	SH8, SC14, BC15	2.50
0.13	Cz, Kf, P	SC21, BC21	1.67
	Amp, Cz, P	SC4, BC6, SC7, SC8, BC7, SQ9, SC9, BC11, BC14, BC18, SH21, BC19, SH24, SQ20, SH27, SQ25, SH29, BC26, SC23, SC25, SC28	17.50
0.08	Kf, P	SC18	0.83
	Cxm, P	SH7	0.83
	Cz, P	SH10, SH22, SH23, SC27	3.33
	Amp, P	BC9, SH12, SC16, SH26, BC24, SQ28	5.00
0.04	P	SC5, BC8, SQ16, SQ18, SQ19, BC20, SC20, SC22, SQ27, BC28, BC31	9.17

[a] BC – Blood clam; SH – Shrimp; SC – Surf clam; SQ – Squid.

From the results, it can be concluded that *V. parahaemolyticus* strains isolated from different types of seafood were found to be highly resistant to beta-lactam class antibiotics, including penicillin and cephalosporins. Therefore, ampicillin, penicillin and cefazolin should be phased-out for treating *V. parahaemolyticus* infections. Likewise, cefotaxime and ciprofloxacin are not a good choice in the treatment regimens for *Vibrio* infections because of its associated intermediate resistance by 60% and 66.67% of *V. parahaemolyticus* isolates, respectively.

Although *V. parahaemolyticus* isolates from this study displayed high levels of resistance to ampicillin, cefazolin, and penicillin, as well as intermediate levels of resistance to cefotaxime and ciprofloxacin, the antibiogram revealed that most of the *V. parahaemolyticus* isolates were susceptible to ampicillin-sulbactam (93.33%), chloramphenicol (100%), doxycycline (98.33%), imipenem (98.33%), meropenem (98.33%), tetracycline (94.17%), and trimethoprim-sulfamethoxazole (95%). These findings are comparable to the results of Letchumanan et al. (2015) in which *V. parahaemolyticus* isolates from shrimp samples were found to be highly susceptible to ampicillin-sulbactam (96%), chloramphenicol (95%), imipenem (98%), tetracycline (82%), and trimethoprim-sulfamethoxazole (93%). Lopatek et al. (2015) also reported that all isolates of *V. parahaemolyticus* isolated from raw shellfish were susceptible to chloramphenicol and tetracycline. Similarly, Xu et al. (2016) demonstrated that majority of *V. parahaemolyticus* isolates from retail aquatic products in North China were susceptible to chloramphenicol (95%), ciprofloxacin (92%), gentamicin (63%), tetracycline (83%), and trimethoprim-sulfamethoxazole (75%). These antibiotics could, therefore, be used effectively in the treatment of *V. parahaemolyticus* infections.

V. parahaemolyticus isolates tested in this study demonstrated MAR indices ranging from 0.04 to 0.71, with an average of 0.22.

One isolate from blood clam exhibited the highest MAR index value of 0.71 which showed resistance to 17 antibiotics. Overall, 90.83% of *V. parahaemolyticus* isolates were multidrug resistance (MDR) which exhibited resistance to more than one antibiotic used in this study. A total 67 of 120 isolates (55.83%) had MAR indices of more than 0.20. The MAR index greater than 0.2 indicates that the bacterial strain tested originated from the high-risk sources where antibiotics are frequently used (Krumperman, 1983; Gufe et al., 2019). According to the study conducted by Ahmed et al. (2018) which reported all *V. parahaemolyticus* isolates showed MDR for at least 7 antibiotics, MAR indices ranging from 0.58 to 1, and an average MAR index value of 0.77. Yu et al. (2016) showed the MAR indices ranging from 0.11 to 0.22 and *V. parahaemolyticus* isolate with the highest MAR index exhibited resistance to 4 antibiotics. From these results, it is noted that MAR indices vary between the studies and are not suitable for comparison due to the number of antibiotics and types of antibiotics used in the tests.

5. Conclusion

High prevalence of *V. parahaemolyticus* was detected in various types of seafood samples collected in Selangor, Malaysia. Although pathogenic *V. parahaemolyticus* strains with hemolysin *tdh* and *trh* genes have not been detected in this study, the risk of *V. parahaemolyticus* infection cannot be disregarded as the pathogenesis of vibriosis caused by *tdh*- and *trh*-negative strains of *V. parahaemolyticus* is still open to question. Infections caused by multidrug-resistant *V. parahaemolyticus* strains also pose a significant risk to human health. Antimicrobial resistance surveillance programmes should be continuous and aggregated at the national level in order to detect the emerging resistance and access the burden of antimicrobial resistance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by Fundamental Research Grant Scheme (FRGS) of the Ministry of Higher Education (MOHE), Malaysia (01-01-18-2015FR), Grant Putra IPS from Universiti Putra Malaysia (GP-IPS 9438703) and partly by the Japan Agency for Medical Research and Development, AMED (JP15km0908001).

References

Ahmed, H.A., El Bayomi, R.M., Hussein, M.A., Khedr, M.H.E., Abo Remela, E.M., El-Ashram, A.M.M., 2018. Molecular characterization, antibiotic resistance pattern and biofilm formation of *Vibrio parahaemolyticus* and *V. cholerae* isolated from crustaceans and humans. Int. J. Food Microbio. 274, 31–37. https://doi.org/10.1016/j.ijfoodmicro.2018.03.013.

Al-Othrubi, S.M., Kqueen, C.Y., Mirhosseini, H., Hadi, Y.A., Radu, S., 2014. Antibiotic resistance of *Vibrio parahaemolyticus* isolated from cockles and shrimp sea food marketed in Selangor, Malaysia. Clin. Microbial. 3, 148–154. https://doi.org/10.4172/2327-5073.1000148.

Amalina, N.Z., Santha, S., Zulperi, D., Amal, M.N.A., Yusof, M.T., Zamri-Saad, M., Ina-Salwany, M.Y., 2019. Prevalence, antimicrobial susceptibility and plasmid profiling of Vibrio spp. isolated from cultured groupers in Peninsular Malaysia. BMC Microbiol. 19, 251. https://doi.org/10.1186/s12866-019-1624-2.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Path. 45, 493.

Bej, A.K., Patterson, D.P., Brasher, C.W., Vickery, M.C.L., Jones, D.D., Kaysner, C.A., 1999. Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of *tlh*, *tdh* and *trh*. J. Microbiol. Methods. 36, 215–225. https://doi.org/10.1016/S0167-7012(99) 00037-8.

Berutti, T., Williams, R., Shen, S., Taylor, M., Grimes, D., 2014. Prevalence of urease in Vibrio parahaemolyticus from the Mississippi Sound. Lett. Appl. Microbiol. 58, 624–628. https://doi.org/10.1111/lam.12237.

Bhoopong, P., Palittapongarnpim, P., Pomwised, P., Kiatkittipong, A., Kamruzzaman, M., Nakaguchi, Y., Nishibuchi, M., Ishibashi, M., Vuddhakul, V., 2007. Variability in properties of *Vibrio parahaemolyticus* strains isolated from single patients. J. Clin. Microbiol. 45, 1544–1550. https://doi.org/10.1128/JCM.02371-06.

Centers for Disease Control and Prevention (CDC), 2013. Signs & symptoms: *Vibrio parahaemolyticus* illnesses associated with consumption of shellfish, United States, 2013 accessed 20 October 2019 https://www.cdc.gov/vibrio/investigations/vibriop-09-13/signs-symptoms.html, .

Centers for Disease Control and Prevention (CDC), 2019. Cholera and Other Vibrio Illness Surveillance (COVIS) accessed 22 October 2019 https://www.cdc.gov/vibrio/investigations/vibriop-09-13/signs-symptoms.html, .

CLSI, 2010. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline – Second Edition. CLSI document M45-A2. Clinical and Laboratory Standards Institute, Pennsylvania.

de Melo, L.M., Almeida, D., Hofer, E., Dos Reis, C.M., Theophilo, G.N., Santos, A.F., Vieira, R.H., 2011. Antibiotic resistance of *Vibrio parahaemolyticus* isolated from pond-reared *Litopenaeus vannamei* marketed in natal, Brazil. Braz. J. Microbiol. 42 (4), 1463–1469. https://doi.org/10.1590/S1517-83822011000400032.

Ghenem, L., Elhadi, N., Alzahrani, F., Nishibuchi, M., 2017. *Vibrio Parahaemolyticus*: a review on distribution, pathogenesis, virulence determinants and epidemiology. Saudi. J. Med. Med. Sci. 5 (2), 93–103. https://doi.org/10.4103/sjmms.sjmms_30_17.

Givens, C.E., Bowers, J.C., Depaola, A., Hollibaugh, J.T., Jones, J.L., 2014. Occurrence and distribution of *Vibrio vulnificus* and *Vibrio parahaemolyticus* - potential roles for fish, oyster, sediment and water. Lett. Appl. Microbiol. 58 (6), 503–510. https://doi.org/10.1111/lam.12226.

Gufe, C., Hodobo, T.C., Mbonjani, B., Majonga, O., Marumure, J., Musari, S., Jongi, G., Makaya, P.V., Machakwa, J., 2019. Antimicrobial profiling of bacteria isolated from fish sold at informal market in Mufakose, Zimbabwe. Int. J. Food Microbiol. https://doi.org/10.1155/2019/8759636. 8759636.

Hongping, W., Jilun, Z., Ting, J., Yixi, B., Xiaoming, Z., 2011. Insufficiency of the Kanagawa hemolytic test for detecting pathogenic *Vibrio parahaemolyticus* in Shanghai, China. Diagn. Microbiol. Infect. Dis. 69 (1), 7–11. https://doi.org/10.1016/j.diagmicrobio.2010.08.016.

Johnson, A.P., 2015. Surveillance of antibiotic resistance. Phil. Trans. R. Soc. B 370, 20140080. https://doi.org/10.1098/rstb.2014.0080.

Johnson, C.N., Bowers, J.C., Griffitt, K.J., Molina, V., Clostio, R.W., Pei, S., Laws, E., Paranjpye, R.N., Strom, M.S., Chen, A., Hasan, N.A., Huq, A., Noriea, N.F.I.I.I., Grimes, D.J., Colwell, R.R., 2012. Ecology of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). Appl. Environ. Microbiol. 78 (20), 7249–7257. https://doi.org/10.1128/AEM.01296-12.

Jones, J.L., Ludeke, C.H., Bowers, J.C., Garrett, N., Fischer, M., Parsons, M.B., Bopp, C.A., DePaola, A., 2012. Biochemical, serological, and virulence characterization of clinical and oyster *Vibrio parahaemolyticus* isolates. J. Clin. Microbiol. 50, 2343–2352. https://doi.org/10.1128/JCM.00196-12.

Jorgensen, J.H., Ferraro, M.J., 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin. Infect. Dis. 49, 1749–1755. https://doi.org/10.1086/647952.

Kalburge, S.S., Whitaker, W.B., Boyd, E.F., 2014. High-salt preadaptation of *Vibrio parahaemolyticus* enhances survival in response to lethal environmental stresses. J. Food Prot. 77 (2), 246–253. https://doi.org/10.4315/0362-028X.JFP-13-241.

Kaysner, C.A., DePaola, A.J., 2004. Bacteriological analytical manual: methods for specific pathogens. U.S. Food and Drug Administration. Chapter 9 Vibrio. https://www.fda.gov/food/laboratory-methods-food/bam-vibrio (accessed 12 March 2018).

Kim, Y.B.U., Okuda, J.U.N., Matsumoto, C., Takahashi, N., Hashimoto, S., Nishibuchi, M., 1999. Identification of *Vibrio parahaemolyticus* strains at the species level by PCR targeted to the toxR gene. J. Clin. Microbiol. 37, 1173–1177.

Kirs, M., DePaola, A., Fyfe, R., Jones, J.L., Krantz, J., Van Laanen, A., Cotton, D., Castle, M., 2011. A survey of oysters (*Crassostrea gigas*) in New Zealand for *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Int. J. Food Microbiol. 147 (2), 149–153. https://doi.org/10.1016/j.ijfoodmicro.2011.03.012.

Rodgers, C., Parveen, S., Chigbu, P., Jacobs, J., Rhodes, M., Harter-Dennis, J., 2014. Prevalence of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in blue crabs (*Callinectes sapidus*), seawater and sediments of the Maryland Coastal Bays. J. Appl. Microbiol. 117 (4), 1198–1209. https://doi.org/10.1111/jam.12608.

Krumperman, P.H., 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl. Environ. Microbiol. 46 (1), 165–170.

Letchumanan, V., Yin, W.F., Lee, L.H., Chan, K.G., 2015. Occurrence and antibiotic resistance of *Vibrio parahaemolyticus* from shellfish in Selangor, Malaysia. Front. Microbiol. 6, 1417. https://doi.org/10.3389/fmicb.2015.01417.

Li, Y., Xie, X., Shi, X., Lin, Y., Qiu, Y., Mou, J., Chen, Q., Lu, Y., Zhou, L., Jiang, M., Sun, H., Ma, H., Cheng, J., Hu, Q., 2014. *Vibrio parahaemolyticus*, southern coastal region of China, 2007–2012. Emerg. Infect. Dis. 20, 685–688. https://doi.org/10.3201/eid2004.130744.

Li, Y., Pei, X., Yan, J., Liu, D., Zhang, H., Yu, B., Li, N., Yang, D., 2019. Prevalence of foodborne pathogens isolated from retail freshwater fish and shellfish in China. Food Control. 99, 131–136. https://doi.org/10.1016/j.foodcont.2018.12.024.

Lopatek, M., Wieczorek, K., Osek, J., 2015. Prevalence and antimicrobial resistance of *Vibrio parahaemolyticus* isolated from raw shellfish in Poland. J. Food Prot. 78 (5), 1029–1033. https://doi.org/10.4315/0362-028x.jfp-14-437.

Mala, W., Alam, M., Angkititrakul, S., Wongwajana, S., Lulitanond, V., Huttayananont, S., Kaewkes, W., Faksri, K., Chomvarin, C., 2016. Serogroup, virulence, and molecular traits of *Vibrio parahaemolyticus* isolated from clinical and cockle sources in northeastern Thailand. Infect. Genet. Evol. 39, 212–218. https://doi.org/10.1016/j.meegid.2016.01.006.

Malcolm, T.T.H., Cheah, Y.K., Radzi, C.W.J.W.M., Kasim, F.A., Kantilal, H.K., John, T.Y. H., Martinez-Urtaza, J., Nakaguchi, Y., Nishibuchi, M., Son, R., 2015. Detection and quantification of pathogenic *Vibrio parahaemolyticus* in shellfish by using multiplex PCR and loop-mediated isothermal amplification assay. Food Control. 47, 664–671. https://doi.org/10.1016/j.foodcont.2014.08.010.

Makino, K., Oshima, K., Kurokawa, K., Yokoyama, K., Uda, T., Tagomori, K., Iijima, Y., Najima, M., Nakano, M., Yamashita, A., Kubota, Y., Kimura, S., Yasunaga, T., Honda, T., Shinagawa, H., Hattori, M., Iida, T., 2003. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V cholerae*. Lancet 361, 743–749. https://doi.org/10.1016/S0140-6736(03)12659-1.

Miranda, C.D., Godoy, F.A., Lee, M.R., 2018. Current status of the use of antibiotics and the antimicrobial resistance in the Chilean salmon farms. Front. Microbiol. 9, 1284. https://doi.org/10.3389/fmicb.2018.01284.

Nair, G.B., Ramamurthy, T., Bhattacharya, S.K., Dutta, B., Takeda, Y., Sack, D.A., 2007. Global dissemination of *Vibrio parahaemolyticus* serotype O3:K6 and its serovariants. Clin. Microbiol. Rev. 2 (1), 39–48. https://doi.org/10.1128/CMR.00025-06.

Nelapati, S., Krishnaiah, N., 2010. Detection of total and pathogenic *Vibrio parahaemolyticus* by polymerase chain reaction using *toxR*, *tdh* and *trh* genes. Vet. World. 3 (6), 268–271.

Newton, A., Kendall, M., Vugia, D.J., Henao, O.L., Mahon, B.E., 2012. Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. Clin. Infect. Dis. 54, S391–S395. https://doi.org/10.1093/cid/cis243.

Noorlis, A., Ghazali, F.M., Cheah, Y.K., Tuan Zainazor, T.C., Ponniah, J., Tunung, R., Tang, J.Y.H., Nishibuchi, M., Nakaguchi, Y., Son, R., 2011. Prevalence and quantification of vibrio species and *Vibrio parahaemolyticus* in freshwater fish at hypermarket level. Int. Food Res. J. 18, 689–695.

Okuda, J., Ishibashi, M., Abbot, S.L., Janda, J.M., Nishibuchi, M., 1997. Analysis of the thermostable direct hemolysin (tdh) gene and the tdh-related hemolysin (trh) genes in urease- positive strains of *Vibrio parahaemolyticus* isolated on the west coast of the United States. J. Clin. Microbiol. 35, 1965–1971.

Otomo, Y., Hossain, F., Rabbi, F., Yakuwa, Y., Ahsan, C.R., 2013. Pre-enrichment of estuarine and fresh water environmental samples with sodium chloride yields in better recovery of *Vibrio parahaemolyticus*. Adv. Microbiol. 3, 21–25. https://doi.org/10.4236/aim.2013.31003.

Pazhana, G.P., Bhowmik, S.K., Ghosh, S., Guin, S., Dutta, S., Rajendran, K., Saha, D.R., Nandy, R.K., Bhattacharya, M.K., Mukhopadhyay, A.K., Ramamurthy, T., 2014. Trends in the epidemiology of pandemic and non-pandemic strains of *Vibrio parahaemolyticus* isolated from diarrheal patients in Kolkata, India. PLoS Negl. Trop. Dis. 8 (5), e2815. https://doi.org/10.1371/journal.pntd.0002815.

Ralston, E.P., Kite-Powell, H., Beet, A., 2011. An estimate of the cost of acute health effects from food- and water-borne marine pathogens and toxins in the USA. J. Water Health 9 (4), 680–694. https://doi.org/10.2166/wh.2011.157.

Schwab, F., Gastmeier, P., Meyer, E., 2014. The warmer the weather, the more Gram-negative bacteria - Impact of temperature on clinical isolates in intensive care units. PLoS One. 9 (3), e91105. https://doi.org/10.1371/journal.pone.0091105.

Srinivasan, P., Ramasamy, P., 2009. Occurrence, distribution and antibiotic resistance patterns of *Vibrio* species associated with viral diseased shrimp of South Indian aquaculture environment. Int. J. Agric. Sci. 1, 1–10. https://doi.org/10.9735/0975-3710.1.2.1-10.

Sterk, A., Schets, F.M., de Roda Husman, A.M., de Nijs, T., Schijven, J.F., 2015. Effect of climate change on the concentration and associated risks of *Vibrio* Spp. in Dutch recreational waters. Risk Anal. 35 (9), 1717–1729. https://doi.org/10.1111/risa.12365.

Su, Y.C., Liu, C., 2007. *Vibrio parahaemolyticus*: a concern of seafood safety. Food Microbiol. 24 (6), 549–558. https://doi.org/10.1016/j.fm.2007.01.005.

Sudha, S., Mridula, C., Silvester, R., Hatha, A.A., 2014. Prevalence and antibiotic resistance of pathogenic vibrios in shellfishes from Cochin market. Indian J. Geo Mar. Sci. 43, 815–824.

Sun, Y., Guo, D., Hua, Z., Sun, H., Zheng, Z., Xia, X., Shi, C., 2019. Attenuation of multiple *Vibrio parahaemolyticus* virulence factors by Citral. Front. Microbiol. 10, 894. https://doi.org/10.3389/fmicb.2019.00894.

Syal, K., Mo, M., Yu, H., Iriya, R., Jing, W., Guodong, S., Wang, S., Grys, T.E., Haydel, S. E., Tao, N., 2017. Current and emerging techniques for antibiotic susceptibility tests. Theranostics 7 (7), 1795–1805. https://doi.org/10.7150/thno.19217.

Tada, J., Ohashi, T., Nishimura, N., Shirasaki, Y., Ozaki, H., Fukushima, S., Takano, J., Nishibuchi, M., Takeda, Y., 1992. Detection of the thermostable direct hemolysin gene (*tdh*) and the thermostable direct hemolysin-related hemolysin gene (*trh*) of *Vibrio parahaemolyticus* by polymerase chain reaction. Mol. Cell. Probes. 6, 477–487. https://doi.org/10.1016/0890-8508(92)90044-X.

Tan, C.W., Malcolm, T., Kuan, C.H., Thung, T.Y., Chang, W.S., Loo, Y.Y., Premarathne, J. M.K.J.K., Ramzi, O.B., Norshafawatie, M.F.S., Yusralimuna, N., Rukayadi, Y., Nakaguchi, Y., Nishibuchi, M., Radu, S., 2017. Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from short mackerels (*Rastrelliger brachysoma*) in Malaysia. Front. Microbiol. 8, 1087. https://doi.org/10.3389/fmicb.2017.01087.

Tran, T., Yanagawa, H., Nguyen, K.T., Hara-Kudo, Y., Taniguchi, T., Hayashidani, H., 2018. Prevalence of *Vibrio parahaemolyticus* in seafood and water environment in the Mekong Delta, Vietnam. J. Vet. Med. Sci. 80 (11), 1737–1742. https://doi.org/10.1292/jvms.18-0241.

Whitaker, W.B., Parent, M.A., Naughton, L.M., Richards, G.P., Blumerman, S.L., Boyd, E.F., 2010. Modulation of responses of *Vibrio parahaemolyticus* O3:K6 to pH and temperature stresses by growth at different salt concentrations. Appl. Environ. Microbiol. 76 (14), 4720–4729. https://doi.org/10.1128/AEM.00474-10.

Wiegand, I., Hilpert, K., Hancock, R., 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. 3, 163–175. https://doi.org/10.1038/nprot.2007.521.

Wu, Y., Wen, J., Ma, Y., Ma, X., Chen, Y., 2014. Epidemiology of foodborne disease outbreaks caused by *Vibrio parahaemolyticus*, China, 2003–2008. Food Control. 46, 197–202. https://doi.org/10.1016/j.foodcont.2014.05.023.

Xu, X., Cheng, J., Wu, Q., Zhang, J., Xie, T., 2016. Prevalence, characterization, and antibiotic susceptibility of *Vibrio parahaemolyticus* isolated from retail aquatic products in North China. BMC Microbiol. 16 (32), 1–9. https://doi.org/10.1186/s12866-016-0650-6.

Yano, Y., Hamano, K., Satomi, M., Tsutsui, I., Aue-umneoy, D., 2014. Diversity and characterization of oxytetracycline-resistant bacteria associated with non-native species, white-leg shrimp (*Litopenaeus vannamei*), and native species, black tiger shrimp (*Penaeus monodon*), intensively cultured in Thailand. J. Appl. Microbiol. 110, 713–722. https://doi.org/10.1111/j.1365-2672.2010.04926.x.

Yu, Q., Niu, M., Yu, M., Liu, Y., Wang, D., Shi, X., 2016. Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shellfish in Shanghai. Food Control. 60, 263–268. https://doi.org/10.1016/j.foodcont.2015.08.005.

Zanetti, S., Spanu, T., Deriu, A., Romano, L., Sechi, L.A., Fadda, G., 2001. In vitro susceptibility of *Vibrio* spp. isolated from the environment. Int. J. Antimicrob. Agents 17, 407–409. https://doi.org/10.1016/s0924-8579(01)00307-7.