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Prevalence of multidrug-resistant *Vibrio* species in fish in a reduction trial using lemon juice and sesame oil

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ABSTRACT

Background: Because of its high nutritional value, excellent taste, and ease of digestion, fish is one of the most popular foods. However, it also serves as a vehicle for various pathogenic microorganisms, particularly *Vibrio* species, which pose a risk to public health.

Aim: This study targeted studying the prevalence of multidrug-resistant *Vibrio* species in three marine fish retained in Egypt. In addition, the antimicrobial activities of lemon juice and sesame oil against *Vibrio parahaemolyticus* were screened.

Methods: For these reasons, this study assessed the prevalence of *Vibrio* species in marine fish (mullet, brush tooth lizard, and coral fish) that are sold in the local market of Zagazig, Egypt. Additionally, certain virulence factors were detected in the recovered *V. parahaemolyticus* isolates. The antimicrobial susceptibility of the recovered *V. parahaemolyticus* isolates was also screened. The antivibrio activities of lemon juice and sesame oil (1% and 2%) were additionally tested.

Results: The acquired data showed that the prevalence of *Vibrio* species was 40% among the tested fish samples. *V. parahaemolyticus* was isolated at 20.8%. *Vibrio fluvialis* (8.3%), *Vibrio Mimicus* (8.3%), and *Vibrio alginolyticus* (2.5%). The recovered isolates were highly resistant to cefazolin (87.5%), ampicillin (77.1%), cephalothin (68.75%), penicillin, and streptomycin (100%, each). Lemon juice and sesame oil had marked antibacterial activities against *V. parahaemolyticus*.

Conclusion: Fish dipping in lemon juice and sesame oil was an effective way to lower the load of *V. parahaemolyticus* in fish. In addition, sanitary measures must be implemented to prevent microbial contamination in fish markets and aquatic environments.

Keywords: Fish, *Vibrio parahaemolyticus*, Antibiotic sensitivity, Virulence genes, Lemon juice, Sesame oil.

Introduction

The nutritional significance of fish has led to a simultaneous increase in consumption during the past ten years (Morshdy *et al.*, 2022a). Fish is a perishable, high-protein food that contains essential amino acids, vitamins, minerals, and Omega-3 fatty acids, all of which are vital to human life (Morshdy *et al.*, 2023). According to Ibrahim *et al.* (2018), polyunsaturated fatty acids found in fish protect consumers from coronary heart disease by lowering

the risk of thrombosis, arrhythmias, fatal heart attacks, and sudden death. Given that fish have lower feed conversion rates, shorter production cycles, and lower rearing and feeding costs than other animal proteins, they may be able to contribute to solving the animal protein shortage issue (Liu *et al.*, 2018). Marine fish, particularly the mullet, brush-tooth lizard, and coral fish, can easily harbor pathogenic microorganisms because of their filter-feeding nature and the microbial

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overcrowded habitat, particularly of the *Vibrio* species (Austin and Austin, 2012; Caburlotto et al., 2016). Regarding human diseases, the three most significant *Vibrio* species are *cholerae*, *parahaemolyticus*, and *vulnificus*. The clinical symptoms of *Vibrio* species vary according to the species in question (Tsai et al., 2013). An important human disease caused by seafood is *Vibrio parahaemolyticus*. Headache, nausea, vomiting, abdominal cramps, diarrhea, and low temperature are symptoms of acute gastroenteritis, which can be caused by raw or undercooked seafood contaminated with *V. Parahaemolyticus* (Chao et al., 2010). The overuse of antibiotics in medicine and agriculture has led to an increase in bacteria resistant to these drugs (Moore et al., 2014). Molecular methods such as polymerase chain reaction (PCR) assays are widely used to identify the harmful *V. parahaemolyticus* strain in environmental and dietary samples (Zaafrane et al., 2022). From a food safety perspective, it is essential to monitor microbiological contamination of seafood continuously.

Food shelf-life, safety, and marketability can all be increased by using natural antibacterial substances to preserve the product's fresh appearance (Jun et al., 2012). Researchers and consumers alike have become interested in natural bacteriostatic agents as concerns over artificial food preservation additive safety and consumer health have grown (Yassin et al., 2022). As secondary metabolites, plants produce phenolic chemicals, and certain plant phenolics or phenolic-rich extracts have been shown to remarkably inhibit a wide spectrum of bacteria (Darwish et al., 2020). Phenolic compounds can be used as seasonings to enhance product flavor and microbial quality (Mahmoud et al., 2021). According to Yasin et al. (2022) and Takó et al. (2020), phenolic compounds can alter the shape of bacteria, weaken bacterial cell walls, and affect biofilm formation. Sesamol is a phenolic compound that is found in sesame oil, meal, and seeds. It has been shown to have antioxidant, immunomodulatory, anticancer, antiangiogenic, and microbiological properties (Xie et al., 2021; Zhou et al., 2021). The principal organic acid in lemon juice, citric acid, is used to flavor and preserve food. Antioxidant and antibacterial properties make it a natural meat preservative (Nawi et al., 2017). The antibacterial activity of sesame oil against *Vibrio* species has not been investigated. Thus, this study assessed the prevalence of *Vibrio* species in fish samples collected from markets in the Sharkia Governorate, Egypt. The study involved biochemical identification, virulence gene detection, and antibiotic resistance profiling in *Vibrio* isolates. The antibacterial properties of sesame oil and lemon juice against *V. parahaemolyticus* were also investigated.

Material and Methods

Collection and preparation of samples

A total of 120 fish samples were collected at random from different fish stores in Zagazig City, Sharkia Governorate, Egypt. The samples included 40 mullets

(*Mugil cephalus*) (average weight 325 ± 40 g), brush-tooth lizards (*Saurida undosquamis*) (average weight 125 ± 30 g), and Coral fish (*Pagrus major*) (average weight 230 ± 40 g) (forty of each). The samples were promptly marked and forwarded to the Faculty of Veterinary Medicine, Laboratory of Meat Hygiene, Zagazig University, Egypt, for bacterial isolation and identification upon collection. The inedible fins were removed, the surface of the fish was sterilized with a hot spatula, and muscle samples were collected from each examined fish.

Isolation of *Vibrio* species

Following guidelines set out by the Food and Drug Administration (FDA, 2004), *Vibrio* species was successfully isolated. After meticulously measuring out 90 ml of sterile alkaline peptone water (APW, Micro Master, India) and 10 g of fish meat, the two were mixed together. Subsequently, according to the International Standardization Organization (ISO/TS, 21872-1, 2007), the mixture was kept at a temperature of $35 \pm 2^\circ\text{C}$ for 24–48 hours. A loopful of each APW broth was placed on thiosulfate citrate bile salt sucrose agar plates (TCBS, Hi-Media, India), and the plates were left to sit at 37°C for an entire day. After being picked, washed, and biochemically identified (arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase), citrate, and D-glucosamine utilization, Voges-Proskauer tests and growth in 8% NaCl were performed), colonies having a yellowish-green or greenish-blue hue were analyzed according to ISO/TS, 21872-1 (2007) and ISO/TS, 21872-2 (2007). The detection of somatic (O) and capsular (K) antigens was performed using *V. parahaemolyticus* antiserum (Hardy Diagnostics Co., USA) in accordance with the methods described by Ansaruzzaman et al. (2005).

Antimicrobial susceptibility testing

In accordance with Adeleye et al. (2008), the Kirby-Bauer disc diffusion method was used to assess the antimicrobial susceptibility of 48 *Vibrio* isolates to 16 different antibiotics: penicillin (P), Streptomycin (S), Cefozolin (CZ), Ampicillin (AM), Cephalothin (CN), ciprofloxacin (CP), Cefotaxime (CF), Tetracycline (T), Sulphamethoxazol (SXT), Gentamicin (G), Azithromycin (AZ), Ofloxacin (O), meropenem (M), and Chloramphenicol (C). Following the National Committee for Clinical Laboratory Standards (NCCLS's) guides (NCCLS, 2001), inhibitory zones were located.

Molecular characterization of *V. parahaemolyticus* isolates

For bacterial DNA extraction, a QIAamp DNA Mini kit (Catalog no. 51304) was used by QIAGEN (GmbH, Hilden, Germany). The thermolabile hemolysin (*tlh*), thermostable direct hemolysin (*tdh*), and thermostable related hemolysin (*trh*) genes amplification was performed using primers supplied by Metabion (Germany). The primer preparation, PCR cycling

conditions, and electrophoresis were according to (Bej et al., 1999).

Lemon juice and sesame oil as natural decontaminants

In the experimental trial, 25 mullet fish of the same size were used. Each fish was inoculated in the back muscle with 1 ml of *V. parahaemolyticus* broth, which was adjusted to 0.5 McFarland (6 log 10 cfu/ml). The fish that inoculated was maintained at room temperature (25°C) for half an hour. The inoculated fish were then split into groups and dipped in solutions made with two concentrations (1% and 2%) of lemon juice and sesame oil (Nawi et al., 2017; Morshdy et al., 2022a). The negative control was dipped in sterile distilled water.

Statistical analysis

The reduction counts were converted into log 10 relevant values. Values represent means \pm SD. On-way analyses of variance followed by the posthock Tukey's Kramer HSD test were performed using JMP

software provided by SAS institute, USA, with $p < 0.05$ being deemed significant.

Results

The prevalence of *Vibrio* species in the analyzed fish species, including mullets, brush-tooth lizards, and coral fish, is presented in Table 1. The bacteriological analysis of the fish samples indicated that *Vibrio* species were found in 40% of all the samples analyzed. *V. parahaemolyticus* was found in 25 of the 120 samples tested (20.8%), *Vibrio fluvialis* was found in 10 samples (8.3%), *Vibrio mimicus* was found in 10 samples (8.3%), and *Vibrio alginolyticus* was found in 3 samples (2.5%). The highest isolation rate was observed in mullets (42.5%), whereas the lowest was recorded in coral fish (37.5%).

Table 2 shows that all of the *Vibrio* isolates tested were completely resistant to penicillin and streptomycin (100%). They are also resistant to cefazolin (87.5%), ampicillin (77.1%), and cephalothin (68.75%).

Table 1. Prevalence of *Vibrio* species isolated from fish samples (N = 40 of each).

<i>Vibrio alginolyticus</i>	<i>Vibrio mimicus</i>	<i>Vibrio fluvialis</i>	<i>Vibrio parahaemolyticus</i>	No. of positive (%)	Fish
_____	4(10%)	3(7.5%)	10 (25%)	17(42.5%)	Mullet
3(7.5%)	_____	3(7.5%)	9 (22.5%)	15(37.5%)	Coral
_____	6(15%)	4(10%)	6 (15%)	16(40%)	Lizard
3(2.5%)	10(8.3%)	10(8.3%)	25 (20.8%)	48(40%)	Total

Table 2. Antimicrobial susceptibility of the *Vibrio* species (N = 48).

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Penicillin (P)	-	-	-	-	48	100
Streptomycin (S)	-	-	-	-	48	100
Cefozolin (CZ)	3	6.25	3	6.25	42	87.5
Ampicillin (AM)	-	-	11	22.9	37	77.1
Cephalothin (CN)	12	25	3	6.25	33	68.75
Amikacin (AK)	18	37.5	-	-	30	62.5
Ciprofloxacin (CP)	18	37.5	3	6.25	27	56.25
Cefotaxime (CF)	20	41.7	6	12.5	22	45.8
Tetracycline (T)	24	50.0	6	12.5	18	37.5
Sulphamethoxazol (SXT)	24	50.0	11	22.9	13	27.1
Gentamicin (G)	30	62.5	4	8.3	14	29.2
Azithromycin (AZ)	36	75	3	6.25	9	18.75
Ofloxacin (O)	39	81.25	-	-	9	18.75
Meropenem (M)	36	75	6	12.5	6	12.5
Chloramphenicol (C)	42	87.5	3	6.25	3	6.25
Imipenem (IPM)	45	93.75	-	-	3	6.25

S = sensitive; I = intermediate; R = resistance.

Concurrently, *Vibrio* species exhibited greater sensitivity to Imipenem (93.75%), Chloramphenicol (87.5%), and Meropenem (75%).

tlh was identified in all examined *V. parahaemolyticus* isolates. The *th* and *trh* virulence genes were identified in 71.5% of the analyzed isolates (Fig. 1).

This study evaluated the antibacterial efficacy of 1% and 2% lemon juice and sesame oil against *V. parahaemolyticus*. The findings indicate that the reduction counts of *V. parahaemolyticus* were 0.53, 0.93, 0.92, and 1.32, with reduction percentages of 8.82%, 15.47%, 15.30%, and 21.96%, respectively, following immersion in fresh lemon juice extract 1% and 2% for 1 and 2 hours. In addition, this study investigated how well sesame oil at 1% and 2% concentrations reduced bacterial counts as a possible method of decontaminating raw fish infected with *V. parahaemolyticus*. The results indicated that the reduction counts of *V. parahaemolyticus* were 0.38, 0.71, 0.81, and 0.91 with reduction percentages of 6.32, 11.81, 13.4, and 15.14 following immersion in fresh sesame oil 1% and 2% for 1 and 2 hours, respectively (Table 3). The results showed a positive correlation between the number of reductions and the time of exposure. In addition, after immersion in either 2% sesame oil or lemon juice for 2 hours, *V. parahaemolyticus* exhibited a significant decrease ($p <$

0.05). Results showed that fish treated with a 2-hours solution of lemon juice and sesame oil had a much lower prevalence of *V. parahaemolyticus*. Lemon juice had a much stronger reduction effect against *V. parahaemolyticus*.

Discussion

The risk of health problems increases when eating seafood contaminated with *Vibrio spp.*, an aquatic pathogen (Morshdy et al., 2023). Two main factors make marine fish susceptible to contamination by different microorganisms: (1) exposure to contaminated water; and (2) post-harvest contamination caused by improper handling, transportation, marketing, and storage. This kind of contamination can make fish very dangerous to eat or even unfit for human consumption (Sugumar et al., 2008). Water, soil, and people who handle the fish are some of the many potential causes of microbial contamination. According to Mahmoud et al. (2022), improper handling of fish during fishing, transportation, and shipping greatly increases the risk of cross-contamination and acts as a stressor, making it easier for germs to move from the fish's stomach to its muscles. Contamination of the tested fish likely occurred during transportation and sale, as evidenced by the isolation of *Vibrio* species from the samples. *Vibrio* species thrive in environments where fish are

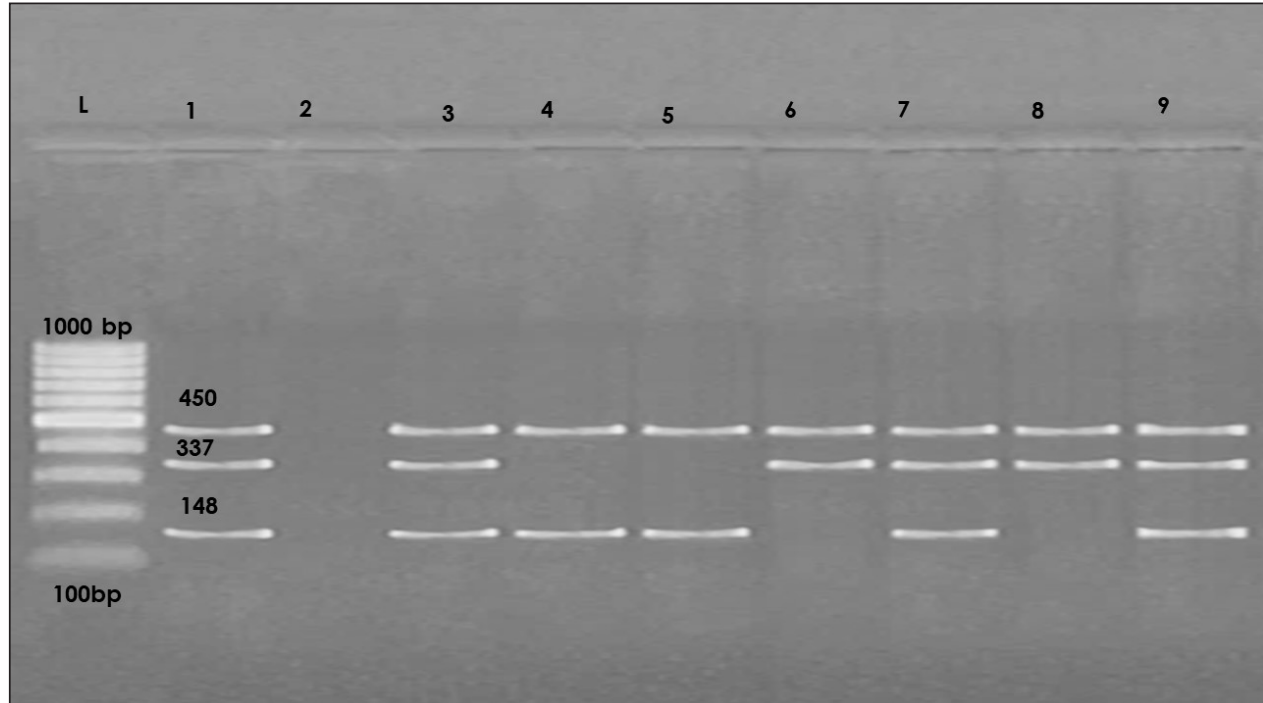


Fig. 1. Representative image for multiplex polymerase chain reaction for 1.5% agarose gel for *V. parahaemolyticus* characterization; *trh* (148 bp), *tdh* (337 bp) and *tlh* (450 bp). M: A ladder of 100 base pairs, 1: Positive control; 2: Negative control, 3, 7, 9: positive strains for *trh*, *tdh* and *tlh* genes, 4 & 5: positive strain for *trh* and *tlh* genes, 6 & 8: positive strains for *tdh*, and *tlh*, genes.

Table 3. Effects of lemon juice and Sesame oil on *Vibrio parahaemolyticus* counts log₁₀ cfu/g in fish after varying exposure periods.

Control	Lemon juice			Lemon juice			Sesame oil			Sesame oil		
	1%			2%			1%			2		
	1hour	2hours	1hour	1hour	2hours	2hours	1hour	2hours	1hour	1hour	2hours	2hours
Minimum	4.23	4.1	4.10	4.69	4	4	3.95	4.77	4.47	4.30	4.30	4.30
Maximum	6.30	5.63	5.4	5.90	5.75	5.75	5.7	5.90	5.96	5.60	5.60	5.60
Mean	6.01 ± 0.021 ^a	5.48 ± 0.069 ^{ab}	5.08 ± 0.041 ^b	5.09 ± 0.059 ^b	4.69 ± 0.064 ^c	4.69 ± 0.064 ^c	5.63 ± 0.036 ^{ab}	5.30 ± 0.041 ^b	5.2 ± 0.073 ^b	5.1 ± 0.087 ^c	5.1 ± 0.087 ^c	5.1 ± 0.087 ^c
R. count	0.53	0.93	0.93	0.92	1.32	1.32	0.38	0.71	0.81	0.91	0.91	0.91
Reduction %	8.82%	15.47%	15.47%	15.30%	21.96%	21.96%	6.32%	11.81%	13.47%	15.14%	15.14%	15.14%

R. Count = Before treatment (log₁₀) - After treatment (log₁₀), R.% = (Before treatment - After treatment) ÷ Before treatment = x100.

maintained at room temperature. The prevalence rates of *Vibrio* species in the current investigation were consistent with studies done in other countries. While 72.7% of the fish samples examined in Turkey tested positive for *Vibrio* species, only 59% of the fish samples in Malaysia tested positive (Radu *et al.*, 2003; Yu *et al.*, 2016). *V. parahaemolyticus*, *V. fluvialis*, and *V. mimicus* were the three most studied species of *Vibrio* species in fish-related studies (Morshdy *et al.*, 2022b). One hundred sixty-six (44%) of the fish samples tested positive for *Vibrio* species in Morshdy *et al.* (2021b). *Vibrio cholerae* was found in just one sample (0.7%), while *V. parahaemolyticus* was present in 28 samples (18.6%). Similarly, El-Sharaby *et al.* (2018) recorded an isolation rate of 49% for the *Vibrio* species in Egypt's freshwater fish. *Vibrio* species were found in 52% of the fish samples sold in Egypt, according to Morshdy *et al.* (2022a), among which 42.3% were *V. parahaemolyticus*, 26.92% were *V. mimicus*, 9.62% were *V. alginolyticus*, and 1.92% were *V. cholera*. According to Suresh *et al.* (2018), the majority of freshwater fish tested positive for *V. parahaemolyticus*, whereas a small percentage tested positive for *V. vulnificus*, 4.6% for *V. alginolyticus*, and 3.45% for *V. cholera*. Crustaceans sold in Egypt's Sharkia Governorate tested positive for 36 *Vibrio* isolates, representing 16% of the total. Among them, *V. parahaemolyticus* accounted for 15.1% and *V. cholerae* for 0.9% (Ahmed *et al.*, 2018). Freshwater fish in China were found to contain 10.33% *V. cholerae*, 3.89% *V. parahaemolyticus*, 1.24% *V. alginolyticus*, and 0.76% *V. vulnificus*, as reported by Yan *et al.* (2019). According to Jakšić *et al.* (2002), shrimp samples from Croatia had lower prevalence rates of *V. parahaemolyticus* (7.14%), *V. alginolyticus* (3.57%), and *V. vulnificus* (10.71%). The highest prevalence of *V. parahaemolyticus* in Selangor shrimp samples, Malaysia, was reported by Tan *et al.* (2020) at 88.57%. In Thailand, 38% of farmed shrimp samples tested positive for *V. parahaemolyticus*, whereas 26.67% of Chinese seafood and shrimp tested positive, according to research by Yano *et al.* (2014) and Jiang *et al.* (2019), respectively. In addition, Yücel and Balci (2010) discovered that *V. parahaemolyticus*, *V. vulnificus*, and *V. mimicus* were present in marine fish from Turkey at proportions of 40%, 18%, and 6%, respectively. Salinity, geographical location, seasonal fluctuations, and isolation procedures can affect the prevalence of different *Vibrio* species (Morshdy *et al.*, 2023). *Correlations of Vibrio species with environmental factors like temperature and salinity may shed light on why these organisms are so common. The prevalence of V. parahaemolyticus increased during the warmer months, which can be attributed to the higher temperatures (Morshdy et al., 2022a). In addition, microbes may enter a latent state in the bottom sediments and then reemerge into the water column when high temperatures resume (Pfeffer et al., 2003).*

Antibiotic residues in aquaculture products are associated with serious public health risks. Consistent with previous research, this investigation demonstrated that *V. parahaemolyticus* is resistant to many medications in Malaysia (Letchumanan *et al.*, 2015). A majority of these antibiotics were ampicillin (82%), followed by amikacin (51%), cefotaxime (37%), gentamicin (11%), ceftazidime (15%), levofloxacin (9%), and imipenem (2%). A large portion of the seafood imported into China was discovered to be resistant to the following antibiotics: ampicillin (95.46%), amikacin (30%), tetracycline (17.78%), sulphamethoxazole/trimethoprim (17.78%), and ciprofloxacin (2.22%), according to Jiang *et al.* (2019). Tan *et al.* (2020) reported that *V. parahaemolyticus* strains in Selangor, Malaysia, were not sensitive to ampicillin (84.17%), cephalothin (54.17%), amikacin (37.50%), ciprofloxacin (13.33%), gentamicin (6.67%), cefotaxime (5%), ceftazidime (5%), or levofloxacin (1.67%). A study was conducted in the region. In the same way, Jiang *et al.* (2019) found that imipenem and meropenem worked well to treat all *V. parahaemolyticus* isolates obtained from different types of Chinese seafood. According to Tan *et al.* (2020), *V. parahaemolyticus* is resistant to tetracycline (94.17%), imipenem and meropenem (98.33%), levofloxacin (73.33%), ceftazidime (70.83%), gentamicin (64.17%), and levofloxacin (73.33%). The results demonstrate the importance of regularly testing different types of *Vibrio* found in nature against a large number of antimicrobial drugs and treatments that are used in medicine. In this way, useful baseline data can be collected in the laboratory, which can be used in real-life therapy (Baker-Austin *et al.*, 2008).

Several studies from around the world have found a link between *V. parahaemolyticus* isolates that have *tdh* and/or *trh* genes and their ability to cause disease (Nordstrom *et al.*, 2007; Ahmed *et al.*, 2018). According to Morshdy *et al.* (2023), the vast majority of *V. parahaemolyticus* strains found in the environment do not cause disease. However, when it comes to clinical isolates, 99% are harmful because they include the *tdh* and/or *trh* genes. Although a small percentage of environmental isolates carrying the *tdh* and/or *trh* genes are considered hazardous, this number is still rather low (Letchumanan *et al.*, 2014). For several reasons, it is important to identify *tdh* and/or *trh* *V. parahaemolyticus* in freshwater and marine fish samples. These infectious microbes may initially cause gastrointestinal illness (Jun *et al.*, 2012). Additionally, according to Thongjun *et al.* (2013), pathogenic *V. parahaemolyticus* causes significant economic losses in the aquaculture sector due to disease propagation and contamination of seafood. Unlike a previous study by Letchumanan *et al.* (2015), which found the presence of *trh* and *tdh* in *V. parahaemolyticus* isolates from Malaysian shrimp sales 10% of the time, the present investigation revealed a higher number of virulent genes

(*tdh* and *trh*) in *V. parahaemolyticus* strains. Although Tan *et al.* (2020) reported that *V. parahaemolyticus* strains found in seafood samples from Selangor, Malaysia, did not have the harmful *tdh* and *trh* genes, Jiang *et al.* (2019) also detected virulence-associated genes in the recovered *V. parahaemolyticus* isolates from Chinese seafood, with a frequency of 1.11% for *tdh* and 5.56% for *trh*. Among 165 *V. parahaemolyticus* isolates from marine and freshwater fish in Selangor, Malaysia, in 2018, only 4 (2.4%) exhibited evidence of the *trh* gene. They were all negative for *tdh*. One in fourteen *V. parahaemolyticus* isolates from Egyptian crustaceans harbored the *tdh* and/or *trh* genes (Ahmed *et al.*, 2018). Among the *V. parahaemolyticus* strains detected in Chinese seafood, 28.4% carried the *trh* gene and 2.1% carried the *tdh* gene. Xie *et al.* (2017) reported that no isolate possessed both genes simultaneously. In China, researchers conducted two separate experiments. Xie *et al.* (2015) reported that among all the *V. parahaemolyticus* isolates that tested positive for the *tdh* gene, 45.9% possessed the *trh* gene. Some genes, like *OmpU*, *hlyA*, and *rtxA-D*, make *V. cholerae* more harmful (Rivera *et al.*, 2001; Ruenchit *et al.*, 2017; Morshdy *et al.*, 2023). An infection with ambient *V. cholerae* can cause mild gastroenteritis if the *hlyA* gene is present; however, most of these isolates are not lethal (Saravanan *et al.*, 2007). Two samples of *V. cholerae* isolated from Egyptian crustaceans included the *tdh* and *hlyA* genes. According to Ahmed *et al.* (2018), these samples did not originate from the O1 or O139. The *rtxC* and *hlyA* virulence genes of *V. cholerae* were detected in abundance in freshwater fish from Shanghai, China, according to Xu *et al.* (2019). Both were present at 95.8% and 87.8% of the time.

Lemon juice demonstrated promising results for lowering *V. parahaemolyticus* counts in the experiment. Lemon Juice reduced *V. parahaemolyticus* counts at 8.82%, 15.47%, 15.30%, and 21.96%, respectively, following immersion in 1% and 2% juice for 1 and 2 hours, respectively. In the same way, fish infected with *V. parahaemolyticus* were immersed in 5% lemon juice for 0.5, 1, and 2 hours, respectively. This led to much higher decrease rates of 45.08%, 51.23%, and 62.08% (Morshdy *et al.*, 2022). At the same time, Sushmita (2022) and Tsai *et al.* (2021) proved that lemon juice may treat *V. cholerae*, while Nawi *et al.* (2017) proved that lemon juice was effective against *V. parahaemolyticus*, making it a natural decontaminant for seafood. In addition, when tilapia filets were marinated in lime juice at 25°C for 30 minutes and 120 minutes, Ibrahim *et al.* (2018) and Kato *et al.* (2018) discovered that the counts of *V. parahaemolyticus* were dramatically reduced. The effects of 1% lemon essential oil were studied by Morshdy *et al.* (2021b), who reported its notable antibacterial activities. The main reasons for that lemon is so popular is its low cost, ease of availability, and lack of negative adverse effects. The high levels of bioflavonoids,

limonene, pectin, calcium, magnesium, and vitamins in lemon juice improve its antiviral, antibacterial, and immune-boosting properties (Alsaraf *et al.*, 2016). Sesame oil was used in this investigation because sesame oil is inexpensive and has inherent antioxidant properties. Using different amounts of vegetable oil, the antioxidant and oxidation activities were evaluated over several hours. Adding more vegetable oil boosts antioxidant activity, which makes fish less oxidatively stable and allows for longer storage times. The obtained results indicated that the reduction rates of *V. parahaemolyticus* were 6.32%, 11.81%, 13.4%, and 15.14% following immersion in fresh sesame oil 1% and 2% for 1 and 2 hours, respectively. The sesame oil was likely to have a clear antimicrobial effect in several reports. On days 4, 7, and 15, Anwer *et al.* (2021) found values (log 10 cfu/g) of 4.19 ± 0.85 , 4.89 ± 0.75 , and 5.69 ± 0.53 , respectively. For 1, 2, and 5 days, the values recorded by Vanitha *et al.* (2015) were 3.91, 4.67, and 6.1, respectively. The results of this investigation are consistent with those of Yerlikaya *et al.* (2005) and Yazgan (2013), who observed that plant oils can effectively improve the microbiological, sensory, and chemical qualities of fish. Future studies are needed to investigate the antimicrobial activities of other plant oils and the mechanisms underlying these effects.

Conclusion

The present study revealed that the marketed mullet, brush-tooth lizard, and coral fish might act as potential sources of *Vibrio* species. In particular, *V. parahaemolyticus* was found at a higher prevalence rate of 20.8%. Using lemon juice and sesame oil could reduce the load of *V. parahaemolyticus*, particularly at 2%. Efficient sanitary measures are highly recommended during fish handling, starting from catching, storage, and marketing.

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Conflict of interest

None.

Authors' contributions

All authors made an equal contribution.

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