



Incidence of *Vibrio* species in fish with special emphasis on the effect of heat treatments

Saad Mahmoud Saad¹, Maha Mohammed Samir², Hania El Sayed Abd El Maksod

¹Food Control Department, Faculty of Veterinary Medicine, Benha University, ²Food Control Department, Animal Health Research Institute, Zagazig Branch

ABSTRACT

A grand total of 150 random samples of fresh water fish (*Tilapia nilotica*), marine water fish (*Mugil cephalus*), and farm water fish (*Tilapia nilotica*) fish (50 of each) were collected from Sharkia governor during summer of 2014 to investigate the incidence of *Vibrio* spp. as well as studying the effect of heat treatments (frying and roasting). The obtained results revealed that incidence of *Vibrio* spp. in fresh water fish were 13 (26%), the overall incidence in the samples was 4(8%) for *V. vulnificus*, and 2(4%), 2(4%), 2(4%), 2(4%) and 1(2%) for, *V. mimicus*, *V. fluvialis*, *V. damsela*, *V. furnissi*, and *V. alginolyticus*, respectively. In marine fish *Vibrio* spp. were 24(48%), the overall incidence in the samples was for *V. parahaemolyticus* 5(10%), *V. vulnificus* 4(8%), *V. fluvialis* 4 (8%), *V. mimicus* 7(14%), *V. alginolyticus* 2(4%), and *V. damsela* 2(4%). In farm water fish *Vibrio* spp. were 17(34%) while the overall incidence in the samples was *V. parahaemolyticus* 1(2%), *V. vulnificus* 3(6%), *V. fluvialis* 3(6%), *V. mimicus* 5(10%), *V. alginolyticus* 2(4%), and *V. damsela* 3(6%). Ten pieces of fish fillet (100g of each and 5 pieces for each treatment) were used to study the effect of frying with cotton seed oil (1900 C) for 10 minutes and roasting in oven at 1500C for 10 minutes after their inoculation with 106 cfu/g *V. parahaemolyticus*. After roasting and frying, the microbial counts of *V. parahaemolyticus* were decreased by 98.2% and 100%, respectively.

Keywords: *Vibrio* spp., fresh water fish, marine fish, farm water fish, *V. parahaemolyticus*, heat treatment.

(<http://www.bvmj.bu.edu.eg>)

(BVMJ-29(1): 38-44, 2015)

1. INTRODUCTION

Fish is a nutrient-rich part of a healthful diet, and its consumption is associated with potential health benefits (Hibbeln et al., 2007) Fish and their products are responsible for a substantial proportion of foodborne diseases worldwide pathogen such as *Campylobacter*, *Salmonella*, *Vibriosis*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 have been found to be responsible for major food borne outbreaks worldwide (Velusamy et al., 2010). *Vibriosis* are Gram-negative, rod-shaped bacteria that occur naturally in estuarine or marine environments. Infection is usually occur

from exposure to seawater or consumption of raw or undercooked fish (Altekruse et al., 2000). *Vibriosis* is characterized by diarrhea, primary septicemia, wound infections, or other extra-intestinal infections (Daniels et al., 2000). Numerous studies have been conducted to determine the relationship between *Vibrio* spp. abundance and environmental factors such as temperature, salinity, nutrients and dissolved oxygen. As a result, these water quality characteristics can be used in a predictive manner to determine when these pathogens may be present (Gayatri, 2011). Once consumers eat undercooked or

contaminated fish, illness is inevitable (Rahimi et al., 2010). The typical clinical symptoms of *V. parahaemolyticus* poisoning are acute dysentery and abdominal pain, accompanied by diarrhea, nausea, vomiting, fever, chills, and water-like stools (Shimohata and Takahashi, 2010). The feces of patients are mixed with mucus or blood, and their blood pressure decreases dramatically, leading to shock (Broberg et al., 2011). *V. parahaemolyticus* is very sensitive to heat (killed at 47^o- 60^o C) and to ionizing radiation, as well as to halogens (Adams and Moss, 2008). Thermal processing is one of the most common methods for achieving safe convenience fish products with an extended shelf life. The aim of this study was to investigate the incidence of *Vibrio* spp. in marine fish, fresh water fish and farm water fish as well as studying the effect of heat treatments (frying and roasting) on it.

2. Material and methods:

Part I: Isolation of *Vibrio* species

2.1. Collection of the samples

A grand total of 150 random samples of marine (Mullet, Sea Bream, Altobar), fresh water (*Tilapia nilotica*) and farm water (Mullet and Tilapia) fish (50 of each) were collected from Sharkia governorate during summer of 2014. All samples were collected and transferred with a minimum of delay to the laboratory in ice box. All samples were subjected to the bacteriological examination.

2.2. Preparation of samples:

The scales and fins of the fish were removed, the skin was sterilized by alcohol and flamed by sterile spatula. The muscles above the lateral line were removed, five grams were taken under aseptic conditions to sterile homogenizer containing 45ml of sterile alkaline peptone water (3%Nacl and pH 8).

2.3. Screening of *Vibrio* spp.

It was done according to FDA (2004)

Isolation: Loopfuls from each previous cultured tubes were separately streaked onto Thiosulfate citrate bile and sucrose agar (TCBS), then the medium was incubated at 37^o C for 24hrs. Typical colonies of *V. mimicus*, *V. parahaemolyticus* and *V. vulnificus* were appeared as smooth and green (sucrose negative), while colonies of *V. cholerae*, *V. furnissii*, *V. alginolyticus* and *V. fluvialis* were appeared as smooth and yellow (sucrose positive).

Presumptive identification: This was done according to the protocol recommended by ISO/ TS 21872-1 (2007) and ISO/ TS 21872-2 (2007).

Part II: Effect of Heat Treatment on *V. parahaemolyticus* count

2.4. Bacterial strain

V. parahaemolyticus was obtained from the Food Microbiology Laboratory. *V. parahaemolyticus* was maintained on trypticase soy agar slants (containing 3%Nacl) at 4^oC. A loopful of *V. parahaemolyticus* was transferred aseptically into 10 ml sterile Alkaline Peptone Water plus 3% Na cl and followed by cultivating separately at 37^oC for 24hrs in shaker incubator. After incubation *V. parahaemolyticus* was counted by using spread plate method (FDA, 2001) and then adjusted to ~ 10⁶ CFU/ml (Shirazinejad and Ismail, 2010) with tube dilution method.

2.5. Fish fillet Samples

A total of 2 groups of fish fillet (5 pieces for each). All samples were washed in sterile distilled water and disinfected with alcohol.

2.6. Artificial Contamination of fish fillet samples with *V. parahaemolyticus*

Samples were dipped in 100ml Tryptic Soy Broth containing a 24hrs-old culture (with ~ 10⁶ CFU/ml) (Shirazinejad and Ismail, 2010) and left for 30min. at room temperature to allow attachment. The

contaminated samples were stored in sterile glass beakers covered with glass lids at ambient temperature ($30\pm 2^{\circ}\text{C}$). *V. parahaemolyticus* in the samples was enumerated to get the initial load before treatments was performed according to Terzi and Gucukoglu (2010).

2.7. Heat treatment :(roasting and frying) (Pearson and Tauber,1984)

Five pieces of contaminated fish fillet with known *V. parahaemolyticus* load (100g of each) were wrapped separately with aluminum foil then put into oven at 150°C for 10 minutes. Another five pieces were used to study the effect of frying with cotton seed oil (190°C) for 10 minutes. After roasting and frying , the microbial counts of *V. parahaemolyticus* were done. The initial counts of control raw samples were also recorded and calculated.

2.8. Bacteriological Analysis

From each sample 10g were taken under aseptic conditions to sterile homogenizer containing 90ml peptone water (3%Nacl) then the contents were homogenized at 3000 rpm for 2.5 minutes The mixture was allowed to stand for 15 minutes at room temperature under aseptic conditions. The content of the flask were thoroughly mixed by shaking and 1ml was transferred into separated tubes each containing 9ml peptone water (3%Nacl), from which tenfold serial dilutions up to 10^{-6} were prepared. From the prepared sample 0.1 ml of each prepared serial dilutions were streaked over the surface of thiosulphate citrate bile sucrose agar plates (TCBS) and incubated at 37°C for 24hrs (Thatcher and Clark,1978). Rounded colonies 2-3mm in diameter , with green and/or blue centers were recorded as *V. parahaemolyticus*.

3. RESULTS

Incidence of *Vibrio* spp. isolated from the examined samples of fish recorded in Table (1) were 26% ,48% and 43% for freshwater

, marine and farm water fish respectively. Incidence of *Vibrio* sp. in examined Freshwater Fish samples recorded in Table (2) revealed that incidence in the samples was 1(2%) for *V. alginolyticus* and were 2(4%) for each of *V. damsela*, *V. fluvialis* ,*V. furnissi* and *V. mimicus* and for *V. vulnificus* was 4(8%), while *V. cholerae* and *V. parahaemolyticus* failed to be detected biochemically. Incidence of *Vibrio* spp. in examined fish samples collected from marine water was illustrated in Table (3) which revealed that incidence in the samples were 2(4%) for each of *V. alginolyticus* and *V. damsela*, while, for *V. vulnificus* and *V. fluvialis* were 4 (8%) for each. For *V. parahaemolyticus* was 5 (10%) and for *V. mimicus* was 7 (14%), while, *V. cholerae* failed to be detected biochemically. The incidence of *Vibrio* spp. in examined Farm water Fish samples recorded in Table (4) revealed that incidence in the samples was 1(2%) for *V. parahaemolyticus* and was 2(4%) for *V. alginolyticus* and was 3 (6%) for each of *V. damsela*, *V. fluvialis* and *V. vulnificus* and was 5 (10%) for *V. mimicus*, while, *V. cholerae* and failed to be detected biochemically. The influence of cooking using oven and frying on the count of *V. parahaemolyticus* (1×10^6) inoculated into fish fillet samples are shown in Table (5). Before cooking, the obtained results revealed that the count of *V. parahaemolyticus* was 105 cfu/g. After roasting, the maximum count was 3×10^3 cfu/g and the minimum count was 1×10^3 cfu/g with mean value of $1.8\times 10^3 \pm 3.3\times 10^2$. Therefore, the reduction percent in total count of *V. parahaemolyticus* was 98.2%. While after frying *V. parahaemolyticus* was completely destroyed and the reduction % was 100%.

Table (1): Incidence of *Vibrio* species isolated from the examined samples of fish (n = 50 of each type)

Fish type	Positive samples	%
Fresh water fish	13	26
Marine fish	24	48
Farm water fish	17	34

Table (2): Incidence of *Vibrio* species isolated from Fresh Water Fish

Isolate	Number	%
<i>V. vulnificus</i>	4	8
<i>V. mimicus</i>	2	4
<i>V. fluvialis</i>	2	4
<i>V. damsela</i>	2	4
<i>V. furnissi</i>	2	4
<i>V. alginolyticus</i>	1	2

Table (3): Incidence of *Vibrio* species isolated from Marine Water Fish

Isolate	Number	%
<i>V. parahaemolyticus</i>	5	10
<i>V. vulnificus</i>	4	8
<i>V. fluvialis</i>	4	8
<i>V. mimicus</i>	7	14
<i>V. alginolyticus</i>	2	4
<i>V. damsela</i>	2	4

Table (4): Incidence of *Vibrio* species isolated from Farm Water Fish

Isolate	Number	%
<i>V. parahaemolyticus</i>	1	2
<i>V. vulnificus</i>	3	6
<i>V. fluvialis</i>	3	6
<i>V. mimicus</i>	5	10
<i>V. alginolyticus</i>	2	4
<i>V. damsela</i>	3	6

Table (5): Influence of Heat Treatment on the count of *V. parahaemolyticus*. (n=5)

Count (cfu/gm)	Before treatment	After treatment			Reduction %
		Min.	Max.	Mean \pm SE	
Roasting (oven- 150° C/10 min)	10 ⁵	1X10 ³	3X10 ³	1.8X10 ³ \pm 3.3X10 ²	98.2
Frying (cottonseed oil-boiling 190° C/10 min)	10 ⁵	ND	ND	ND	100

4. DISCUSSION

It is evident from the results recorded in table (1) the high level of *Vibrio* spp. in marine fish when compared with samples from fresh and farm water fish this may be due to high salinity. Nearly similar results were obtained by Yücel and Balci (2010) and UCLA (2004).

Sanjeev (2002) reported the incidence of *V. parahaemolyticus* in fresh, marine and brackish water fish varied from 35 to 55%. Also Todar (2005) mentioned that Vibriosis was wide spread in marine and freshwater habitats. Incidence of *Vibrio* spp. in

examined Freshwater Fish samples recorded in Table (2) revealed that the incidence of *Vibrio* spp. was 13 (26%), *V. parahaemolyticus* was detected in samples from Portugal (35%) by Andrew et al. (2003). These results lower than those reported by Noorlis et al. (2011) who found that *Vibrio* spp. could be detected at a prevalence of 98.67%, whereas *V. parahaemolyticus* was detected at a prevalence of 24% from examined fresh water fish. The presence of *Vibrio* spp. in samples of freshwater fish suggests that foodborne illness could arise if these fish are consumed in the uncooked or

undercooked state. They could also cross-contaminate ready-to-eat foods that are in the same environment. Incidence of *Vibrio* spp. in examined Fish samples collected from Marine water fish was illustrated in Table (3) which revealed that the incidence of *Vibrio* spp. were 24 (48%). These results nearly similar results those reported by Abd Ellatif (2013), John et al. (2011) and Slavica et al. (2002). On the other hand, higher results were reported by Engy (2006) and Jaksic et al. (2002) who isolated *V. alginolyticus*, *V. fluvialis* and *V. mimicus* from 14%, 9% and 28% of the examined samples of marine fish, respectively.

Lower results were recorded by Raissy et al. (2013) who revealed that 29.3 % of the examined fish were *Vibrio* positive. This high incidence probably reflects the nature of *Vibrio* spp. which is known as a halophilic waterborne bacterium that commonly inhabits environmental water sources worldwide. The incidence of *Vibrio* spp. in examined farm water fish samples recorded in Table (4) revealed that the incidence of *Vibrio* spp. was 17(34%). These results nearly similar to those of Abd-El-Latif et al. (2008) who isolated *Vibrio* spp. with a percentage of 33.75% from farm water fish. Gaber and Samy (2014) also reported that 32% of farm water fish were positive for *Vibrio* spp. These results are higher than results reported by Anwar et al. (2010) who detect *Vibrio* spp. in 16.8% of the total examined fish. On the other hand, higher results were recorded by Ahmed and Naim (2005) who found that 58% of the total isolates were *Vibrio* spp. This may indicate bad management practices (inadequate nutrition, overcrowding and overfeeding) in fish farms which can cause stress to the fish being cultured and thus make them more susceptible to microbial infection. Aquaculture in Egypt remains a growing, vibrant and important production sector for high-protein animal food that is easily digestible and of high biological value. However, a major setback in aquaculture is the outbreak of diseases,

especially those caused by *Vibrio* spp. which considered significant economic and public health problems.

The influence of cooking using oven and frying on the count of *V. parahaemolyticus* (1×10^6) inoculated into fish fillet samples were shown in Table (5). Before cooking, the obtained results revealed that the count of *V. parahaemolyticus* was 10^5 cfu/g. After roasting, the maximum count was 3×10^3 cfu/g and the minimum count was 1×10^3 cfu/g with mean value of $1.8 \times 10^3 \pm 3.3 \times 10^2$. Therefore, the reduction percent in total count of *V. parahaemolyticus* was 98.2%. While after frying *V. parahaemolyticus* was completely destroyed and the reduction % was 100%. Accordingly, the best and fast method for heat treatment of fish was by frying for 10min. at 190^0 C. Such results agree with those reported by Abd Ellatif (2013) who recorded that *V. parahaemolyticus* count reduced by 99.2% after cooking in oven 120^0 C/35min. and 100% after frying. Also, ICMSF (1996) which stated that *V. parahaemolyticus* can be killed when boiling at least 64^0 C for more than 90 seconds.

5. REFERENCES

- Abd-El-Latif, M.M., Moustafa, A.H. and Adawy, R.S.M. 2008. Some studies on Vibriosis in farmed Mugil cephalus in Dakahlia governorate. Assiut Veterinary Medical Journal, 19(1). 54(118):177-185.
- Abd Ellatif, Z.A. 2013. *Vibrio parahaemolyticus* in marine fish. Department of food control ,Faculty of Veterinary Medicine, Benha University, pp. 41-43
- Adams, M.R. and Moss, M.O. 2008. Bacterial agents of food borne illness In: Food Microbiology (3rd Ed.) RSC, Surrey UK, pp. 182–269.
- Ahmed, H. Al-Harbi and Naim Uddin, 2005. Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in

- brackish water in Saudi Arabia Aquaculture. 250(3–4): 566–572
- Altekruse, S.F.; Bishop, R.D. and Baldy, L.M. 2000. *Vibrio gastroenteritis* in the US Gulf of Mexico region, the role of raw oysters. *Epidemiol. Infect* 124:489-95
- Andrews, L.; Jahncke, M .and Mallikarjunan, K. 2003. Low dose gamma irradiation to reduce pathogenic *Vibrio* in live oysters (*Crassostrea virginica*). *J. Aquat. Food Prod. Technol.* 12:71–82.
- Anwar E. Al-Sunaiher; Abdelnasser S.S. Ibrahim and Ali A. Al-Salamah 2010. Association of *Vibrio* Species with Disease Incidence in Some Cultured Fishes in the Kingdom of Saudi Arabia. *World Applied Sciences Journal* 8(5):653-660.
- Broberg, C. A.; Calder, T. J. and Orth, K. 2011. *Vibrio parahaemolyticus* cell biology and pathogenicity determinants. *Microbes Infect.* 13:992–1001.
- Daniels, N. A.; Mackinnon, L.; Bishop, R.; Altekruse, S.; Ray, B.; Hammond, R. M.; Thompson, Sh.; Wilson, S.; Bean, N, H.; Griffin, P. M. and Slutsker, L. 2000. *Vibrio parahaemolyticus* Infections in the United States, 1973-1998. *J. Infect. Dis.*, 181(5):1661-1666.
- Engy, F. A. El-Bahy 2006. *Vibrio parahaemolyticus* and some halophilic bacteria associated with fish and shellfish. Department of food control, Faculty of Veterinary Medicine, Alexandria University, pp. 35.
- Food and Drug Administration (FDA) 2001. Methods for Specific Pathogens: *Escherichia coli* ,Salmonella and *Vibrio*, 8th Ed. FDA Center for Food Safety and Applied Nutrition ,Bacteriological Analytical Manual. Available at www.cfsan.fda.gov/~ebam/bam-toc.html
- Food and Drug Administration (FDA) 2004. Bacteriological Analytical Manual, *Vibrio* Chapter-9 (BAM).
- Gaber, S. Abdellrazeq and Samy A. Khaliel 2014. Molecular Characterization and Antimicrobial Susceptibility of *Vibrios* Isolated from Healthy and Diseased Aquacultured Freshwater Fishes, Department of Microbiology, Faculty of Veterinary Medicine, Alexandria University, Edfina, Rosetta-line, Egypt. *Global Veterinaria* 13(3):397-407
- Gayathri, V. 2011. The prevalence and public health significance of human pathogenic *Vibrio* species in Hawai'i's diverse tropical coastal water environments.
- Hibbeln, J. R.; Davis, J. M. ; Steer, C.; Emmett, P.; Rogers, I.; Williams, C. and Golding, J. 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet* 369:578-585.
- International Commission on Microbiological Specifications for Foods (ICMSF) 1996. *Vibrio cholerae*. In: *Microorganisms in Foods 5. Characteristics of Microbial Pathogens*. London: Blackie Academic & Professional; pp. 414-425.
- 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. cholerae*.
- 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. cholerae*. Include *V. fluvialis*, *V. mimicus* and *V. vulnificus*.
- Jaksic, S.; Uhtil, S.; Patrak, T.; Bazulic, D. and Karolyi, L.G. 2002. Occurrence of *Vibrio* spp. In sea fish, shrimps and bivalve mollusks from the Adriatic Sea. *Food Control*; 13:491-493.
- John, M.O., Odu, N.N., Nwanze, J.C., Adebayo-Tayo, B.C., Okonko, I.O. and Ezediokpu, N. 2011. Occurrence of Potentially Pathogenic *Vibrio* Species in Sea Foods Obtained from Oron Creek *Advances in Biological Research* 5(6):356-365.
- Noorlis, A.; Ghazali, F. M.; Cheah, Y. K.; Tuan Zainazor, T. C.; Ponniah, J.; Tunung, R.; Tang, J. Y. H.; Nishibuchi, M.; Nakaguchi, Y. and Son, R. 2011. Prevalence and quantification of *Vibrio* species and *Vibrio parahaemolyticus* in freshwater fish at hypermarket level. *International Food Research Journal* 18: 689-695
- Pearson, P.A and Tauber, W.F. 1984. *Processed meat* 2nd Ed. AVI publication, INC., West Port. CT.
- Rahimi, E.; Ameri, M.; Doosti, A. and Gholampour, A. R. 2010. Occurrence of toxigenic *Vibrio parahaemolyticus* strains in shrimp in Iran. *Foodborne Pathogen and Diseases*. (7):1107-1111.
- Raissy, M.; Rahimi, E.; Azargun, R. ; Moumeni, M.; Rashedi, M. and Sohrabi, H. R. 2013. Molecular Detection of *Vibrio* spp. in Fish and Shrimp from the Persian Gulf. *Journal of Food Biosciences and Technology, Islamic Azad University, Science and Research Branch*, 5(2):49-52.
- Sanjeev, S. 2002. Pathogenic halophilic *Vibrios* in seafoods. In: *Quality Assurance in Seafood Processing*, 252pp. T.S. Gopala Krishna Iyer, M.K. Kandoran, Mary Thomas and P.T Mathew (Eds). Central Institute of Fisheries Technology and Society of Fisheries Technologists (India), Cochin.
- Shimohata, T. and Takahashi, A. 2010. Diarrhea induced by infection of *Vibrio parahaemolyticus*. *J. Med. Invest.*, 57:179–182.
- Shirazinejad, A. and Ismail, N. 2010. Effect of lactate treatments on survival of foodborne pathogens in frozen shrimp. *American J. Agricultural and Biological Sciences*, 5:242-246.
- Slavica Jak, Si. C. ; Suncica, U.; Petrak, T.; Bazulic, D. and Lada, G. K. 2002. Occurrence of *Vibrio* spp. in sea fish, shrimps and bivalve mollusks harvested from Adriatic sea. www.elsevier.com/locate/foodcont *Food Control* 13:491–493.
- Terzi, G. and Gucukoglu, A. 2010. Effects of lactic acid and chitosan on the survival of *V. parahaemolyticus* in mussel samples. *J. Animal and Veterinary Advances*, 9:990-994.
- Thatcher, F.S. and Clark, 1978. *Microorganisms in foods. Their Significance and methods of enumeration*. 2nd Ed. Academic Press, New York.
- Todar, K. 2005. *Vibrio cholerae* and Asiatic cholera. <http://textbookofbacteriology.net/cholera.html>.
- University of California, Los Angeles Marine science center (UCLA) 2004. *Density and salinity, A Curriculum in Marine Sciences*, 3-6
- Velusamy, V.; Arshak, K.; Korastynska, O.; Oliwa, K. and Adley, C. 2010. An overview of food borne pathogen detection in the perspective of Biosensors. *Biotechnology Advances*, 28: 232-254.
- Yücel, N. and Balci, Ş. 2010. Prevalence of *Listeria*, *Aeromonads*, and *Vibrio* Species in Fish used for Human Consumption in Turkey. *Journal of Food Protection*, 2: 212-404