INCREASED INCIDENCE AND ANTIMICROBIAL RESISTANCE AMONG *VIBRIO PARAHAEMOLYTICUS* IN SHELLFISHES FROM MAJOR FISH MARKETS IN COCHIN, SOUTH INDIA: SEAFOOD RISK ASSESSMENT

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Abstract

India remains as a top supplier of value-added seafoods to the global markets. Cochin is one of the leading fishing ports in India contributing to country's major seafood export. As part of risk assessment, we analyzed the prevalence of multi-drug resistant *Vibrio parahaemolyticus*, seafood borne gastroenteritis-causing pathogen, in shellfishes collected from major fish markets in Cochin. This bacterial species was initially isolated on thiosulfate-citrate-bile salts-sucrose agar and HiCrome™ *Vibrio* agar, further confirmed by detection of *V. parahaemolyticus* species-specific *toxR* gene and by 16S rRNA sequencing. A total of 113 confirmed *V. parahaemolyticus* were recovered. Almost all the strains exhibited resistance towards three or more antibiotics (multiple-drug resistant) and harbored virulence related exoenzymes especially hemolysin (Kanagawa phenomenon), which is indeed a matter of concern. The multiple antibiotic resistance (MAR) index of the isolates ranged from 0.21 to 0.57. Furthermore, our results also indicate a substantial increase in the incidence of the pathogen in seafood from the area compared to previous years. This may pose a risk to the local and international consumers of the seafood. Practicing complete hygiene and adequate cooking is strongly recommended. Regular monitoring of aquaculture areas and fish markets is urged to reduce dissemination of pathogenic *V. parahaemolyticus* in seafood and to prevent fatal cases of human infection induced by this species in the country.

Key words: *V. parahaemolyticus*, seafood, risk assessment, antimicrobial resistance, India

Indian fisheries and aquaculture is an important food sector providing nutritional security, besides livelihood support and gainful employment to more than 14 million people of the country (Dash et al., 2020). Besides, fish and fish products form the largest group in agricultural exports from India and constitute the major protein source of the country. Fisheries has a major role in India's food export basket contributing to 49% of animal product exports from the country (Statistical Year Book India, 2017). The Kerala state of India is abundantly rich with marine, brackish water and fresh water resources. The State with a long coast line is a significant contributor to the total marine fish landings of the country and a major seafood exporter from the country (Kulkarni, 2005).

One of the major threats to the Indian fishery sector is the bacterial contamination of the seafood and presence of prohibited drugs; leading to rejection of tons of export consignment each year (Henson et al., 2004).

Among the various diseases, vibriosis caused by pathogenic *Vibrio* spp. is the most common which has led to mass mortality among fish and shellfish in Asian countries (Ananda Raja et al., 2017 a, b, c; Ina-Salwany

et al., 2019). Shellfish and other aquatic organisms are frequently associated with pathogens such as *Vibrio parahaemolyticus* and they are known as vehicles for the transmission of this bacterium (Malcolm et al., 2015; Yu et al., 2016; Ananda Raja et al., 2017 a, b). Ingestion of seafood contaminated with *V. parahaemolyticus* may lead to seafood-borne gastroenteritis, and the common symptoms include watery diarrhea, abdominal cramps, nausea, vomiting, fever, headache and/or bloody diarrhoea (Humphries and Linscott, 2015; Ananda Raja et al., 2017 a).

The major factors involved in pathogenicity of *V. parahaemolyticus* are thermostable direct haemolysin (TDH) and TDH-related haemolysin (TRH) (Honda et al., 1987 a, b). However, absence of *tdh* and *trh* was reported in clinical strains of *V. parahaemolyticus* (Pazhani et al., 2014). This suggests that pathogenicity of this bacterium is rather complex and involves multiple factors.

India is the leading antibiotic consumer in the world closely followed by China and the USA (Van Boeckel et al., 2014) and is also one among the top five consumers of antimicrobials in food animals. Antibiotics are widely

used by Indian farmers to control and treat diseases. Tetracycline, quinolones, oxytetracycline, trimethoprim and sulphonamides are among the antibiotics commonly used in the Asian aquaculture industry (Rico et al., 2012; Yano et al., 2014). The unnecessary or overuse of antibiotics in aquaculture sector results in the emergence of multi-drug resistant 'superbugs' and the dissemination of the antibiotic-resistant genes in the environment. It also leads to accumulation of antibiotic residues in aquatic organisms such as fish (Miranda et al., 2018).

The unsustainable practices have taken the aquaculture industry on a rollercoaster ride, bringing it close to collapse. The present situation is such that the world is running short of effective drugs for critical treatments. If not dealt seriously, this could lead to a post-antibiotic era where people will start dying even due to simple bacterial infections. Hence, regular surveillance and reporting of antibiotic resistance is particularly important.

In India drug-resistant infections are the highest in the world (Laxminarayan and Chaudhury, 2016). Every year, many pathogenic *Vibrio* spp. are reported to develop high degree of resistance to many clinically important antibiotics (Letchumanan et al., 2015). The increasing emergence and spread of antibiotic-resistant *V. parahaemolyticus* strains is a matter of major concern in many countries (Ahmed et al., 2018; Elmahdi et al., 2016; Letchumanan et al., 2014).

The consumption of seafood contaminated with these multi-drug resistant pathogens can cause serious health

risks for the consumers. Seafoods are among the major commercially valuable food items for local and export trade. Previous study has reported incidence of the pathogen in seafood from Cochin market, India (Sudha et al., 2014). However, regular monitoring is required to look for any increasing trend in the prevalence of the pathogen so as to ensure public health safety.

Taking into consideration the epidemiological importance of *V. parahaemolyticus* and seafood risk assessment, the present study aimed to verify antibiotic susceptibility and virulence factors among *V. parahaemolyticus* isolated from shellfishes commercialized in the major fish markets in Cochin, South India.

Material and methods

Sampling

Shrimp and crab samples (n=21, 21) were collected from three major fish markets (Munambam, Varapuzha and Thoppumpady) in Cochin located in Kerala, South India. The map displaying the location of the study area is shown in Figure 1. High prevalence of *V. parahaemolyticus* is usually reported during the summer months. Hence, sampling was conducted during the summer of May, 2019. The samples (whole; 7 samples of shrimp/crab from each location) were collected aseptically in sterile ziplock bags and transported to the laboratory in an icebox.

Figure 1. Map showing the details of the study locations

Sample processing and enrichment

All samples were processed within 4 hours after the collection. Samples were washed thoroughly with sterile normal saline prior to bacteriological examination. The whole of shrimps and flesh from the main body part of crabs were blended in a stomacher. For enrichment, 25 g of the sample was added to 225 ml of alkaline peptone water (APW) and incubated at 37°C for 18–24 hours for isolation of *V. parahaemolyticus* (Ananda Raja et al. 2017 a).

Isolation and identification of *V. parahaemolyticus*

A loopful of enrichment broth was aseptically streaked onto sterile surface dried thiosulphate citrate bile salt sucrose (TCBS; Himedia, India) agar plates, and incubated overnight at 37°C. About 3–4 colonies having a green or bluish-green colour were picked from each TCBS plate (Atlas and Snyder, 2013). The preliminary identification of *Vibrio* species was performed using Gram staining, oxidase test and oxidative-fermentative test as previously described (Silvester et al., 2015).

A total of 158 presumptive *Vibrio* isolates (Gram negative, oxidase positive and fermentative without gas production) were obtained from TCBS agar. These isolates were streaked onto a chromogenic medium for further confirmation (HiCrome™ *Vibrio* agar, Himedia). The isolates producing species-specific bluish-green colonies on HiCrome™ *Vibrio* agar (Supplementary Figure 1) was considered as *V. parahaemolyticus* as previously described (Silvester et al., 2015). The colonies were maintained on nutrient agar slants supplemented with 3% NaCl for further identification.

Doxycycline hydrochloride (DO) – 10 µg, Erythromycin (E) – 15 µg, Gentamycin (GEN) – 10 µg, Meropenem (MRP) – 10 µg, Nitrofurantoin (NIT) – 100 µg, Penicillin (P) – 10 µg, Streptomycin (ST) – 10 µg and Tetracycline (TET) – 30 µg were used. After overnight incubation at 37°C, the inhibition zone was measured and compared with the standard Hi-Media interpretation chart based on recommendations of Clinical Laboratory Standards Institute (CLSI, 2015) and classified as Resistant, Intermediate and Susceptible. The MAR index value of the isolates was calculated by taking the ratio of the number of antibiotics to which the isolate is resistant to the total number of antibiotics to which the isolate is exposed (Krumperman, 1983).

Phenotypic virulence screening

The isolated *V. parahaemolyticus* strains were further screened for their virulence potential by verifying the phenotypic production of extracellular virulence enzymes. The enzymes screened were amylase, gelatinase, lipase, phospholipase, hemolysin (β-hemolysis) and urease. β-hemolysis (Kanagawa phenomenon) test was done on Wagatsuma blood agar (Himedia, India) (Su and Lee, 1997). Plate assay was used for all the enzymes except for urease which was screened by tube test. Enzyme screening methods previously described in Silvester et al. (2017) were followed.

Statistical analyses

IBM SPSS version 22 (IBM Corporation, New York, USA) was used for the analysis. Pearson's Chi-squared test was applied to test significant differences in the prevalence of antibiotic resistance and extracellular enzymes among isolates from various sources. Statistical significance level was set at 5%.

Results

Isolation of *V. parahaemolyticus* from shrimp and crab samples

V. parahaemolyticus was present in all the tested shrimp and crab samples (n=42). Those isolates which produced *V. parahaemolyticus* specific bluish-green-coloured colonies on HiCrome™ *Vibrio* agar and gave positive amplification for species-specific *toxR* gene were confirmed as *V. parahaemolyticus*. It is to be noted that all the isolates which gave species-specific coloured colonies on HiCrome agar were positive for *toxR* gene, thus indicating the efficacy of the chromogenic media for accurate identification of the species. Further, 16S rRNA sequence of TC7 strain exhibited 99.46% identity to 16S rRNA sequence of *V. parahaemolyticus* strain GH289 (id: MG593218.1) present in the GenBank database thus confirming our strain to be *V. parahaemolyticus*. The sequence of TC7 was submitted and allotted with a GenBank accession number MW326758.

Among the shrimp samples 60 isolates out of 72 pre-

sumptive colonies (85%; 18 isolates from Munambam, 30 isolates from Varapuzha and 12 from Thoppumpady) were *V. parahaemolyticus*. Similarly, 53 out of 86 isolates from crab samples (61.6%; 9 isolates from Munambam, 28 from Varapuzha and 16 from Thoppumpady) were found to be *V. parahaemolyticus*. Overall, 113 *V. parahaemolyticus* were isolated from the tested samples, among which highest prevalence was observed in samples collected from Varapuzha (51.3%).

Prevalence of antibiotic resistance among *V. parahaemolyticus* isolates from crab

Table 1 gives the percentage of antibiotic resistance among the crab isolates from the three stations. Majority of the *V. parahaemolyticus* strains (94%) from crabs were multiple drug resistant (MDR). Thoppumpady isolates showed 100% resistance to three of the antibiotics namely AMC, CTX and P and 68.75% were resistant towards ST. They were completely sensitive to six of the total antibiotics used; C, CL, COT, DO, NIT and TET. Isolates obtained from Munambam station showed complete resistance (100%) to P, 77.8% and 66.6% were resistant to ST and CTX respectively. All the isolates (100%) tested from this station showed susceptibility to six of the antibiotics namely C, CL, COT, DO, NIT and TET. All strains isolated from Varapuzha showed resistance towards AMC, CTX, P and ST. One hundred percent sensitivity was shown by the strains towards six of the antibiotics, namely C, CL, COT, DO, NIT and TET. By comparing the results, we could conclude that all the isolated strains of *V. parahaemolyticus* from all the three stations were completely sensitive to six of the drugs: C, CL, COT, DO, NIT and TET. Few strains showed varying degrees of intermediate resistance to few of the drugs tested (Table 1).

The MAR index of isolates ranged from 0.21 to 0.43. Comparing between the stations, the MAR index of Thoppumpady isolates ranged from 0.21 to 0.36, that of Munambam ranged from 0.21 to 0.43 and from Varapuzha ranged from 0.29 to 0.43. From the present study, a total of ten different antibiotic resistance patterns were obtained among isolates from crab samples. Majority of the isolates were resistant to 3 or more antibiotics. Quadruple resistance was exhibited by about 70% of the isolates (n=37). Few isolates showed resistance to five, six, or even seven antibiotics (n=7). AMC, CTX, P, ST (n=20) was the most repeated antibiotic resistance phenotype pattern observed followed by AMC, CTX, E, P, ST (n=8) and AMC, CTX, E, MRP, P, ST (n=6).

Prevalence of antibiotic resistance among *V. parahaemolyticus* isolates from shrimp

Table 2 represents the percentage of antibiotic resistance among the shrimp isolates from three stations. All the *V. parahaemolyticus* isolates from shrimp were found to be multiple drug resistant. The isolates from Thoppumpady were resistant to AMC, CTX and P, and sensitive towards C, NIT and TET. A high percentage (83.3%)

of resistance was also exhibited towards ST and CL. Isolates from Munambam showed 100% resistance to AMX, CTX, P and ST. The isolates were 100% sensitive towards C, CL, COT, DO, NIT and TET. A high percentage of resistance was also exhibited towards MRP (83.3%). The isolates from Varapuzha showed 100% resistance to the antibiotics AMC, CTX, MRP and P. High level of resistance (83.3%) was observed towards ST also. The isolates were highly sensitive to NIT (100%) and CIP (83.3%). Thus, we found that all the shrimp isolates from three stations showed resistance to AMC, CTX and P and all were sensitive to NIT.

The overall MAR index of the isolates from the three sampling stations ranged from 0.28 to 0.57; Munambam 0.28–0.42, Varapuzha 0.35–0.57 and Thoppumpady 0.28–0.57. All the isolates showed resistance to 3 or more antibiotics thus revealing them to be multi-drug resistant. A total of 11 different antibiotic resistant phenotypes were observed among shrimp isolates. The most repeated antibiotic resistance pattern observed among the isolates was AMC, CTX, E, MRP, P, ST (n= 16) followed by AMC, E, MRP, P, ST (n=6) and AMC, CTX, CL, P, ST (n=6).

Phenotypic virulence enzyme screening

Extracellular virulence enzyme profiling revealed that majority of the isolates produced most of the virulence enzymes. The most potential virulence phenotypes of *V. parahaemolyticus* were hemolysin, lipase, amylase and phospholipase production, from both crab and shrimp samples (Figure 2). Urease and gelatinase production were the least expressed phenotypes from both the samples. The Kanagawa phenomenon (β -hemolysis) was observed in almost all the strains from both shrimp and crabs. This confirms the presence of *tdh* (thermostable direct hemolysin) gene in the *V. parahaemolyticus* isolates because only TDH protein induces beta-hemolysis in Wagatsuma medium. There was significant difference in hemolysin, gelatinase and phospholipase production among isolates from crab and shrimp samples ($P<0.05$) (Figure 2). However, there was no significant difference in lipase, urease and amylase production ($P>0.05$). The extracellular enzyme profile of the *V. parahaemolyticus* from crab showed the following dominance pattern: hemolysin>lipase> phospholipase>amylase>gelatinase>urease. The isolates from shrimp exhibited the following extracellular enzyme profile: hemolysin>amylase>lipase>phospholipase>gelatinase=urease.

Table 1. Antibiotic resistance profiles of *V. parahaemolyticus* isolates from crab samples represented in percentage as Resistant (R), Intermediate (I), Susceptible (S)

Antibiotics	Thoppumpady (%) (n=16)			Munambam (%) (n=9)			Varapuzha (%) (n=28)			P-value
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	
AMC 10	16 (100)	0	0	5 (55)	4 (45)	0	28 (100)	0	0	.000
CTX 30	16 (100)	0	0	6 (67)	3 (33)	0	28 (100)	0	0	.000
C 10	0	0	16 (100)	0	0	9 (100)	0	0	28 (100)	–
CIP 5	0	16 (100)	0	0	5 (55)	4 (45)	0	28 (100)	0	–
CL 10	0	0	16 (100)	0	0	9 (100)	0	0	100	–
COT 25	0	0	16 (100)	0	0	9 (100)	0	0	100	–
DO 10	0	0	16 (100)	0	0	9 (100)	0	0	100	–
E 15	8 (50)	50	0	3 (33)	6 (67)	0	9 (33)	19 (67)	0	.017
GEN 10	3 (19)	8 (50)	5 (31)	0	5 (55)	4 (45)	5 (17)	19 (67)	4 (14)	.000
MRP 10	5 (31)	11 (69)	0	3 (33)	6 (67)	0	9 (33)	19 (67)	0	–
NIT 100	0	0	16 (100)	0	0	9 (100)	0	0	28 (100)	–
P 10	16 (100)	0	0	9 (100)	0	0	28 (100)	0	0	–
ST 30	11 (69)	5 (31)	0	7 (78)	0	2 (22)	28 (100)	0	0	.000
TET 30	0	0	16 (100)	0	0	9 (100)	0	0	28 (100)	–

Table 2. Antibiotic resistance profiles of *V. parahaemolyticus* isolates from shrimp samples represented in percentage as Resistant (R), Intermediate (I), Susceptible (S)

Antibiotics	Thoppumpady (%) (n=12)			Munambam (%) (n=18)			Varapuzha (%) (n=30)			P-value
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	
AMC 10	12 (100)	0	0	18 (100)	0	0	30 (100)	0	0	–
CTX 30	12 (100)	0	0	18 (100)	0	0	30 (100)	0	0	–
C 10	0	0	12 (100)	0	0	18 (100)	0	20 (67)	10 (33)	–
CIP 5	2 (17)	10 (83)	0	0	15 (83)	3 (17)	5 (17)	25 (83)	0	.000
CL 10	10 (83)	0	2 (17)	0	0	18 (100)	15 (50)	0	15 (50)	.000
COT 25	0	2 (17)	10 (83)	0	0	18 (100)	0	10 (33)	20 (67)	–
DO 10	0	2 (17)	10 (83)	0	3 (17)	15 (83)	0	10 (33)	20 (67)	–
E 15	6 (50)	6 (50)	0	6 (33)	12 (67)	0	15 (50)	15 (50)	0	0.20
GEN 10	0	10 (83)	2 (17)	0	18 (100)	0	0	20 (67)	10 (33)	–
MRP 10	6 (50)	6 (50)	0	15 (83)	3 (17)	0	30 (100)	0	0	.000
NIT 100	0	0	12 (100)	0	0	18 (100)	0	0	30 (100)	–
P 10	12 (100)	0	0	18 (100)	0	0	30 (100)	0	0	–
ST 30	10 (83)	2 (17)	0	18 (100)	0	0	25 (83)	5 (17)	0	.000
TET 30	0	0	12 (100)	0	0	18 (100)	0	15 (50)	15 (50)	–

Figure 2. Graph showing the relative extracellular virulence enzyme production among *V. parahaemolyticus* strains from crab and shrimp samples

Figure 3. Graph showing the percentage of extracellular enzyme production of *V. parahaemolyticus* strains from crab samples

Figure 4. Graph showing the percentage of extracellular enzyme production of *V. parahaemolyticus* strains from shrimp samples

Comparing the station-wise enzyme production, a significant difference was observed in production of all enzymes ($P < 0.05$) except lipase among crab isolates (Figure 3). Similarly, a significant difference was observed between shrimp isolates from three stations ($P < 0.05$) (Figure 4). Hemolysin producers dominated in shrimps and crabs from Varapuzha and the least produced was gelatinase and urease. All the shrimp isolates from Thoppumpady produced amylase and hemolysin and all crab isolates produced hemolysin. Urease producing isolates were absent in samples from this station. Among the Munambam isolates, all from the crabs and majority from shrimp produced hemolysin. Urease producers were least

expressed among crab samples and absent among shrimp samples.

Discussion

In the present study, *V. parahaemolyticus* was detected in all the shrimp and crab samples (100%) collected from major fish markets in Cochin. In a previous study on finfish and shellfish collected from Cochin during the year 2015–16, 90% of the samples were positive for *V. parahaemolyticus* (Narayanan et al., 2020). Whereas in another study on shellfishes collected from

the same study area during 2010–11, only 68% samples harboured *V. parahaemolyticus* (Sudha et al., 2014). This clearly shows that the incidence of the pathogen has increased over these years. In a study from Malaysia, 44% of the shellfish samples collected was contaminated with *V. parahaemolyticus* (Letchumanan et al., 2015). Tran et al. (2018) reported that 86.2% of the seafood samples collected from Vietnam were found to be positive for *V. parahaemolyticus*. In a recent study from China, *V. parahaemolyticus* was isolated from fresh aquatic products including 19 species of shellfish, 2 species of crustaceans, and 2 species of fishes collected from Shanghai fish markets (Su and Chen, 2020).

The rise in multiple antibiotic resistance and antibiotic resistance genes (ARGs) among pathogenic vibrios in seafood is a threat to the public health. This poses a serious health risk as the seafood may act as vehicles for transmission of resistant strains from environment to consumers *via* ingestion of contaminated food. In the present study, majority of the *V. parahaemolyticus* strains isolated from seafoods exhibited multi-drug resistance. The MAR index of the isolates from shrimp samples ranged from 0.28 to 0.57 and those from crab ranged from 0.25 to 0.43. MAR index >0.2 is said to be originated from high risk sources of contamination. The antibiotics usually recommended for *Vibrio* infections are cephalothin, cefuroxime, cefotaxime, ceftazidime, tetracycline, doxycycline, fluoroquinolone, amikacin, gentamicin and trimethoprim-sulfamethoxazole (CDC, 2013; Letchumanan et al., 2015). All the shrimp isolates from three stations exhibited resistance to amoxycylav, cefotaxime and penicillin and all were sensitive to nitrofurantoin. Our results are in agreement with previous reports stating that *V. parahaemolyticus* is becoming resistant toward third and fourth generation cephalosporins (Narayanan et al., 2020; Park et al., 2018; Vaseeharan et al., 2005; Yu et al., 2016). In our study in 2015, all *V. parahaemolyticus* isolates from shrimp farms along the Cochin estuary demonstrated sensitivity towards streptomycin, gentamicin and netillin and a high percentage of resistance was also observed towards beta lactams, sulphamethoxazole and erythromycin and low-level resistance to nitrofurantoin (Silvester et al., 2015). However, in the present study, a high level of resistance was observed towards streptomycin and complete sensitivity to nitrofurantoin, thereby clearly indicating a change in the antibiotic resistance pattern. In another recent study from Malaysia *V. parahaemolyticus* isolates displayed high levels of resistance to ampicillin, cefazolin, and penicillin, as well as intermediate levels of resistance to cefotaxime and ciprofloxacin (Tan et al., 2020). In a study from Palk Bay, the *Vibrio* isolates exhibited resistance towards beta-lactams, vancomycin, nitrofurantoin, gentamicin, azithromycin, oxytetracycline, tetracycline and chloramphenicol (Sneha et al., 2016). A rising trend is observed in the presence of multi-drug resistant *V. parahaemolyticus* from aquatic origin (Xie et al., 2016). Multiple drug resistant *V. parahaemolyticus* was previously isolated from fish landing centers

and retail markets from South India (Reyhanath and Kutty, 2014; Sudha et al., 2014). All *Vibrio* isolates from previous study were susceptible towards chloramphenicol, tetracycline and nalidixic acid (Sudha et al., 2014). The isolates from our study areas showed high level of susceptibility towards chloramphenicol, co-trimoxazole (trimethoprim-sulfamethoxazole), doxycycline, nitrofurantoin and tetracycline. These findings are in agreement with previous reports from various countries highlighting susceptibility of *V. parahaemolyticus* from shrimp and other seafoods towards chloramphenicol, cotrimoxazole, doxycycline, tetracycline (Letchumanan et al., 2015; Narayanan et al., 2020; Tan et al., 2020). Hence, these drugs could be used to treat *V. parahaemolyticus* infections from our area. Recent studies have highlighted the role of bacteriophages as potential antibiotic alternatives against vibrios (Letchumanan et al., 2016). Natural bacteriophages do not affect the health of fish or consumers. Phages pose significant advantages, such as being environmentally friendly and easily available in the environment and having greater host specificity and cheaper than antibiotics (Kalatzis et al., 2018).

Detection of exoenzymes in microorganisms isolated from foods represents a risk to the communities that consume the food because they tend to be more exposed to foodborne infections (Silva et al., 2018). Majority of the *V. parahaemolyticus* isolates in our study produced the virulence related exoenzymes. This highlights the potential of the bacterium to turn into pathogenic under favorable conditions. The urease and hemolysin enzymes are considered the typical virulence markers for *V. parahaemolyticus* from clinical sources. Urea hydrolysis is known to be directly linked with the presence of *trh* gene and beta-hemolysis (Kanagawa) with presence of *tdh* gene (Nair et al., 2007). Even though urease producers were least in our samples, hemolysin producers dominated in both shrimp and crab samples, revealing the presence of *tdh* gene and virulence potential of the *V. parahaemolyticus* strains. This shows the capability of *V. parahaemolyticus* strains to cause beta-hemolysis of human erythrocytes, thereby posing a risk to consumers of these seafoods. In a previous study among *V. parahaemolyticus* from Cochin estuary, around 42.3% of the isolates produced hemolysin and 23.5% produced urease (Silvester et al., 2017). Lipases and phospholipases were also produced by our strains; these enzymes mainly contribute in nutrient acquisition and degrading the membrane lipid of the host, thus involving in the pathogen's virulence (Costa et al., 2013). The role of amylases and gelatinases in the human bacteremia also need to be confirmed (Vergis et al., 2002).

Conclusion

The study highlights the presence of multiple antibiotic resistant *V. parahaemolyticus* with virulence potential in the seafood samples. Further studies have to be conducted to screen the strains for the presence of various antibiotic resistant genes and also virulence genes such as *tdh*, *trh*, *t3ss*, *t6ss* etc. Our study reveals that seafoods may act as potential vehicle of transfer of antibi-

otic resistant *Vibrio* to humans via ingestion of such contaminated food, thus risking consumer health. Though localised in nature these findings may have international significance since seafoods from our area are exported worldwide. In addition, it is to be noted that seafoods contaminated with banned antibiotics may lead to export rejection thus affecting our economy too. Surveillance and monitoring of the seafoods for multi-drug resistant *Vibrio* with virulence potential are needed on regular basis to guarantee the safety of the public health and for food security of the country.

Acknowledgement

The authors would like to thank Head, Dept. of Marine Biology, Microbiology and Biochemistry, CUSAT and J and J Biotech, Kerala, India for the facilities provided.

Conflict of interest

The authors declare that there is no potential conflict of interests.

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Received: 13 IV 2021

Accepted: 22 IX 2021