

# Virulence and Antibiotic and Heavy Metal Resistance of *Vibrio parahaemolyticus* Isolated from Crustaceans and Shellfish in Shanghai, China

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## ABSTRACT

*Vibrio parahaemolyticus* can cause serious human seafoodborne gastroenteritis and even death. In this study, we isolated and characterized 208 *V. parahaemolyticus* strains from 10 species of commonly consumed crustaceans and shellfish available in fish markets in Shanghai, People's Republic of China, in 2014. Most of these aquatic species had not been detected previously. The results revealed an extremely low occurrence of pathogenic *V. parahaemolyticus* carrying the toxin gene *trh* (1.9%). However, a high level of resistance to the antibiotics ampicillin (94.2%), rifampin (93.3%), and streptomycin (77.9%) was found. Approximately 74.5% of the isolates had multidrug-resistant phenotypes. Tolerance to the heavy metals Cu<sup>2+</sup>, Pb<sup>2+</sup>, and Cd<sup>2+</sup> was detected in the majority of antibiotic resistant isolates. The resistance patterns differed depending on the tested samples. The crustaceans *Penaeus monodon* and *Marsupenaeus japonicus* harbored more antibiotic-resistant bacteria, whereas the isolates from the crustacean *Litopenaeus vannamei* and the shellfish *Busycon canaliculatus* had high tolerance to eight heavy metals tested. In contrast to the wide distribution of multidrug resistance and tolerance to heavy metals, lower percentages of plasmid DNA (22.6%) and SXT/R391-like integrative and conjugative elements (4.8%) were detected in the isolates, suggesting that *V. parahaemolyticus* in these aquatic species may have adopted some other molecular mechanisms that mediated the high prevalence of resistance determinants. The results of this study support the need for food safety risk assessment of aquatic products.

Key words: Antibiotic resistance; Aquatic products; Heavy metal tolerance; *Vibrio parahaemolyticus*; Virulence

*Vibrio parahaemolyticus* is a gram-negative bacterium that is virtually ubiquitous in marine, estuarine, and aquaculture environments worldwide (21). The bacterium is commonly found free swimming, attached to underwater surfaces, or commensally associated with various types of aquatic animal products (8). Consumption of raw, undercooked, or mishandled seafood contaminated with pathogenic *V. parahaemolyticus* can lead to serious gastroenteritis or even death (21). *V. parahaemolyticus* has two major virulence factors, thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH), that play rival roles in the pathogenesis of the bacterium (21). These pore-forming proteins contribute to the invasion of the bacterium in the human gastrointestinal tract, resulting in diarrheal disease (21). The coding genes (*tdh* and *trh*) are used as diagnostic targets to identify pathogenic isolates of *V. parahaemolyticus* (6).

Antimicrobial agents are commonly applied in the animal breeding industry to effectively prevent or treat disease outbreaks caused by pathogenic organism. Never-

theless, the extensive use of drugs in aquaculture has contributed to the development of antibiotic-resistant bacteria and posed serious problems in aquatic ecosystems (1, 17, 40). Water contaminated with industrial pollutants such as heavy metals may enhance selection for antibiotic resistance and vice versa (4, 7, 26). The exchange of resistance genes between foodborne and human gastrointestinal tract microflora has been recognized as a potential threat to human health (40).

The People's Republic of China is the world's largest producer, consumer, and exporter of aquatic products, including fish, crustaceans, and shellfish (10, 28, 36). Along with the fast growing aquaculture industry, aquatic animal diseases caused by waterborne pathogens have also rapidly increased, which has led to huge economic losses in the past decades (42). Numerous previous studies have focused on the prevalence and characterization of antibiotic resistance in pathogenic *V. parahaemolyticus* isolates of clinical origins (11, 22, 38, 44). Information on the potential role of aquatic products in the dissemination of resistant bacteria, particularly *V. parahaemolyticus*, is very limited (12, 16, 34, 43). In this study, we investigated virulence and antibiotic and heavy metal resistance of *V. parahaemolyticus* isolated from

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crustaceans and shellfish in Shanghai, People's Republic of China, to address the lack of molecular ecological data concerning this bacterium in aquatic products.

## MATERIALS AND METHODS

### Isolation and identification of *V. parahaemolyticus* strains.

Ten species of commonly consumed fresh aquatic products were sampled from fish markets in Shanghai in 2014: five species of crustaceans (*Litopenaeus vannamei*, *Macrobrachium nipponense*, *Marsupenaeus japonicus*, *Penaes monodon*, and *Procambarus clarkii*) and five species of shellfish (*Busycon canaliculatus*, *Haliotis discus hannai*, *Moerella iridescens*, *Scapharca subcrenata*, and *Tegillarca granosa*). The crustacean samples were collected from August to September 2014, and the shellfish samples were collected from October to November 2014. *V. parahaemolyticus* was isolated and identified according to instructions in Chinese government standard GB17378-2007 (35) and the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (39) as described previously (34). The bacteria were cultured in tryptic soy broth (Beijing Land Bridge Technology Co., Beijing, People's Republic of China) (pH 8.5, 3% NaCl) at 37°C. *V. parahaemolyticus* ATCC 33847 (*tdh*<sup>+</sup> *trh*<sup>-</sup>) (14) and ATCC 17802 (*tdh*<sup>-</sup> *trh*<sup>+</sup>) (5), which were isolated from clinical and food poisoning cases, respectively, were used as positive control strains as described previously (16).

**Determination of antimicrobial and heavy metal resistance.** *V. parahaemolyticus* isolates were measured for in vitro resistance to 10 antimicrobial agents and eight heavy metals according to methods described previously (25, 34). Examined antimicrobial agents (Oxoid, UK) were 10 µg of ampicillin (AMP), 30 µg of chloramphenicol (CHL), 10 µg of gentamicin (CN), 30 µg of kanamycin (KAN), 5 µg of rifampin (RIF), 100 µg of spectinomycin (SPT), 10 µg of streptomycin (STR), 30 µg of tetracycline (TET), 5 µg of trimethoprim (TM), and 25 µg of sulfamethoxazole (23.75 µg) plus trimethoprim (1.25 µg) (SXT). Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial categories (24). The heavy metals used in this study were copper (CuCl<sub>2</sub>), cadmium (CdCl<sub>2</sub>), chromium (CrCl<sub>3</sub>), mercury (HgCl<sub>2</sub>), manganese (MnCl<sub>2</sub>), nickel (NiCl<sub>2</sub>), lead (PbCl<sub>2</sub>), and zinc (ZnCl<sub>2</sub>) (Sinopharm Chemical Reagent Co., Shanghai, China). The assays were performed in triplicate experiments. *Escherichia coli* strains ATCC 25922 and K-12 (Institute of Industrial Microbiology, Shanghai, China) were used as quality control strains in antibiotic and heavy metal resistance tests, respectively (26, 34).

**Molecular biology methods.** Genomic DNA was prepared using the TIANamp Bacteria DNA Kit (Beijing Tiangen Biotech, Beijing, China) and plasmid DNA (<23 kb) was isolated using the TaKaRa MiniBEST Plasmid Purification Kit, version 4.0 (Japan TaKaRa BIO, Dalian Company, Dalian, People's Republic of China) according to the manufacturers' instructions. Oligonucleotide primers targeting the toxin genes (*tdh* and *trh*) and the highly conserved core genes (*int*, *traC*, *traI*, and *setR*) of the SXT-R391 family of integrative and conjugative elements (ICEs) are listed in Supplementary Table S1 (all supplemental materials are available at <http://spxy.shou.edu.cn>). ICEs are self-transmissible mobile genetic elements that mediate horizontal gene transfer between bacteria (41). The *int* gene encodes an integrase that mediates site-specific recombination between attP and attB sites on the circular form of ICEs and its respective host chromosome. The *traC* gene encodes a typical conjugation transfer protein involved in mating-

pair formation, and the *traI* gene encodes a relaxase and participates in ICE DNA processing and single-strand DNA mobilization to the recipient cell. As an important regulator, the *setR* gene inhibits the expression of the *setDC* operon that encodes the master transcriptional activators required for SXT transfer (34, 41). The *tlh* gene encodes a thermolabile hemolysin, which is used for *V. parahaemolyticus* identification. The primers were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co. (Shanghai, China). PCRs were performed according to the method described previously (34) in a Mastercycler pro PCR thermal cycler (Eppendorf, Hamburg, Germany). Amplified products were detected and analyzed as described previously (34).

## RESULTS

**Isolation and identification of *V. parahaemolyticus* strains.** The 10 species of commonly consumed aquatic animals were collected from fish markets in Shanghai in 2014. Pure cultures of 208 randomly selected *V. parahaemolyticus* isolates were obtained and analyzed in this study. All isolates were positive for the *tlh* gene; 104 isolates were derived from the five shellfish species (*B. canaliculatus*, *H. discus hannai*, *M. iridescens*, *S. subcrenata*, and *T. granosa*), and the remaining isolates were from the five crustaceans (*L. vannamei*, *M. nipponense*, *M. japonicus*, *P. monodon*, and *P. clarkii*). Four species, the crustaceans *P. monodon* and *M. nipponense* and the shellfish *B. canaliculatus* and *M. iridescens*, appeared to be contamination reservoirs; approximately 14.9 to 18.8% of the isolates were recovered from these four species, whereas only 2.9 to 7.7% of the isolates were recovered from the other six species.

**Virulence of the *V. parahaemolyticus* isolates.** Virulence-associated genes (*tdh* and *trh*) in the 208 isolates were examined by PCR assay. Most of the isolates were considered not virulent, based on no amplification of the toxin gene *tdh*. Nevertheless, four isolates (1.9%) were positive for the *trh* gene, three isolates from shellfish (*B. canaliculatus*, *H. discus hannai*, and *M. iridescens*) and one isolate from a crustacean (*L. vannamei*).

**Antimicrobial resistance of the *V. parahaemolyticus* isolates.** Antimicrobial resistance of the 208 *V. parahaemolyticus* isolates was determined using the Kirby-Bauer disk diffusion method (33) (Fig. 1). All isolates were susceptible to CHL, and most were also sensitive to TET (95.2%) and SXT (94.7%). In contrast, AMP resistance was the most common profile (94.2%) among the isolates. Resistance to other antimicrobial agents was also observed, including RIF (93.3%) and STR (77.9%). Approximately 89.4, 88.9, and 53.4% of the isolates also exhibited intermediate susceptibility to KAN, SPT, and CN. The trend of resistance of the isolates was AMP > RIF > STR > KAN > TM > SPT > SXT > TET = CN > CHL. A total of 155 isolates (74.5%) were resistant to more than three antimicrobial agents tested in this study, and one isolate was resistant to eight antimicrobial agents (Table 1).

Our results also revealed distinct antibiotic-resistant patterns for the *V. parahaemolyticus* isolates from the two types of aquatic species (Fig. 2). A higher incidence of

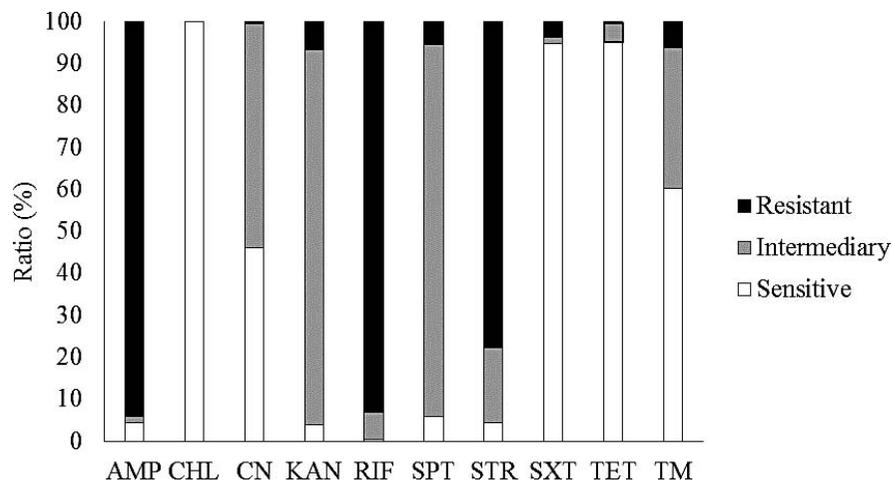


FIGURE 1. Antimicrobial resistance of 208 *V. parahaemolyticus* isolates recovered from 10 aquatic species collected from Shanghai fish markets in 2014. AMP, ampicillin; CHL, chloramphenicol; CN, gentamicin; KAN, kanamycin; RIF, rifampin; SPT, spectinomycin; STR, streptomycin; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline; TM, trimethoprim.

multidrug-resistant isolates was observed in the shellfish samples (87.5%) than in the crustaceans (70.2%). The isolates recovered from shellfish had a higher level of resistance to STR (85.6%) than did those isolates recovered from crustaceans (70.2%). In contrast, the crustacean isolates were resistant to TM (12.5%), SXT (7.7%), CN (1.0%), and TET (1.0%), whereas none of the shellfish isolates were resistant to these four drugs. Similar high percentages (>90%) of resistance to AMP and RIF were detected in both classes of aquatic species.

Although only a small number of isolates were analyzed, different antibiotic resistance patterns were observed among the 10 species (Table S2). The *V. parahaemolyticus* isolates from *P. monodon* and *M. japonicus* were resistant to the maximum number of antimicrobial agents (8 of 10 agents), whereas isolates from *S. subcrenata* had the opposite pattern (resistant to 3 of 10 agents). The highest incidences of resistance to TM (36.4%; 4 of 11 isolates), SXT (36.4%; 4 of 11 isolates), and SPT (18.2%; 2 of 11 isolates) were found in the isolates recovered from *M. japonicus*, compared with isolates from the other nine species (TM, 0 to 16.1%; SXT, 0 to 13.3%; SPT, 0 to 10.0%). Similarly, the isolates from *T. granosa* had the highest resistance against KAN (33.3%; 2 of 6 isolates), whereas the unique pattern of resistance to TET or CN alone was detected in only a few isolates from *M.*

*japonicus* (9.1%; 1 of 11 isolates) and *B. canaliculatus* (2.6%; 1 of 39 isolates), respectively. High levels of resistance to AMP (80.0 to 100%), RIF (83.3 to 100%), and STR (71.0 to 91.9%) were observed among the 10 species.

**Heavy metal tolerance of the *V. parahaemolyticus* isolates.** In this study, tolerance of the *V. parahaemolyticus* isolates in response to eight heavy metals was also examined. The maximum MICs observed in the tested isolates were 3,200 µg/mg for Cu<sup>2+</sup>, Pb<sup>2+</sup>, Cr<sup>3+</sup>, Ni<sup>2+</sup>, and Mn<sup>2+</sup>, 1,600 µg/mg for Zn<sup>2+</sup> and Cd<sup>2+</sup>, and 25 µg/mg for Hg<sup>2+</sup> (Table 2) when compared with the quality control strain *E. coli* K-12 (25). The majority of the isolates were tolerant to Cu<sup>2+</sup> (89.4%), Pb<sup>2+</sup> (80.3%), and Cd<sup>2+</sup> (80.3%), and a few isolates were resistant to Ni<sup>2+</sup> (1.9%), Mn<sup>2+</sup> (2.4%), and Zn<sup>2+</sup> (2.4%). The trend of tolerance of the isolates was Cu<sup>2+</sup> > Pb<sup>2+</sup> = Cd<sup>2+</sup> > Cr<sup>3+</sup> > Hg<sup>2+</sup> > Mn<sup>2+</sup> = Zn<sup>2+</sup> > Ni<sup>2+</sup>.

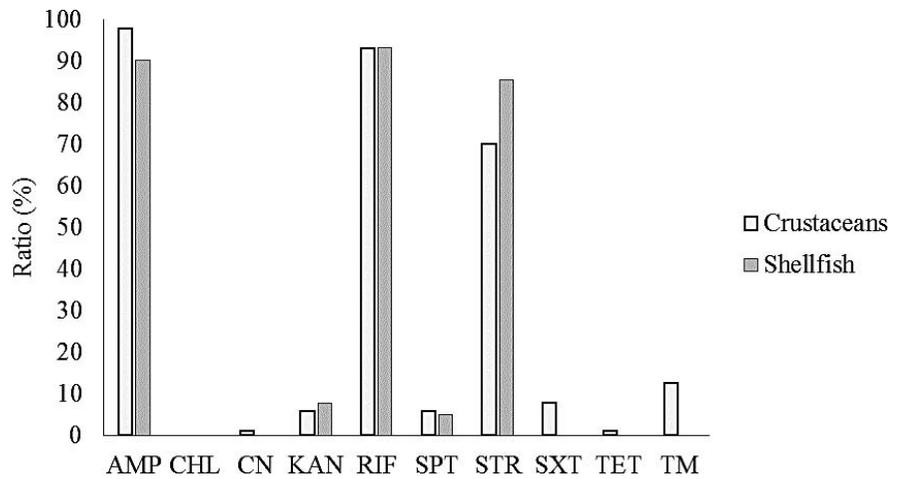
*V. parahaemolyticus* isolates recovered from the shellfish and crustacean samples had similar heavy metal tolerance patterns (Fig. 3). However, higher resistance to Cu<sup>2+</sup>, Pb<sup>2+</sup>, Cr<sup>3+</sup>, and Mn<sup>2+</sup> were observed in the isolates from shellfish than in the isolates from crustaceans, whereas Cd<sup>3+</sup>, Hg<sup>2+</sup>, and Zn<sup>2+</sup> had the opposite pattern. All isolates recovered from crustaceans were sensitive to Ni<sup>2+</sup>.

The heavy metal resistance patterns in this study differed depending on the samples analyzed. The *V. parahaemolyticus* isolates recovered from *L. vannamei* and *B. canaliculatus* were tolerant to all eight heavy metals tested in this study, whereas isolates from *P. clarkii* and *T. granosa* were resistant to the minimum number of heavy metals (4 of 8) (Table S3). All isolates recovered from *H. discus hannai*, *M. japonicus*, and *L. vannamei* were tolerant to Cu<sup>2+</sup>, Pb<sup>2+</sup>, and Cd<sup>2+</sup>, respectively, albeit high incidences of tolerance to these three heavy metals were found in all the tested samples. The Cr<sup>3+</sup> and Hg<sup>2+</sup> contamination appeared most seriously in *L. vannamei* (86.7%; 13 of 15 isolates) and *H. discus hannai* (93.8%; 15 of 16 isolates), respectively. Various degrees of tolerance to Cr<sup>3+</sup> and Hg<sup>2+</sup> were also observed in the isolates from the other species, except *T. granosa* and *P. clarkii*, respectively. In addition, few isolates from *L. vannamei* (6.7%; 1 of 15 isolates), *B. canaliculatus*

TABLE 1. MDR patterns among 208 *V. parahaemolyticus* isolates

No. of antimicrobial agents	No. (%) of resistant isolates	Resistance pattern
3	120 (57.7)	AMP, RIF, STR
	1 (0.5)	AMP, RIF, TM
4	1 (0.5)	RIF, STR, TM, SXT
	6 (2.9)	AMP, RIF, STR, TM
	8 (3.8)	AMP, RIF, STR, SPT
	10 (4.8)	AMP, RIF, STR, KAN
5	2 (1.0)	AMP, RIF, STR, KAN, SPT
	1 (0.5)	AMP, RIF, STR, KAN, CN
	5 (2.4)	AMP, RIF, STR, TM, SXT
8	1 (0.5)	AMP, RIF, STR, KAN, SPT, SXT, TET, CN

FIGURE 2. Prevalence of antibiotic resistance among *V. parahaemolyticus* isolates recovered from five crustacean and five shellfish species. AMP, ampicillin; CHL, chloramphenicol; CN, gentamicin; KAN, kanamycin; RIF, rifampin; SPT, spectinomycin; STR, streptomycin; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline; TM, trimethoprim.



(5.3%; 2 of 38 isolates), and *P. monodon* (2.6%; 1 of 39 isolates) were resistant to  $\text{Ni}^{2+}$ . All isolates from *M. nipponense*, *P. clarkii*, *M. iridescens*, *H. discus hannai*, *S. subcrenata*, and *T. granosa* were susceptible to  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$ .

**Plasmids and the SXT/R391-like ICEs of the *V. parahaemolyticus* isolates.** All 208 *V. parahaemolyticus* isolates were examined for the presence of plasmids. electrophoresis in a 0.7% agarose gel revealed that 47 isolates (22.6%) harbored plasmid DNA (<23 kb). Of these 47 isolates, 29 were recovered from crustaceans and 18 were from shellfish. The occurrence of plasmids was the highest in the isolates recovered from *L. vannamei* (46.7%; 7 of 15 isolates), followed by *P. monodon* (33.3%; 13 of 39 isolates), *T. granosa* (33.3%; 2 of 6 isolates), *S. subcrenata* (23.1%; 3 of 13 isolates), *M. nipponense* (22.6%; 7 of 31 isolates), and *B. canaliculatus* (18.4%; 7 of 38 isolates). Very low percentages of plasmids (3.1 to 0.1%) were observed in the isolates from *H. discus hannai*, *M. japonicus*, and *M. iridescens*.

Analysis aimed at the highly conserved core genes of SXT/R391-like ICEs revealed that 10 isolates (4.8%) recovered from the crustaceans harbored the *int*, *traI*, *traC*, and *setR* genes; 7 of these 10 isolates were from *M. nipponense* and 3 were from *M. japonicus*.

## DISCUSSION

Our results revealed that *V. parahaemolyticus* was present in the 10 species of aquatic animal products sold in Shanghai fish markets in 2014. To our knowledge, the majority of these aquatic species have not been detected previously. Our data also revealed a very low occurrence (1.9%) of pathogenic *V. parahaemolyticus* (*trh*<sup>+</sup>) in the detected samples, consistent with some previous reports that approximately 90 to 99% of *V. parahaemolyticus* isolates of environmental origin were negative for the two major toxin genes (*tdh* and *trh*) (12, 15, 16). For example, Haley et al. (15) recovered 170 *V. parahaemolyticus* isolates from water and plankton samples collected along the Georgian coastal zone of the Black Sea and found that none of the isolates were positive for the *tdh* and *trh* genes.

TABLE 2. Tolerance of 208 *V. parahaemolyticus* isolates to heavy metals

Heavy metal	No. of isolates with a maximum observed MIC ( $\mu\text{g/ml}$ ) of:										Resistant isolates	
	6.25	12.5	25	50	100	200	400	800	1,600	3,200	No.	%
$\text{Cd}^{2+}$							MIC <sup>a</sup>					
							41	166	1		167	80.3
$\text{Cr}^{3+}$												
								1	79	128	128	61.5
$\text{Cu}^{2+}$								MIC				
							2	20	179	7	186	89.4
$\text{Hg}^{2+}$	MIC											
	120	87	1								88	42.3
$\text{Mn}^{2+}$									MIC			
							133	69	1	5	5	2.4
$\text{Ni}^{2+}$									MIC			
								121	83	4	4	1.9
$\text{Pb}^{2+}$									MIC			
									41	167	167	80.3
$\text{Zn}^{2+}$								MIC				
								203	5		5	2.4

<sup>a</sup> MIC for the quality control strain *E. coli* K-12.

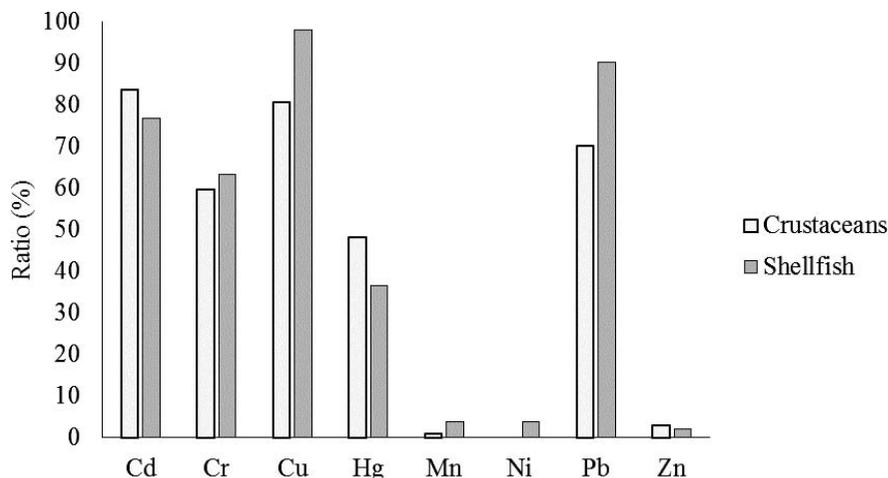


FIGURE 3. Prevalence of tolerance to heavy metals among *V. parahaemolyticus* isolates recovered from the five crustacean and five shellfish species.

AMP (94.2%) and RIF (93.3%) resistance was very prevalent among the *V. parahaemolyticus* isolates analyzed in this study, the majority of which also were resistant to STR (77.9%). Recently, high incidences of resistance to these three drugs have also been reported in *V. parahaemolyticus* isolates from some aquatic products in Asian and European countries, such as southern China (43), Korea (20), Italy (30), and Poland (23). The widespread occurrence of AMP- and STR-resistant bacteria may be attributed to the abuse of drugs and the inappropriate release of industrial wastes into environment (37). Runoff from pastures grazed by animals treated with antibiotics also may contribute to the problem of resistant bacteria. In the present study, all isolates were susceptible to CHL. This drug and its salts and esters (including chloramphenicol succinate) have been banned from the animal breeding industry in China (31), which may explain the results obtained in this study. Our findings are consistent with a recent report (43) that all 150 *V. parahaemolyticus* isolates recovered from aquatic products collected from South China markets were also susceptible to CHL. Although TET, sulfonamides, and quinolones are widely used in aquaculture (17), resistance to TET was not detected in most of the *V. parahaemolyticus* isolates in the present study. High percentages of intermediate susceptibility to KAN, SPT, and CN were found among the isolates tested in this study, suggesting a potential resistance trend for these drugs.

Environmental pollution is a challenging issue, particularly in developing countries with rapid industrialization (29). High incidences among bacteria of resistance to heavy metal contamination have been reported in various environments (e.g., marine, river, and agricultural soil) (3, 25, 33). The East China Sea has been a major fishing ground, especially within the Yangtze River plume and the surrounding sea along China's coast (13). However, the Yangtze River estuary area in Shanghai is one of the most rapidly developing economic areas in China and thus has suffered from heavy metal contamination (2, 47). Estuaries are zones of complex interaction between fluvial and marine process that act as a geochemical trap for heavy metals (9, 19). Yin et al. (46) reported higher health risks from exposure to Cu, Ni, and Pb in the near shore zones and to Zn, Cr, Cd, and Hg in the estuarine channel water. Yang et

al. (45) also reported that Hg, Pb, Zn, and Cu were present in significantly higher concentrations in topsoil than in deep soil layers along the Huangpu River in Shanghai. Consistent with these reports, in our study 89.4, 80.3, and 80.3% of the *V. parahaemolyticus* isolates derived from aquatic products from Shanghai fish markets in 2014 were tolerant to Cu, Pb, and Cd. High tolerance to Cr (61.5%) and Hg (42.3%) were also observed among these isolates. High levels of heavy metal tolerance also were found in 97 gram-negative bacterial isolates (*E. coli*, *Aeromonas hydrophila*, and *Stenotrophomonas maltophilia*) recovered from shrimp along the south coast of Turkey (27).

The abundance of bacteria resistant to two or more antimicrobial agents, particularly the *V. parahaemolyticus* isolate identified in this study that was resistant to eight agents, could be a cause of serious concern owing to the potential health impacts of consuming contaminated products (17). In this study, the *V. parahaemolyticus* isolate recovered from the crustaceans *P. monodon* and *M. japonicus* was resistant to the highest number of antimicrobial agents (8 of 10), suggesting that these species may be important reservoirs of antibiotic resistant bacteria. The two classes of aquatic species had similar heavy metal resistance patterns, suggesting that inappropriate release of industrial wastes may affect aquaculture environments.

Previous research indicated that the dissemination of antimicrobial and heavy metal resistance determinants could be a result of gene transfer, resulting in the increasing prevalence of mobile genetic elements (e.g., SXT/R391 family of ICEs) in marine aquaculture environments (32). The SXT/R391-like ICEs have been reported in *Vibrio* spp., and all of these ICEs possessed highly conserved core genes and three mode structures (41). Individual ICEs bestow a wide range of phenotypes on their hosts, such as resistance to antibiotics and heavy metals (41). In our study, MDR and tolerance to heavy metals were very prevalent among the *V. parahaemolyticus* isolates. However, only 4.8% of the isolates harbored the four core genes of SXT/R391-like ICEs, consistent with previous reports (34). The transfer of these ICEs will be further investigated via conjugation experiments and DNA sequencing analysis in our future research. Our data also revealed a low percentage of plasmid-positive isolates (22.6%), considering the consider-

ably higher incidence of antibiotic resistance. The accessory genes (e.g., antibiotic resistance genes) carried on plasmids can be highly beneficial to bacteria for increasing fitness in various environments (18). Nevertheless, only four antibiotic resistance-associated genes (*SullI*, *StrA*, *StrB*, and *aadA*) were found in this study in a few isolates harboring the plasmids (data not shown). These results suggested that *V. parahaemolyticus* in these aquatic species may have adopted some other molecular mechanisms that mediated the high prevalence of resistance determinants.

In summary, we isolated and characterized 208 *V. parahaemolyticus* strains from 10 species of commonly consumed crustaceans and shellfish that were collected from Shanghai fish markets in 2014. An extremely low occurrence of pathogenic *V. parahaemolyticus* (*trh*<sup>+</sup>, 1.9%) was detected. However, high incidences of antibiotic resistance were observed among the isolates, especially to AMP (94.2%), RIF (93.3%), and STR (77.9%). Approximately 74.5% of the isolates had MDR phenotypes. Tolerance to the heavy metals Cu<sup>2+</sup>, Pb<sup>2+</sup>, and Cd<sup>2+</sup> was found in the majority of antibiotic resistant isolates. However, the resistance patterns differed among the tested species. The *V. parahaemolyticus* isolates from *P. monodon* and *M. japonicus* were resistant to the highest number of antimicrobial agents (8 of 10), whereas isolates from *S. subcrenata* had the opposite pattern (resistant to 3 of 10 agents). Isolates recovered from *L. vannamei* and *B. canaliculatus* were tolerant to all eight heavy metals tested in this study, whereas those from *P. clarkii* and *T. granosa* were resistant to four of the eight heavy metals. Inconsistent with the wide distribution of MDR and heavy metal resistance, lower prevalences of plasmid DNA (22.6%) and SXT/R391-like ICEs (4.8%) were detected among the isolates, suggested that the *V. parahaemolyticus* isolates in these aquatic species may have adopted some other molecular mechanisms to mediate the high prevalence of resistance determinants. This study provided data on the molecular ecological of *V. parahaemolyticus* that highlight the need for food safety risk assessments of aquatic products.

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