Prevalence of *Vibrio parahaemolyticus* in seabass (*Dicentrarchus Labrax*) and seabream (*Sparus aurata*) and Detection of Streptomycin-resistant Strains

Adel M. El-Gamal\(^1\) and Engy F. EL-Bahi\(^2\)

\(^1\)Bacteriology unit, Animal Health Research Institute, Kafr El-Sheikh branch, Agriculture research center (ARC), Egypt.
\(^2\)Food hygiene unit, Animal Health Research Institute, Kafr El-Sheikh branch, Agriculture research center (ARC), Egypt.

*Corresponding author’s Email: adelegamal5544@yahoo.com; DOI: https://dx.doi.org/10.36380/scil.2020.wvj42*

**ABSTRACT**

*Vibrio* species are the most common and serious pathogens in fish and shellfish marine aquaculture worldwide. The present study aimed to determine the prevalence of *Vibrio* spp. in seabass and seabream in fish markets, especially streptomycin-resistant strains that have great public health importance. A total of 30 seabass (*Dicentrarchus Labrax*) and 30 seabream (*Sparus aurata*) were purchased from fish markets at Kafr El Sheikh Governorate and subjected to bacteriological examination. The PCR assay was used for the detection of virulence genes (*tdh* and *trh*), aminoglycoside resistance gene (*aadA1*), and *toxR* gene. The results indicated that the total prevalence of *Vibrio* spp. was 26.66%, including *V. parahaemolyticus* (8.3%), *V. alginolyticus* (8.3%), *V. mimicus* (3.3%), *V. harveyi* (5%) and *V. vulnificus* (1.6%). The *toxR*, *trh*, and *aadA1* genes were found in all *V. parahaemolyticus* isolates while *tdh* gene was found in 80% of isolates. Antimicrobial sensitivity test of *V. parahaemolyticus* isolates showed sensitivity to ciprofloxacin, norfloxacin, cefotaxime, and chloramphenicol. *Vibrio parahaemolyticus* isolates were resistant to ampicillin, erythromycin, streptomycin, and gentamycin. The present results indicated that good hygienic measures should be taken to avoid infection with *Vibrio* species, especially *V. parahaemolyticus* that can pose a great risk to human health.

**Keywords:** Antibiotic resistance, Seabass, Seabream, Streptomycin, *Vibrio parahaemolyticus*.

**INTRODUCTION**

*Vibrio* genus contains Gram-negative, halophilic, rod-shaped, non-spore forming, oxidase-positive bacteria, which are widespread in the coastal and estuarine environments (Austin and Austin, 2007). *Vibrio parahaemolyticus* is the most recorded pathogenic species of *Vibrio* genus and affects persons who consume improperly cooked or raw seafood (Raissy et al., 2015). This foodborne bacteria is reported as the main cause of seafood-borne illness in Egypt and many other countries around the world such as United States, Malaysia, Thailand, Korea, China, and Japan (Yoon et al., 2008; Iwahori et al., 2010; Abdel-Azeem et al., 2016). Infection with *V. parahaemolyticus* may cause acute human gastroenteritis, the major symptoms of which are headache, diarrhea, abdominal pain, and in some cases, septicemia (Broberg et al., 2011; Wang et al., 2015; Su and Liu, 2017). In coastal areas of the world, like Japan, *V. parahaemolyticus* has been regularly recognized as the main cause of sporadic cases of gastroenteritis (Qadri et al., 2005; Wang et al., 2017). In China, about 322 gastroenteritis outbreaks due to *V. parahaemolyticus* infection were reported from 2003 to 2008 (Wu et al., 2014). Multiplication of *V. parahaemolyticus* is related to water temperature and season (Deepanjali et al., 2005; Angela et al., 2006), with the highest prevalence in summer due to the higher salinity of water than other seasons (Zulkifi et al., 2009).

The pathogenicity of bacteria depends mainly on some virulence factors and virulence genes, which act together as major orchestrators. The most virulence genes leading to pathogenicity of *V. parahaemolyticus* are hemolysin genes (*tdh* and *trh*) (Hiyoshi et al., 2010). Molecular epidemiological studies demonstrated a clear relation between the hemolysin genes and disease-causing ability of *V. parahaemolyticus* (Kishishita et al., 1992; DePaola et al., 2003; Vongxay et al., 2008; Chao et al., 2009; Han et al., 2015; Hasrimi et al., 2018). These two genes were recorded in the most isolates from clinical cases of *V. parahaemolyticus* infections (Bej et al., 1999; Rojas et al., 2011). The *tdh* and *trh* genes encode virulence factors of thermostable direct hemolysin (TDH), and TDH-related hemolysin (TRH), respectively, which are involved in important pathogenic activities, such as enterotoxemia, hemolytic activity, cytotoxicity and cardiotoxicity (Shirai et al., 1990; Osaka et al., 1996).

The *toxR* gene is a pandemic marker gene for all *V. parahaemolyticus* strains either pathogenic or nonpathogenic one, and it was recorded in some other *Vibrio* species (Kim et al., 1999). The sequence of *toxR* gene can be used for molecular identification of *V. parahaemolyticus* (Yung et al., 1999; Hubbard et al., 2016). The *aadA1* and *aadA2* encode
Materials and Methods

Samples Collection
Thirty seabass and 30 seabream with a weight range of 100-250 g were purchased from fish markets at Kafr El Sheikh Governorate from February to August 2019. All samples were transferred in ice box to Animal Health Research Institute, Kafr El Sheikh laboratory, Egypt.

Bacteriological Examination
Bacteriological examinations were done according to ISO/TS 21872-1 (2007) and ISO/TS 21872-2 (2007).

Samples Preparation
After skin sterilization with alcohol, the muscles above the lateral line were removed, 25 g of each fish sample were mixed with 225 ml of alkaline saline peptone water (APW, pH 8.6) in a Stomacher bag. After that, these mixtures were incubated at 37 °C for 8-16 hours.

Isolation of Vibrio Species
After the incubation period, the upper layer of the alkaline saline peptone water (APW) enrichment broth was inoculated on Thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Oxoid, UK), and then these plates were incubated at 37 °C for another 18-24 hours. After that, growing colonies were used for further screening tests including Gram staining, oxidase and catalase tests.

Biochemical Identification
Suspected colonies of Vibrio spp. on TCBS media and positive oxidase test were subjected to further identification by Microbact GNB kit (Oxoid, UK).

Polymerase Chain Reaction
Suspected isolates of the V. parahaemolyticus were examined by using PCR for the detection of virulence genes (tdh and trh), toxR gene, and aadA1 gene, DNA extraction were performed according to the manufacturer’s recommendations by using the QIA amp DNA Mini kit (Qiagene, Germany, GmbH). Oligonucleotide primers were supplied from Metabion (Germany). The primers were utilized in a 25-µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan) using an Applied Biosystem 2720 thermal cycler. Primers used and PCR conditions are presented in Table 1. The products of PCR were separated by electrophoresis on 1% Agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5 V/cm. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Antimicrobial Susceptibility Test
Antimicrobial disk susceptibility test were performed as described by the Clinical and Laboratory Standards Institute (CLSI, 2012).

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

| Target genes | Primers Sequence (5’-3’) | Amplicon Size (base pair) | Primary Denaturation | Secondary Denaturation | Annealing  | Extension  | Final Extension | Reference |
|--------------|--------------------------|--------------------------|----------------------|------------------------|------------|------------|----------------|----------|
| toxR         | F GTCTTCTGACGCAAATGTTTG  | 368                      | 94°C 5 min.          | 94°C 30 sec.           | 55°C 40 sec. | 72°C 40 sec. | 72°C 10 min.   | Kim et al., 1999 |
|              | R ATACGAGTGGTCGCTGATGG    |                          |                      |                        |            |            |                |          |
| aadA1        | F TATCAGGAGGTGTTGCGCTCATT| 484                      | 94°C 5 min.          | 94°C 30 sec.           | 54°C 40 sec. | 72°C 45 sec. | 72°C 10 min.   | Randall et al. 2004 |
|              | R GTCACATGGCTGTTAAGTTTCA  |                          |                      |                        |            |            |                |          |
| trh          | F GGCTCAAATGGTGTTAACG    | 250                      | 94°C 5 min.          | 94°C 30 sec.           | 54°C 30 sec. | 72°C 30 sec. | 72°C 7 min.    | Mustapha et al., 2013 |
|              | R CATTGCCCTCTCATATGCC     |                          |                      |                        |            |            |                |          |
| tdh          | F CCAATCGTCCTTCTTCTG     | 373                      | 94°C 5 min.          | 94°C 30 sec.           | 54°C 30 sec. | 72°C 40 sec. | 72°C 7 min.    |          |
|              | R CCAAATACATTATTATTGG     |                          |                      |                        |            |            |                |          |
RESULTS AND DISSCUSION

Vibrio spp. commonly inhabit the marine environment and can be found in the fresh water (Sujeewa et al., 2009). Seafood may be a vehicle for most of the bacterial pathogens such as Vibrio spp. (Huss, 1997). Various outbreaks of bacterial disease associated with seafood consumption have been reported (Friesema et al., 2012). Recently, V. parahaemolyticus recoded as an important species causing seafood infection associated with gastroenteritis illness in humans.

Table 2 shows that the total incidence of Vibrio spp. isolated from the examined seabass and seabream samples is 26.66% (16 out of 60 samples). Raissy et al. (2015) and Azwail et al. (2016) recorded nearly similar results (22% and 22.9%, respectively). However, the result of present study is lower than that recorded by Pal and Das (2010), Saad et al. (2015), Abdel-Azeem et al. (2016), Fri et al. (2017), and Hammat et al. (2018). These differences may be due to differences in the types of examined fish, the method of bacterial isolation, or differences in the hygienic state of fish sources. Additionally, the difference in results can be attributed to differences in season sampling, as Vibrio spp. has been reported to have higher concentrations in summer seasons due to higher water salinity levels than other seasons (Zulkifli et al., 2009). As presented in Table 2, several Vibrio strains were isolated from examined seabass and seabream, including V. parahaemolyticus (8.3%), V. alginolyticus (8.3%), V. mimicus (3.3%), V. harveyi (5%) and V. vulnificus (1.6%). Vibrio cholera was not detected in the studied samples. The examined seabass fish were more infected with V. parahaemolyticus than the examined seabream fish which may be due to the hygienic state of each fish source.

Similarly, Saad et al. (2015) isolated V. parahaemolyticus (10%), V. fluvialis, V. vulnificus, V. alginolyticus, V. mimicus, and V. damsel from Tilapia nilotica and Mugil cephalus. Hemmat et al. (2018) isolated V. parahaemolyticus (12%), V. mimicus, V. alginolyticus, V. cholera, V. vulnificus, and V. fluvialis from Oreochromis niloticus, Mugil cephalus, shrimp, and crab. Raissy et al. (2015) isolated V. harveyi that was the most frequent species isolated, followed by V. parahaemolyticus (3.5%), V. mimicus, V. vulnificus, and V. alginolyticus from some marine fish and shrimps. Fri et al. (2017) isolated V. fluvialis, Vibrio vulnificus, and V. parahaemolyticus (5.45%) from dusky kop fish and sea water. Pal and Das (2010) isolated Vibrio parahaemolyticus with a high prevalence (35%) from shrimp, prawn, bhetki, pamfret, and hillsa. According to the Egyptian Organization for Standardization and Quality Control (EOSQC, 2005), any seafood products must be free from V. parahaemolyticus.

As shown in Table 3 and Figure 1, all examined Vibrio parahaemolyticus isolates were positive for toxR gene. This result supports the findings of Yung et al. (1999); Pal and Das (2010), who reported that toxR-targeted PCR protocol can be used for V. parahaemolyticus detection. Also, all examined Vibrio parahaemolyticus isolates were positive for aadA1 gene (Figure 2). Taviani et al. (2008) stated that aadA1 gene is responsible for antibiotic resistance against aminoglycoside group including streptomycin in Vibrio spp. isolates from shellfish and other marine fish.

Pathogenicity of V. parahaemolyticus is conferred either by tdh, and/or trh (Yamaichi et al., 1999). As shown in Table 3, all examined V. parahaemolyticus isolates were positive for trh gene (Figure 3), and 80% were positive for tdh gene (Figure 4). The results did not match with that reported by Rojas et al. (2011) who detected tdh gene in 10.5% of V. parahaemolyticus isolates, while trh gene was not found. Also, Pal and Das (2010) recorded tdh gene in 35% of V. parahaemolyticus isolated from fish samples while trh gene was found only in 1.7% of V. parahaemolyticus isolates. Wang et al. (2017) recorded the virulence genes; tdh and trh with 87.9% and 3.7% of examined V. parahaemolyticus strains, respectively. Fri et al. (2017) recorded trh gene as 9.46% in examined V. parahaemolyticus strains, while Wong et al. (2000) recorded only one V. parahaemolyticus isolate (1.4%) harboring trh gene, but did not detect tdh gene among the examined V. parahaemolyticus isolates.

Antimicrobial susceptibility test showed that V. parahaemolyticus isolates were sensitive to ciprofloxacin, norfloxacin, cefotaxime, and chloramphenicol while they were resistant to ampicillin, erythromycin, streptomycin, and gentamycin (Table 4). These results indicate that the examined strains were resistant to most members of the aminoglycoside group, which may be due to the fact that aadA1 gene was detected in all examined V. parahaemolyticus isolates. This result is nearly similar to that recorded by Rojas et al. (2011), who reported that V. parahaemolyticus had resistance to streptomycin and ampicillin with intermediate susceptibility to gentamicin.

Table 2. Prevalence of Vibrio species in examined seabass and seabream fish, Egypt.

| Vibrio spp. | Seabass (n=30) | Seabream (n=30) | Total (%) |
|-------------|----------------|----------------|-----------|
| V. parahaemolyticus | 1 | 4 | 5 (8.3) |
| V. alginolyticus | 3 | 2 | 5 (8.3) |
| V. mimicus | 0 | 2 | 2 (3.3) |
| V. harveyi | 1 | 2 | 3 (5) |
| V. vulnificus | 1 | 0 | 1 (1.6) |
| Total | 6 | 10 | 16 (26.6) |
Table 3. Distribution of virulence genes among examined isolates of *Vibrio parahaemolyticus* isolated from seabass and seabream fish.

| Sample No. | toxR | tdh | trh | aadA1 |
|------------|------|-----|-----|--------|
| 1          | +    | +   | +   | +      |
| 2          | +    | -   | +   | +      |
| 3          | +    | +   | +   | +      |
| 4          | +    | +   | +   | +      |
| 5          | +    | +   | +   | +      |

Table 4. Results of agar disc diffusion test of *Vibrio parahaemolyticus* isolated from marine fish.

| Antibiotic | Disc symbol & concentration (μg/disc) | Result |
|------------|---------------------------------------|--------|
| Norfloxacin | Nor (10)                              | S      |
| Erythromycin | E (15)                                | R      |
| Ampicillin   | AMP (10)                               | R      |
| Amoxicillin + clavulanic acid | AMC (30) | S      |
| Cefotaxime   | CTX(30)                                | S      |
| Doxycycline  | DO (30)                                | R      |
| Streptomycin | S(10)                                  | R      |
| Sulfamethazol + Trimethoprim | SXT(25) | R      |
| Chloramphenicol | C (30)                        | S      |
| Gentamycin   | CN(10)                                 | R      |
| Ciprofloxacin | Cip (5 )                              | S      |

S: Sensitive  R: Resistant

Figure 1. Agarose gel electrophoresis of PCR amplification of toxR gene (368 bp) of *Vibrio parahaemolyticus*. Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1-5: Positive samples.

Figure 2. Agarose gel electrophoresis of PCR amplification of aadA1 gene (484 bp) of *Vibrio parahaemolyticus*. Lane L: 100-600 bp DNA Ladder. Neg.: Negative control. Pos.: Positive control. Lane 1-5: Positive samples.
To cite this paper: Adel M. El-Gamal and Engy F. EL-Bahi (2020). Prevalence of Vibrio parahaemolyticus in seabass (Dicentrarchus Labrax) and seabream (Sparus aurata) and Detection of Streptomycin-resistant Strains. World Vet. J., 10 (3): 325-331. DOI: https://dx.doi.org/10.36380/wvj.2020.wvj42

CONCLUSION

Vibrio spp. especially V. parahaemolyticus, V. alginolyticus, V. mimicus, and V. vulnificus are commonly isolated from seabass and seabream fish, which affects persons who consume improperly cooked or raw seafood. Most of these bacteria have antibiotic resistance genes that pose a great risk to human health; therefore, good hygienic measures should apply to avoid such infections.

DECLARATIONS

Acknowledgments
The authors would like to thank all member of Animal Health Research Institute, Egypt, for their kindly help during the investigation and paper preparation.

Authors’ contributions
All authors participated equally in study design, data collection, data analysis, writing, and approving the final manuscript.

Competing interests
The authors declare that they have no competing interests.
REFERENCES

Angela D, Schulzea Abayomi O, Alabib Adele R, Sheldrake T and Miller KM (2006). Bacterial diversity in a marine hatchery: Balance between pathogenic and potentially probiotic bacterial strains. Aquaculture, 256: 50–73. DOI: https://doi.org/10.1016/j.aquaculture.2006.02.008

Austin B and Austin DA (2007). Bacterial fish pathogens, disease of farmed and wild fish, 4th ed. Springer Praxis, Godalming. Available at: https://www.springer.com/gp/book/9781840260687

Azwai SM, Alfallani EA, Abolghait SK, Garbaj AM, Naas HT, Moawad AA, Gammoudi FT, Raysen HM, Barbieri I and Eldaghayes IM (2016). Isolation and molecular identification of Vibrio spp. by sequencing of 16S RNA from seafood, meat and meat products in Libya. Open Veterinary Journal, 6(1): 36-43. DOI: https://doi.org/10.4314/ovj.v6i1.6

Bej AK, Patterson DP, Brasher CW, Vickery MCL, Jones DD, and Kaysner CA (1999). Detection of total and hemolysin-producing Vibrio parahaemolyticus in shellfish using multiple PCR amplification of tlih, tlih, and trh. Journal of Microbiological Methods, 36: 215 – 225. DOI: https://doi.org/10.1016/S0167-7012(99)00037-8

Brooks CA, Calder TJ and Orth K (11). Vibrio parahaemolyticus cell biology and pathogenicity determinants. Microbes and infection / Institute Pasteur, 13(12–13): 992-1001. DOI: https://doi.org/10.1016/j.micinf.2011.06.013

Chao G, Jiao X, Zhou X, Yang Z, Huang J, and Pan Z (2009). Serodiversity, pandemic O3:K6 clone, molecular typing, and antibiotic susceptibility of food borne and clinical Vibrio parahaemolyticus isolates in Jiangsu, China. Foodborne Pathogenic Disease, 6(8): 1021–1028. DOI: https://doi.org/10.1089/fpd.2009.0295

Clinical and Laboratory Standards Institute (2013). Performance standards for antimicrobial disk susceptibility tests; approved standard, 11th ed (M02-A11). Clinical and Laboratory Standards Institute, Wayne, Pennsylvania. Available at: http://www.facs.ucal.edu/intranet/CLSI/CLSI-M100S22

Dalsgaard A, Forslund A, Sandvang D, Arntzen L and Keddy K (2001). Vibrio parahaemolyticus pathogenes in the Marine Environment. Edited by Belkin and Colwell, Springer, New York, 2005.

Deepanjali A, Sanath K and Karunasagar I (2005). Seasonal variation in abundance of total and pathogenic Vibrio parahaemolyticus bacteria in oysters along the Southwest coast of India. Applied and Environmental Microbiology, 71: 3575–3580. DOI: https://doi.org/10.1128/AEM.71.7.3575-3580.2005

DePaola A, Nordstrom JL, Bowers JC, Wells JG and Cook DW (2003). Seasonal Abundance of Total and Pathogenic V. parahaemolyticus in Alabama Oysters. Journal of Applied and Environmental Microbiology, 69 (3): 1521 –1526. DOI: https://doi.org/10.1128/AEM.69.3.1521-1526.2003

Egyptian Organization for Standardization and quality control (EIOSC) (2005). The Egyptian standard number 2005/3494. Available at: www.egyptian-organization-standards.gov.eg

Taviani E, Ceccarelli D, Lazoro N, Bani S, Cappuccinelli P, Colwell RR and Colombo MM (2008). Environmental Vibrio spp., isolated in Mozambique, contain a polymorphic group of integrative conjugative elements and class 1 integrons. FEMS Microbiology Ecology, 64: 45–53. DOI: https://doi.org/10.1111.j.1574-6941.2008.00455.x

Qadir F, Chowdhury NR, Takeda Y and Nair BG (2005). Vibrio parahaemolyticus-Seafood Safety and Associations with Higher Organisms. Oceans and Health: Pathogenes in the Marine Environment. Edited by Belkin and Colwell, Springer, New York, 2005. DOI: https://doi.org/10.1007/3-587-237097-711

Fri J, Nip RN, Njom HA and Clarke AM (2017). Occurrence of virulence genes associated with human pathogenic Vibrio isolated from two commercial Dusky Kob (Argyrosomus japonicus) farms and Kareiga Estuary in the Eastern Cape Province, South Africa. International Journal of Environmental Research Public Health, 14: 1111. DOI: https://doi.org/10.3390/ijerph141110111

Friesema IH, De Jong AE, Fitz James IA, Heck ME, Hidayat MK and Health: Pathogenes in the Marine Environment. Edited by Belkin and Colwell, Springer, New York, 2005.

Taviani E, Ceccarelli D, Lazoro N, Bani S, Cappuccinelli P, Colwell RR and Colombo MM (2008). Environmental Vibrio spp., isolated in Mozambique, contain a polymorphic group of integrative conjugative elements and class 1 integrons. FEMS Microbiology Ecology, 64: 45–53. DOI: https://doi.org/10.1111.j.1574-6941.2008.00455.x

Hasrini AN, Budhirajo A and Jananna SN (2018). Detection of tlih and tlih genes in Vibrio parahaemolyticus inhabiting farmed water ecosystem used for L. Vannamei aquaculture. Journal of Physics: Conference Series, 1025: 012058.

Hemmata M, Ibrahim, Reham A, Amin1, Nesreen Z, Elewa and Hanan RM Ghanaym (2018). Vibrio species in fish and shell fish . Benha Veterinary Medical Journal, 34 (2): 246-254. DOI: https://doi.org/10.1007/bf0309.6487

Hiyoshi H, Kodama T, Ida T, and Honda T (2005). Contribution of Vibrio parahaemolyticus virulence factors to cytotoxicity, enterotoxicity, and lethality in mice. Infection and Immunity, 78: 1772-1780. DOI: https://doi.org/10.1128/IAI.01051-09

Hubbard TP, Chao MC, Abel S, Blondel CJ, zur Wieschdel PA, Zhoue X, Davis BM and Waldor MK (2016). Genetic analysis of Vibrio parahaemolyticus intestinal colonization, Proceedings of the National Academy of Sciences, 113 (22): 6283–6288. DOI: https://doi.org/10.1073/pnas.1601718113

Huss HH (1997). Control of indigenous pathogenic bacteria in seafood. Food Control, 8(2): 91-98. DOI:https://doi.org/10.1016/S0956-7135(96)00079-5

International Commission on Microbiological Specifications for Foods (ICMSF) (1996). Vibrio cholera. In: Microorganisms in Foods 5. Characteristics of Microbial Pathogens. London: Blackie Academic & Professional, pp. 414-425. Available at: https://www.iso.org/organization/5260.html

International Organization for Standardization/technical specification (ISO/TS21872-1) (2007). Specifies a horizontal method for the detection of the two main pathogenic Vibrio species causing intestinal illness in humans: V. parahaemolyticus and V. cholera. Available at: https://www.iso.org/standard/38278.html

International Organization for Standardization/technical specification (ISO/TS21872-2) (2007). Specifies a horizontal method for the detection of the enteropathogenic Vibrio species, causing illness in or via the intestinal tract , other than V. parahaemolyticus and V. cholera. Include V. fluvialis , V. mimicus and V. vulnificus. Available at: https://www.iso.org/standard/38279.html

Iwahori J, Yamamoto A, Suzuki H, Yamamoto T, Tsutsumi T and Motoyama K (2010). Quantitative risk assessment of Vibrio parahaemolyticus in finfish: a model of raw horse mackerel consumption in Japan. Risk Analysis, 30(12): 1817–1832. DOI: https://doi.org/10.1111/j.1539-6924.2010.01444.x

Kim YB, Okuda J, Matsumoto C, Takahashi T, Hashimoto S and Nishibuchi M (1999). Identification of Vibrio parahaemolyticus Strains at the Species Level by PCR Targeted to the toxR Gene. Journal of Clinical Microbiology, 37 (4): 1173-1177. DOI: https://doi.org/10.1128/JCM.37.4.1173-1177.1999

Kishishita M, Matsuoka N, Kumagai K, Yamashita S, Takada Y and Nishibuchi M (1992). Sequence variation in the most stable direct hemolysin related hemolysin (thh) gene of Vibrio parahaemolyticus. Applied and Environmental Microbiology, 58: 2449–2457. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC195802/
