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PREVALENCE OF ANTIBIOTIC RESISTANT *V. parahaemolyticus* AND *V. cholerae* IN FISH AND HUMANS WITH SPECIAL REFERENCE TO VIRULOTYPING AND GENOTYPING OF *V. parahaemolyticus*

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Abstract: This study aimed to investigate prevalence, virulence determinants, antibiogram and genotyping of *Vibrio* isolates from retail shrimp and tilapia fish as well as stool samples from gastroenteritis patients in Sharkia Governorate, Egypt. *Vibrio* spp were molecularly confirmed in 25.5% and 3% of fish and human stool samples, respectively. *V. parahaemolyticus* was isolated from 8.9%, 5% and 3% of shrimp, tilapia and stool samples, respectively. However, 0.7% of shrimp and 1.7% of tilapia were found to harbor *V. cholera*. *trh* and *tdh* virulence related genes were assessed in 34 *V. parahaemolyticus* isolates (25 from shrimp, 6 from tilapia and 3 from human stool). The *tdh* gene alone was recorded in 4 (16%) isolates from shrimp and 2 (66.7%) isolates from human stool. However, *trh* gene was detected alone in one (4%) isolate from shrimp. Moreover, both genes were detected simultaneously in one shrimp (4%) and one human stool (33.3%). Tilapia fish isolates were negative for both virulence genes. The resistance of the examined isolates were 100% (each of nalidixic acid and erythromycin), 81.6% (sulphamethoxazol), 73.7% (chloramphenicol), However, susceptibilities to gentamicin (81.6%), ciprofloxacin (73.7%) and 71.1% for each ampicillin/sulbactam and amikacin were observed. Multiple drug resistance was recorded in *V. parahaemolyticus* and *V. cholerae* isolates. Out of 38 isolates, 6 (15.8%) were resistant to all 14 antibiotics with MAR index of 1. Twenty of the isolates (52.6%) were resistant to 5-13 drugs with MAR index higher than 0.286. ERIC-PCR fingerprinting revealed five distinct profiles namely E1-E5 and the discriminatory index of the reaction was 0.5107, indicating low discrimination of the technique. In conclusion, this study revealed the contamination of tilapia and shrimp in fish markets with potentially virulent *V. parahaemolyticus* strains in the study area. Moreover, the presence of human and fish isolates in the cluster indicated the potential of the environmental isolates to cause human infection.

Key words: *Vibrio* spp.; prevalence; antimicrobial resistance; genotyping; ERIC-PCR

Introduction

Fish is highly nutritious food and has various health benefits; however, it also threatens human health because it is an important source of foodborne diseases (1). *Vibrio* spp. naturally inhabit both marine and estuarine environments and are considered as one of the main causes of

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gastroenteritis in humans. They can also inhabit fresh water and different studies reported the presence of the pathogen in fresh water fish (2-4). Currently, there are 72 species of *Vibrio*, 12 of them are zoonotic to humans (5). The most pathogenic strains of *Vibrio* spp. are *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* due to their contribution in foodborne illness related to seafood products (6). Vibriosis is attributed to the consumption of raw or insufficiently cooked seafood (7). *V. parahaemolyticus* causes at least 30000 food borne infections per year (8). It causes epidemic and sporadic cases of gastroenteritis after ingesting raw or insufficiently cooked seafood, while, *V. cholerae* causes mainly water borne outbreaks and sometimes sporadic cases of diarrhea following eating of food harboring the organism (9-11). *V. parahaemolyticus* gastroenteritis could be self-limited but still life threatening because infection may lead to septicemia (12).

Toxigenic serotypes O1 and O139 of *V. cholerae* are frequently incriminated in the majority of epidemics (13). Meanwhile, non-O1/non-O139 strains inhabit water environment and cause diarrhea in sporadic cases due to consumption of contaminated seafood (10). Thermo stable direct hemolysin (TDH) encoded by *tdh* and the TDH-related haemolysin (TRH) encoded by *trh* genes are used for determination of pathogenic strains of *V. parahaemolyticus*. (14,15). Hemolysis of red blood cells and cytotoxicity in the host cells are caused by the *tdh* and *trh* genes.

Antimicrobials are commonly used as an effective therapy for infectious diseases in humans and as therapy and prophylaxis in aquaculture, however, some pathogens developed antimicrobial resistance due to extensive use of those drugs (16). This constitutes risk to humans because resistant bacteria are directly transmitted through food to consumers or due to the transfer of resistance associated genes to other pathogens by portable genetic elements (17,18).

Molecular methods are useful for epidemiological aspects such as the identification of genetic relatedness of isolates from diverse sources, tracing the source of infection and studying the host range and geographical distribution of a pathogen (19). Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reaction (ERIC-PCR) amplifies a specific sequence in the genome of 126 bp which is restricted to transcribed regions (20). ERIC-PCR has been proven in previous studies as a successful method in genotyping different bacterial pathogens (21-23).

This study aimed to determine the occurrence of *Vibrio* spp. in retail fish samples at Sharkia Governorate, Egypt, and in stool swabs from gastroenteritis patients. The virulence, antibiogram and genotyping of *V. parahaemolyticus* isolates were investigated.

Material and methods

Sampling

A total of 400 fish samples (280 shrimps (*Panaeus semisulcatus*) and 120 tilapia (*Tilapia nilotica*) were collected from different retail markets in Sharkia Governorate, Egypt. Brackish water shrimp samples were collected from the Gulf of Suez, while, tilapia samples originated from Nile River. Stool samples (n=100) from patients attending the outpatient clinics at different hospitals in the same study area were also examined. The samples were collected during the period from July 2017 to April 2018. Approval of the study was obtained from the Animal Welfare and Research Ethics Committee, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Isolation and biochemical identification

Vibrio spp. isolation was conducted according to the recommendation of FDA's Bacteriological Analytical Manual (BAM) (24). For enrichment of *Vibrio* spp. muscles from shrimp or tilapia (10 gm) were transferred to 90 ml of sterile alkaline peptone water (Oxoid CM1028B) having pH 8.6, thoroughly homogenized and then incubated at 35ºC±2ºC for 24-48 h (25). A loopful from the enriched homogenate was streaked onto Thiosulfate Citrate Bile Sucrose (TCBS) agar plates (Oxide CM0333B) and then incubated at 35ºC±2ºC for 24 h. Presumptive green or blue green colonies of *V. parahaemolyticus* and yellow colonies of

V. cholerae (5) were purified and then biochemically identified using Oxidase test, TSI agar test, Ornithine decarboxylase (ODC), L- lysine decarboxylase (LDC), Arginine dehydrolase (ADH), β- galactosidase (ONPG), Indole test and Halotolerance test (25,26).

Molecular identification

DNA extraction from biochemically suspected *V. parahaemolyticus* and *V. cholerae* isolates was performed using the QIAamp DNA Mini kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instruction. Isolates suspected to be *Vibrio* species were molecularly confirmed using primers targeting 663 bp of the 16S rRNA specific for *Vibrio* species: F: 5'- CGG TGA AAT GCG TAG AGA T-3', R: 5'- TTA CTA GCG ATT CCG AGT TC-3' (5). Then, PCR targeting 368 bp of *tox*R gene specific for *V. parahaemolyticus* using the primers F: 5'- GTC TTC TGA CGC AAT CGT TG -3', R: 5'- ATA CGA GTG GTT GCT GTC ATG-3' (27) and 304 bp of the *omp*W gene specific for *V. cholerae* with the primers F: 5'- CAC CAA GAA GGT GAC TTT ATT GTG-3', R: GGT TTG TCG AAT TAG CTT CAC C-3') (28).

PCR master mix (25 µl) consisted of 12.5 µl of 2X Dream Taq Green mastermix kit, 5.5 µl PCR grade water, 1 µl of both forward and reverse primers (20 pmol, each), 5µl Template DNA were used. The used primers were supplied from Metabion (Germany). The amplification conditions were; 5 min of primary denaturation at 94ºC, 35 cycles of secondary denaturation for 30 sec at 94ºC, annealing (16S rRNA PCR: 40 sec at 50ºC; toxR PCR: 40 sec at 55ºC; ompW PCR: 40 sec at 59ºC) and extension at 72ºC for 45 sec (16S rRNA and *omp*W PCR) and 50 sec for *tox*R PCR. A final extension was adjusted for 10 min. Positive controls were kindly supplied by the Biotechnology Unit, Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt.

Viruoltyping

The amplification of *trh* (250 bp) and *tdh* (373 bp) virulence genes of molecularly identified *V. parahaemolyticus* isolates was carried out using *trh* (F: 5'- GGC TCA AAA TGG TTA AGC G-3', R: 5'-CAT TTC CGC TCT CAT ATG C-3') and *tdh* (F: 5'- CCA TCT GTC CCT TTT CCT GC -3', R: 5'- CCA AAT ACA TTT TAC TTG G -3') genes specific primers (29). The reaction conditions were 35 cycles of primary denaturation at 94ºC for 5 min, secondary denaturation for 30 sec at 94ºC, annealing for 30 sec at 54ºC, extension at 72ºC for 1.5 min and final extension at 72ºC for 12 min.

Antibiotic susceptibility test

The antibiotic susceptibility of *V. parahaemolyticus* and *V. cholerae* isolates was carried out by Kirby-Bauer disc diffusion method on Mueller Hinton agar. The inhibition zone was measured based on the guidelines of Clinical and Laboratory Standards Institute (CLSI) (30), except for nalidixic acid, ampicillin/salbactam and kanamycin, The *Enterobacteriaceae* interpretation criteria were used. Fourteen antibiotic disks were used and they included; ampicillin (AM, 10 µg), kanamycin (K, 30 µg), nalidixic acid (NA, 30 µg), ciprofloxacin (CP, 5 µg), chloramphenicol (C, 30 µg), amikacin (AK, 30 µg), gentamicin (CN, 10 µg), tetracycline (T, 30 µg), cephalothin (KF, 30 µg), sulfamethoxazole (SXT, 25 µg), cefotaxime (CTX, 30 µg), ampicillin/sulbactam (AS, 20 µg), ceftazidime (CAZ, 10 µg) and erythromycin (E, 15 µg).

E. coli ATCC 25922 was used as a quality control isolate. Multiple antibiotic resistance (MAR) index defined as the ratio of the number of the antibiotics to which *Vibrio* isolates displayed resistance to the total number of antibiotics tested was determined (31). Multidrug resistance (MDR) was defined as resistance of an isolate to at least one agent in three or more antibiotic classes (32).

ERIC-PCR fingerprinting

ERIC-DG111-F 5'-ATG TAA GCT CCT GGG GAT TCA C-3'and ERIC-DG112-R 5'-AAG TAA GTG ACT GGG GTG AGC G-3' primers were used for amplification of repetitive sequences in the chromosomal DNA of *V. parahaemolyticus* isolates using a single amplification profile (20). Based on presence or absence of each band, ERIC-PCR fingerprinting data were presented as a binary code. Dendrogram was constructed by unweighed pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering routine using SPSS, Inc. version 22 (IBM Corp. 2013, Armonk, NY). The Simpson’s index of diversity (*D*) was used to measure the discriminatory power of ERIC-PCR as previously described (33). *D* value of more than 0.9 indicates good distinction.

Results

Vibrio spp were molecularly confirmed in 25.5% fish sample and 3% human stool samples (Figure 1A-C). The prevalence rates of *V. parahaemolyticus* versus *V. cholerae* were (8.9% Vs 0.7%) in shrimp samples and (5% Vs 1.7%) in tilapia fish samples. *V. parahaemolyticus* was the only isolated *Vibrio spp.* from 3% of human stool (Table 1).

Table 1: Proportion of *Vibrio* species isolated from shrimp, tilapia and human samples

Samples	Number examined	*Vibrio* spp.*	*V. parahemolyticus***	*V. cholera***
Shrimp	280	78 (27.9%)	25 (8.9%)	2 (0.7%)
Tilapia	120	24 (20%)	6 (5%)	2 (1.7%)
Total	400	102 (25.5%)	31 (7.8%)	4 (1%)
Humans	100	3 (3%)	3 (3%)	0

*The isolates were identified using PCR targeting 16S rRNA specific for *Vibrio* spp.

**The isolates were confirmed by species specific PCR

The *trh* and *tdh* virulence associated genes were molecularly identified in 34 *V. parahaemolyticus* isolates; 25 from shrimp, 6 from tilapia and 3 from human stool (Figure 1D-E). The results revealed the presence of *tdh* gene alone in 4 (16%) isolates from shrimp and 2 (66.7%) isolates from human stool. However, *trh* gene was detected alone in one (4%) isolate from shrimp. Moreover, both genes were noticed simultaneously in one shrimp (4%) and one human stool (33.3%). All isolates from tilapia fish did not harbor any of the investigated virulence genes.

The antibiotic susceptibility testing (Tables 2 and 3) was performed on 38 isolates (34 *V. parahaemolyticus* and 4 *V. cholerae*) against 14 antibiotics. All isolates were found to resist nalidixic acid and erythromycin, while, 81.6% and 73.7% were resistant to sulphamethoxazol and chloramphenicol, respectively. However, susceptibilities to gentamicin (81.6%), ciprofloxacin (73.7%) and ampicillin/ sulbactam and amikacin (71.1%, each) were observed. Multiple drug resistance was recorded in *V. parahaemolyticus* and *V. cholerae* isolates. Out of 38 isolates, 6 (15.8%) were resistant to all 14 antibiotics with MAR index of 1. Twenty of the isolates (52.6%) were resistant to 5-13 drugs with MAR index higher than 0.286 ranging from 0.357-0.928. The average MAR index was 0.678.

Table 2: Results of antibiotic susceptibility tests on *Vibrio* isolates (n=38)

Antibiotic class	Antibiotics	S	I	R
Penicillin	**Ampicillin**	10 (26.3%)	7 (18.4%)	21 (55.2%)
	Ampicillin/Sulbactam	27 (71.1%)	2 (5.3%)	9 (23.7%)
Cephalosporin	**Cefotaxim**	25 (65.8%)	3 (7.9%)	10 (26.3%)
	Ceftazidime	20 (52.6%)	3 (7.9%)	15(39.5%)
	Cephalothine	13 (34.1%)	4 (10.5%)	21 (55.2%)
Aminoglycosides	**Amikacin**	27 (71.1%)	5 (13.1%)	6 (15.8%)
	Gentamicin	31 (81.6%)	1 (2.6%)	6 (15.8%)
	Kanamycin	19 (50%)	4 (10.5%)	15 (39.5%)
Tetracycline	**Tetracycline**	12 (31.6%)	3 (7.9%)	23 (60.5%)
Quinolones	**Nalidixic acid**	0	0	38 (100%)
	Ciprofloxacin	28 (73.7%)	0	10 (26.3%)
Sulfonamide	**Sulphamethoxazol**	5 (13.2%)	2 (5.3%)	31 (81.6%)
Phenolics	**Chloramphenicol**	7 (18.4%)	3 (7.9%)	28 (73.7%)
Macrolide	**Erythromycin**	0	0	38 (100%)

S: Sensitive, I: Intermediate, R: Resistant

Table 3: Antibiotic resistance pattern and MAR index of *Vibrio* spp.

Resistance pattern	Resistance profile	Number of isolates	Number of antibiotics	MAR
I	NA, E, SXT, AM, C, AS, CZ, T, CN, K, CF, G, CP, AK	$6^{*\#}$	14	1
II	NA, E, SXT, AM, C, AS, CZ, T, CN, K, CF, G, CP	1	13	0.928
III	NA, E, SXT ,AM ,C,AS ,CZ, T,CN,K ,CF ,G ,AK	1	13	0.928
IV	NA, E, SXT, AM, C, AS, CZ, T, CN, K, CF, G	2	12	0.857
V	NA, E, SXT, AM, C,AS,,CZ, T, CN, K ,CP,AK	$1^{\#}$	12	0.857
VI	NA, E, SXT, AM, C, AS, CZ, T, CN, K, CF	2	11	0.786
VII	NA, E, SXT, AM, C, AS, CZ, T, CN,K	2	10	0.714
VIII	NA, E, SXT, AM, C, CZ, T, CN, K, CF	1	10	0.714
IX	NA, E, SXT, AM, C, AS, CZ, T, CN	$2^{\#}$	9	0.643
X	NA, E, SXT, AM, C, AS, CZ, T	1	8	0.571
XI	NA, E, SXT, AM, C, CZ, T, CN	1	8	0.571
XII	NA, E, SXT, AM, C, CZ, T	1	7	0.500
XIII	NA, E, SXT, AM, C, AS	2	6	0.428
XIV	NA, E, SXT, AM, C, CN	1	6	0.428
XV	NA, E, SXT, AM, C	1	5	0.357
XVI	NA, E, SXT, C, CN	1	5	0.357
XVII	NA, E, SXT, AM	2	4	0.286
XVII	NA, E, SXT, C	2	4	0.286
XIX	NA, E, SXT,CZ	$1^{\#}$	4	0.286
XX	NA,E,SXT	1	3	0.214
XXI	NA ,E	5	2	3.143
XXII	NA	1	1	0.071

Average MAR index= 0.678
* Clinical isolates are included in Pattern I
$^{\#}$*V. cholerae* isolates

The genetic similarity of 38 *V. parahaemolyticus* and *V. cholerae* isolates from shrimp, tilapia and human sources was assessed using ERIC-PCR. The amplified fragments ranged between 244 and 1520 bp. A band of 590 bp was commonly present in all *V. parahaemolyticus* and three of *V. cholerae* isolates (Figure 1F). The reaction showed discriminatory index of 0.5107 in typing the isolates. Five profiles namely E1-E5 and two main clusters and one individual isolate were generated at linkage distance 12.5 (Figure 2). Cluster I included three sub-clusters; Ia sub-cluster consisted of *V. parahaemolyticus* isolates from shrimp, tilapia and humans, while, Ib and Ic sub-clusters included *V. parahaemolyticus* from tilapia and shrimp. Three isolates of *V. cholerae* were allocated in one cluster (Cluster II) and one isolate was separately located.

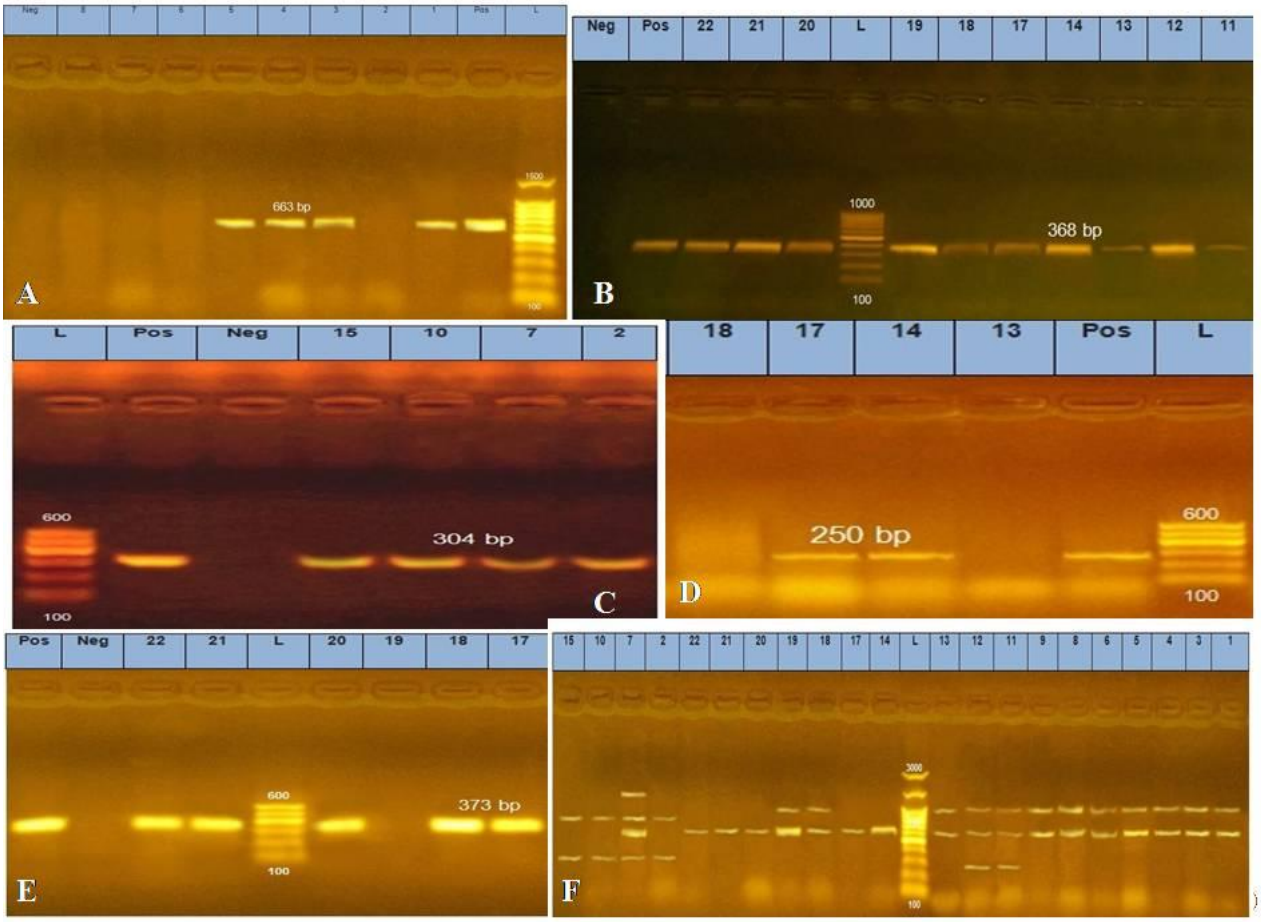

Figure 1: A: 16S rRNA gene amplification for the molecular identification of *Vibrio* isolates from different sources with amplicon size of 663 bp, Ladder: 100 bp. B: *tox*R gene amplification for molecular confirmation of *V. parahaemolyticus* isolates from different sources with amplicon size of 368 bp, Ladder: 100 bp. C: *omp*W gene for the identification of *V. cholerae* isolates with amplicon size of 304 bp, D: *trh* gene in *V. parahaemolyticus* isolates with amplicon size of 250 bp; E: *tdh* gene in *V. parahaemolyticus* isolates with amplicon size of 373 bp. F: ERIC-PCR fingerprinting of *V. parahaemolyticus* and *V. cholerae* isolates, Ladder: 100 bp

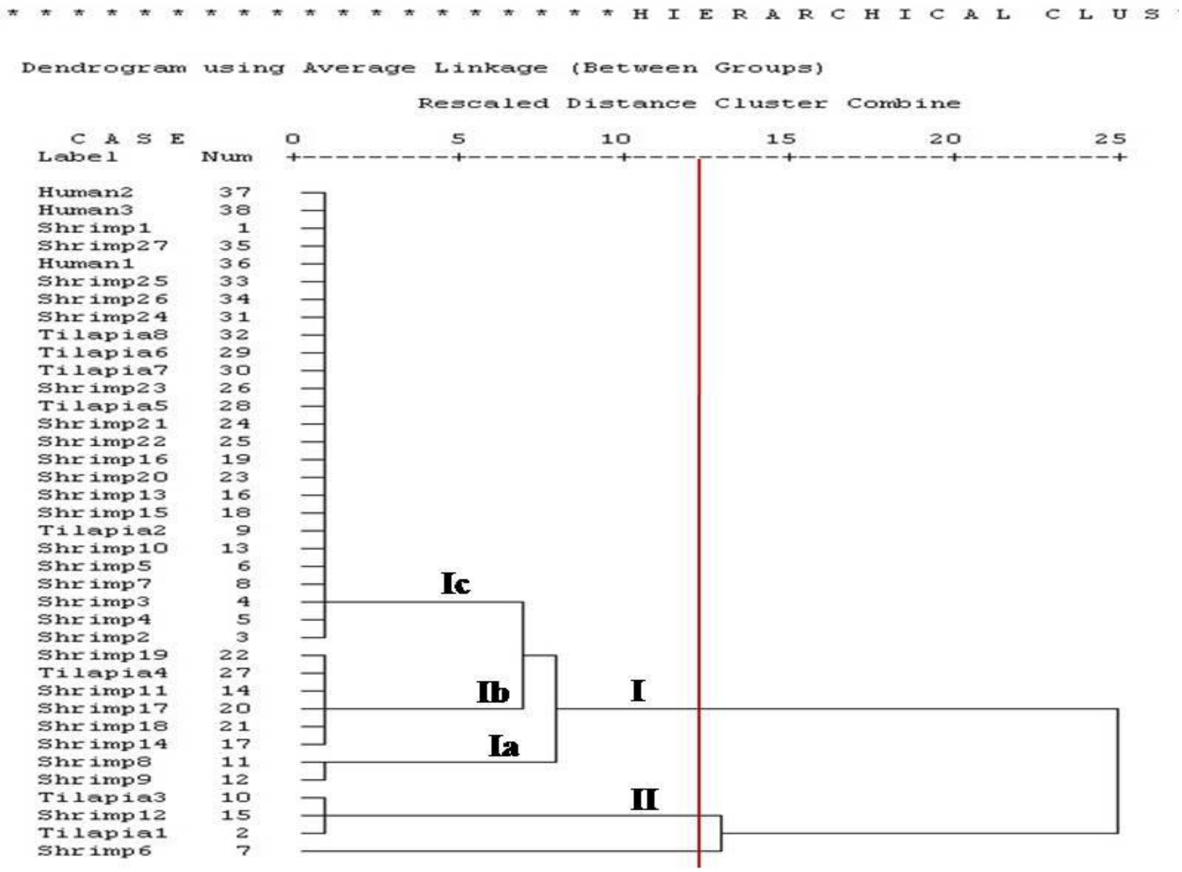

Figure 2: Dendrogram showing the relatedness of *V. parahaemolyticus* and *V. cholerae* isolates from humans and fish sources as determined by ERIC-PCR fingerprinting using the SPSS computer software program

Discussion

Several outbreaks due to ingestion of seafood contaminated with *Vibrio* spp. have been reported worldwide and the source of contamination could be human feces or sewage (34). *V. parahaemolyticus* was isolated in our study from 7.8% of seafood samples (8.9% from shrimp and 5% from tilapia). This was comparable to 8% reported in Netherland (35), 9.3% in Iran (36) and 9.4% in Croatia (37).

Higher isolation rates of *V. parahaemolyticus* from shrimp were reported in different studies; for instance, 32.3% in Senegal (38), 80.8% in Ecuador (39). Moreover, 37.7% (40), 47.9% (41) and 81.7% (8) were reported in China. In Egypt, *V. parahaemolyticus* was isolated from 18.2% of shrimp (42), and 10% from marine water fish (43) in Sharkia Governorate.

V. cholerae was isolated in the current study from 0.7% of shrimp samples compared to 1.5% in Egypt (42) and 2% in Morocco (6). Higher percentages of 9.4% in India (44) and 11.4% in Ecuador (39) were reported for shrimp.

In our study, both species were isolated from freshwater fish samples (tilapia), in accordance, another study in Malaysia reported the isolation of *V. parahaemolyticus* from 24% of freshwater fish samples sold at hypermarkets (3). However, a study in Egypt documented that *Vibrio* spp. other than *V. cholerae* and *V. parahaemolyticus* was recovered from freshwater fish (43).

V. cholerae inhabits some fresh and marine water fish species, and they may spread the bacteria in the aquatic environment and may transmit it to water birds consuming them (45). Hence, fish are considered as a reservoir for *V.*

cholerae and may be responsible for its global distribution. In Burkina Faso, *V. cholerae* were isolated from 6.3% of Tilapia samples (46).

The isolation of *V. parahaemolyticus* from fresh water fish could be due to cross-contamination from marine water fish and ice used for preservation during retail of fish in markets (3). Moreover, improper handling and insufficient hygienic measures in fish markets are also considered risk factors for the contamination of different fish species with *Vibrio* (3). It has been also found that *Vibrio* spp. can survive and grow in freshwater environment as well as in brackish and marine water (47).

The source of samples, methods of identification, study area, season, salinity and temperature during storage or even transportation may influence the variation of *Vibrio* prevalence in seafood (11,48).

Isolation of *V. parahaemolyticus* from 3% of human stool samples coincides with a study in the same geographic area (42). Moreover, isolation of *V. parahaemolyticus* from gastroenteritis patients associated with seafood consumption was previously reported (49,50).

V. parahaemolyticus strains that harbor the *tdh* and/or *trh* genes have a potential of pathogenicity. A proportion of 0.2-3% of *V. parahaemolyticus* strains from environmental sources are considered pathogenic according to studies conducted in different regions (51). In our study, 22.6% of seafood samples harbored *tdh* and/or *trh* genes. In Egypt, 8.3% (42) and 14.3% of *V. parahaemolyticus* from shrimp harbored the *tdh* and/or *trh* genes (48). While, in China, 45.9% isolates were positive for only the *trh* gene (8). The three isolates from clinical cases in our study were positive for the *trh* and *tdh* genes. This was consistent with another study in Egypt in the same study area (42). In China, all the clinical *V. parahaemolyticus* strains were found to harbor the *tdh* gene, and only 77.4% harbored the *trh* gene (41). Isolates lacking hemolysin genes were reported to be pathogenic, thus indicating the presence of other pathogenicity determinants (52).

Antimicrobial resistant bacteria are widely spread due to continuous and extensive use of antibiotics. This is of major concerns in both human and animal health as it reflects the pattern of drug use (8, 53, 54). The bacteria acquired resistance to antibiotics following frequent exposure to antibiotics over time. Resistance is acquired through transfer of horizontal gene mobile genetic elements (55).

In this study we used antibiotics which are mainly used in the treatment of Vibrio infection. High resistance to erythromycin (100%) indicates public health concerns because this drug is used in children treatment (56). The resistance rates were also high to some antibiotics such as sulphamethoxazol, chloramphenicol and tetracycline. This coincides with studies reported in Egypt (42), China (16) and India (9).

Twenty-six of the isolates (68.4%) resisted 5-14 drugs with MAR index more than 0.286 ranging from 0.357-1 with an average MAR of 0.678. The MAR indices of *V. parahaemolyticus* isolates more than 0.2 have been previously reported (42,57,58), thus indicating contamination from high-risk sources including animals and humans causing high risk to fish consumers (15). Hence, antimicrobial resistance testing is important to assess the efficacy of new antibiotics and to guarantee seafood safety. The difference in the MAR indices might be contributed to samples' origin and source as well as the testing methods (59-61).

Different epidemiological studies revealed genetic similarity between clinical and environmental *V. parahaemolyticus* isolates (62,63). ERIC-PCR has been used for typing of *Vibrio* spp. into the species level (63,64).

Our results indicated low discrimination of ERIC-PCR in determining the genetic relationship of *V. parahaemolyticus* and *V. cholerae* isolates. In contrary, ERIC-PCR has been reported as a useful technique for evaluating the genetic relationship of *V. parahaemolyticus* isolates (63). Twenty-seven patterns were reported by ERIC-PCR genotyping of *V. parahaemolyticus* isolates, 12-25 bands with 160-1690 bp size range were discriminated and cluster analysis illustrated close relationship between *V. parahaemolyticus* isolates and discrimination index of 0.98 (23). Bands of sizes 270-, 520-, 660-, and 950-bp bands were detected in all *V. parahaemolyticus* isolates. Another study in Philippine, showed that ERIC-PCR fingerprints of *V. parahaemolyticus* strains included 6 to 8 amplification bands with a molecular weight ranging from 50-2500 bp, and a shared band of 500 bp was observed in all strains (21). Three clusters were generated and most of the strains were found to be genetically unrelated. The high discriminatory power of ERIC-PCR allowed the reaction to be used for tracing the spread of the strains (21). Moreover, the presence of a common band in all the strains can be used in the production of a diagnostic genetic assay.

Human isolates revealed 100% similarity with environmental isolates from shrimp and tilapia, suggests that environmental strains are the causative agents of clinical cases. This was consistent with the findings of another study (65).

ERIC-PCR genotyping of *V. cholerae* revealed genetic diversity and 31 diverse fragments of DNA extending from 250 to 8000 bp were amplified and the discriminatory index was 0.72 (22). Another study showed also diversity among clinical and environmental *V. cholerae* isolates and produced 16 clusters, in 6 clusters, 14 environmental isolates clustered with eight clinical isolates (65). The heterogeneity of *V. cholerae* isolates was observed in other studies (66,67).

Conclusion

The current study revealed the contamination of shrimp and tilapia in Zagazig fish markets are contaminated with *V. parahaemolyticus* and *V. cholerae* isolates. A proportion of *V. parahaemolyticus* isolates are highly pathogenic and show multiple drug resistance, thus posing risk to human consumers. ERIC-PCR has been shown to be of low discriminatory power, therefore, other molecular techniques should be investigated for determining the genetic relatedness of *Vibrio* isolates. The high similarity of human and environmental isolates and their presence in the same cluster indicated the potential of the environmental isolates to cause human infection.

Conflict of interest

The authors declare no conflict of interest.

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