



Enterobacteriaceae in Some Fresh and Marine Fish

Saad, M.S.¹, Hassan, M.A.¹, Hassnien, F.S.¹, Abdel-Aal, M.M.², Zakar, A.H.² and Elshfey, S.A.²

¹ Food Control Department. Faculty of Veterinary Medicine, Benha University

² Central Laboratory for Aquaculture Research, Agriculture Research Center, Egypt.

ABSTRACT

A total of 100 random samples of fresh and marine fishes represented by *Tilapia niloticus*, *Mugil cephalus*, Sardine and Barboni (25 Of each) were collected from the different fish markets located in Gharbia governorate. All collected samples were subjected to bacteriological examination for isolation and identification of Enterobacteriaceae. The results revealed that the total Enterobacteriaceae counts in the examined samples of fresh and marine fish were varied from 1.4×10^3 to 5.7×10^4 with a mean value (1.9×10^4) cfu/g for *Oreochromis niloticus*, 7.0×10^2 to 2.40×10^4 with a mean value (9.06×10^3) cfu/g for *Mugil cephalus*, 4.0×10^2 to 8.9×10^3 with a mean value, 4.25×10^3 cfu/g for Sardine, 1.0×10^2 to 3.30×10^3 with a mean value (1.18×10^3) cfu/g for Barboni. Also, Salmonella species were recovered from 24 %, 20 %, 8% and 8 % of the examined samples of *O. niloticus*, *Mugil cephalus*, Sardine and Barboni, respectively. Enterobacteriaceae count, Coliform count and the isolated Salmonella organisms as well as the public health significance were discussed and the prophylactic measures to reduce contamination in fish were recommended. The work was aimed to study occurrence of Enterobacteriaceae in fish sold in different fish markets located in Gharbia governorate.

Keywords: Enterobacteriaceae, *Oreochromis niloticus*, *Mugil cephalus*, Sardine, Barboni, Salmonella.

(<http://www.bvmj.bu.edu.eg>)(BVMJ-34(1): 261-268, 2018)

1. INTRODUCTION

Fish had long been regarded as a desirable and nutritional source of high quality protein and generous supply of minerals and vitamins constituting the major part of human diet. Also, Fish constitute the cheapest source of animal protein in Africa (Cooper, 2014). Unlike meat and poultry, fish are more liable to contamination with pathogenic bacteria from human reservoir which may contaminate the water depending on the fishing and also may be further contaminated

during handling, processing and packaging. (Bankevich et al., 2012).

Bacterial diseases in fish are a serious threat to aquaculture systems that cause severe damage and mortality in Egypt (Noor El-Deen et al., 2010). Enterobacteriaceae are widely distributed in nature and found in feces of human, poultry and animals (Wogu and Maduakol 2010) and considered as an indicator to sewage pollution and has been reported as opportunistic pathogen in fish

(Rajasekaran 2008). The presence of Enterobacteriaceae in fish farming leads to a serious health public risk. Despite in most cases these microorganisms are part of normal microbiota from fish, when colonizing human sites, they can cause some diseases, like urinary tract infection. (Nagamatsu et al., 2015).

Large quantities of coliform bacteria in water and fish may indicate a higher risk of pathogens being present. Dysentery, typhoid fever, bacterial gastroenteritis and many other water borne disease may coincide with faecal coliform contamination. The presence of faecal coliform may affect human more than it does aquatic organisms (Doyle et al., 2006). Salmonella is a genus of Gram-negative rod-shaped bacteria of the family Enterobacteriaceae (Anonymous 1995). Salmonella is a second leading cause of food borne illness worldwide. The majority of 1.3 billion annual cases of Salmonella cause human gastroenteritis, through the ingestion of shell fish and fish. The wide range of human diseases that caused by it includes, enteric fever, bacteremia and gastroenteritis. Gastroenteritis has the almost adversative effect on children's growth and improvement (Bibi et al., 2015).

Therefore, the present study was planned to determine the contamination level of some marine fish fillet with Enterobacteriaceae to protect the health of the consumers.

2. Materials and methods

2.1. Collection of samples:

A total of 100 random samples of Nile and marine fishes represented by *Tilapia niloticus*, *Mugil cephalus*, Sardine and Barboni (25 Of each) were collected from the different fish markets located in Gharbia governorate. Each sample (10gram) was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue

delay and examined as quickly as possible. The collected samples were subjected to the bacteriological examination to evaluate their quality. To 5 grams of the sample, 45 ml of sterile peptone water 0.1% were added and thoroughly mixed using sterile blender for 1 - 1.5 minutes, from which tenth fold serial dilutions were prepared. The prepared samples were subjected to the following examinations:

- Determination of total Enterobacteriaceae Count (ICMSF, 1996).
- Total Coliform count (ICMS, 1996).
- Identification of family Enterobacteriaceae (Cowan and Steel 1974).
- Isolation and identification of Salmonellae (Rappaport et al., 1956; Harvey and Price, 1981).
- Serological identification of Salmonellae (Kauffman, 1974).

2.2. Statistical Analysis:

The evaluation and interpretation of obtained results were carried out by using of Analysis of Variance (ANOVA) test according to Feldman *et al.* (2003).

3. RESULTS

Table (1 and 2) cleared that, the incidences of enterobacteriaceae among examined samples show a significant differences ($P < 0.01$) among examined fish samples. The higher incidences of enterobacteriaceae observed in *O. niloticus* 25 (100 %), followed by *Mugil cephalus* (92%). While, the lower incidences of enterobacteriaceae observed in Sardine 18 (72 %) and Barboni 17 (68 %). The counts of enterobacteriaceae among examined fish differ significantly ($P < 0.01$) among examined fish. The higher incidences of enterobacteriaceae observed in *O. niloticus* as its level ranged from 1.4×10^3 to 5.7×10^4 with a mean value, 1.91×10^4 while, in *Mugil cephalus* its level ranged from 7.0×10^2 to 2.40×10^4 with a mean value of 9.06×10^3 . While, the lower incidences of enterobacteriaceae observed in Sardine as its level ranged from 4.0×10^2 to

8.9×10^3 with a mean value 4.25×10^3 while, in Barboni its level ranged from 1.0×10^2 to 3.30×10^3 with a mean value of 1.18×10^3 .

Table (3 and 4) cleared that, the incidences of coliforms among examined samples show a significant differences ($P < 0.01$) among examined fish samples.

The higher incidences of coliforms observed in *O. niloticus* 22 (88%), followed by *Mugil cephalus* 19 (76%). While, the lower incidences of coliforms observed in Sardine 15 (60 %) and Barboni 13 (52 %). The counts of coliforms among examined fish differ significantly ($P < 0.01$) among examined fish. The higher incidences of coliforms observed in *O. niloticus* as its level ranged from 3.0×10^2 to 1.5×10^4 with a mean value 6.12×10^3 while, in *Mugil cephalus* its level ranged from 1.0×10^2 to 9.20×10^3 with a mean value of 2.87×10^3 . While, the lower incidences of coliform observed in Sardine as its level ranged from 1.0×10^2 to 4.6×10^3 with a mean value 1.93×10^3 while, in Barboni its level ranged from 1.0×10^2 to 2.10×10^3 with a mean value of 7.66×10^2 .

The recurrent results cleared in table (5) cleared that, the accepted and unaccepted fish samples differ significantly ($P < 0.01$) among the examined fish samples according to its content of coliforms.

The results cleared that, in *O. niloticus* the number of accepted fish samples were 3 (12 %), while, the unaccepted samples were 22 (88), while, in *Mugil cephalus* the number of accepted samples were 8 (32 %), and the number of unaccepted samples were 17 (68 %). While, in sardine the number of accepted samples were 12 (48 %) and the number of unaccepted samples were 13 (52 %), but in barboni the number of accepted samples were 15 (60 %) and the number of unaccepted samples were 10 (40 %).

The present results cleared in table (6), on the incidence of incidences of salmonella from fresh and marine fish cleared that, the incidences of salmonella organisms among examined fish differ significantly ($P < 0.01$). The results cleared that the incidences of *S. Enteritidis* of a higher level in *O. niloticus* 1 (4 %) and *M. cephalus* 1 (4 %) and not isolated from sardine and barboni. The isolates type of *S. Enteritidis* related to group D1 and its antigenic structure were O1, 9, 12 and H g, m : 1, 7.

While, incidences of *S. infantis* of a higher level in *O. niloticus* 1 (4 %) and not recorded in *M. cephalus*, sardine and barboni. They type of *S. infantis* related to group C1 and its antigenic structure were O6, 7, 14 and H r : 1, 5.

The results of the incidences of *S. larochelle* of a higher level in *M. cephalus* 1 (4 %) and not recorded in *O. niloticus*, sardine and barboni. The type of *S. larochelle* related to group C1 and its antigenic structure were O6, 7 and H e, h : 1, 2.

The results of the incidences of *S. heidllberg* of a higher level in *M. cephalus* 2 (8 %) and not recorded in *O. niloticus*, , sardine and barboni. The type of *S.heidllberg* related to group B and its antigenic structure were O1, 4, 5, 12 and H r : 1, 2.

The results of the incidences of *S. malade* of a higher level in Sardine 1 (4 %) and not recorded in *O. niloticus*, *M. cephalus* and barboni. The type of *S. molade* related to group C2 and its antigenic structure were O 8, 20 and H Z10 : Z6.

The results of the incidences of *S. typhimurium* of a higher level in *O. niloticus* 3 (12 %), *M. cephalus* 1 (4 %) and Barboni 1 (4 %) and not recorded in sardine. The type of *S. typhimurium* related to group B and its antigenic structure were O1, 4, 5, 12 and H i : 1, 2.

The results of the incidences of *S. virchow* of a higher level in *O. niloticus* 1 (4 %) and barboni 1 (4 %) and not recorded in *M. cephalus* and sardine. The type of *S. virchow* related to group C1 and its antigenic structure were O6, 7, 14 and H r: 1, 2.

The results of the incidences of *S. wingrove* of a higher level in sardine 1 (4 %) and not recorded in *O. niloticus*, *M. cephalus* and

barboni. The type of *S. wingrove* related to group C2 and its antigenic structure were O 6, 8 and H c : 1, 2.

The total number of isolated salmonella observed in *O. niloticus* 6 (24 %), *M. cephalus* 5 (20 %), sardine 2 (8 %) and barboni 2 (8 %) and the most group related to it is the C2 group and its most antigenic structure related to O 6, 8 and H c: 1, 2.

Table (1): Incidence and counts of Enterobacteriaceae (cfu/g) in the examined samples of fresh and marine fish (n=25).

Fish species	+ve samples		min	max	Mean \pm S.E*
	No.	%			
<i>Oreochromis niloticus</i>	25	100	1.4×10^3	5.7×10^4	$1.91 \times 10^4 \pm 0.38 \times 10^4$
<i>Mugil cephalus</i>	23	92	7.0×10^2	2.4×10^4	$9.06 \times 10^3 \pm 2.15 \times 10^3$
Sardine	18	72	4.0×10^2	8.9×10^3	$4.25 \times 10^3 \pm 0.71 \times 10^3$
Barboni	17	68	1.0×10^2	3.3×10^3	$1.18 \times 10^3 \pm 0.43 \times 10^3$

S.E* = Standard error of mean

Table (2): Analysis of variance (ANOVA) of Enterobacteriaceae counts in the examined fresh and marine fish samples.

Source of variance	D.F	S.S	M.S	F.value
Total	99	269927.48		
Between Species (S)	3	102692.57	34230.86	19.65 ⁺⁺
Error	96	167234.91	1742.03	

D.F = Degrees of freedom, S.S = Sum squares, M.S = Mean squares, ++ = High significant differences (P<0.01)

Table (3): Incidence and counts of coliform (cfu/g) in the examined samples of fresh and marine fish (n=25).

Fish species	+ve samples		Min	Max	Mean \pm S.E*
	No.	%			

Enterobacteriaceae in Some Fresh and Marine Fish

<i>Oreochromis niloticus</i>	22	88	3.0×10^2	1.5×10^4	$6.12 \times 10^3 \pm 1.07 \times 10^3$
<i>Mugil cephalus</i>	19	76	1.0×10^2	9.2×10^3	$2.87 \times 10^3 \pm 0.59 \times 10^3$
Sardine	15	60	1.0×10^2	4.6×10^3	$1.93 \times 10^3 \pm 0.41 \times 10^3$
Barboni	13	52	1.0×10^2	2.1×10^3	$7.66 \times 10^2 \pm 1.19 \times 10^2$

S.E* = Standard error of mean

Table (4): Analysis of variance (ANOVA) of coliform counts in the examined fresh and marine fish samples.

Source of variance	D.F	S.S	M.S	F.value
Total	99	128906.59		
Between Species (S)	3	33154.23	11051.39	11.08 ⁺⁺
Error	96	95752.36	997.42	

D.F = Degrees of freedom, S.S = Sum squares, M.S = Mean squares, ++ = High significant differences (P<0.01)

Table (5): Acceptability of the examined samples of fresh and marine fish based on their coliform count/g (n=25).

Fish species	Coliform count /g*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
<i>Oreochromis niloticus</i>	$> 10^2$	3	12	22	88
<i>Mugil cephalus</i>	$> 10^2$	8	32	17	68
Sardine	$> 10^2$	12	48	13	52
Barboni	$> 10^2$	15	60	10	40

Chi2 = 10.75**

** = Significant at (P < 0.01).

* Egyptian Organization for Standardization "EOS" (2005).

ES 3494-2005 for chilled fish

4. DISCUSSION

The surface of the fish, fish mucus, muscle tissues and body fluids of apparently healthy living fish are usually free from bacteria, but during, transportation, slaughtering and processing contamination occurs and cannot

be avoided leading to introduction of pathogens into the meat. The source of these pathogens may be endogenous from the gastro-intestinal tract or from surrounding environment fish and fish products are the most common food vehicles of human

infection with enteropathogens throughout the world (Rajkowski et al., 2012).

The bacteriological examination of fish meat to determine conformance to the fish meat specification (i.e. bacteriological criteria) is often used, testing for conformance to such criteria provides only limited protection to consumer against food poisoning and/or foodborne diseases, this often in fact the reason for carrying-out the tests to provide assurance of fish meat (Sheen et al., 2012).

The current results in table (1) agreed with those of (Farag, 2006), where they reported that, the incidences of enterobacteriaceae in sardine and barbone, where they bred in sea water that characterized by lower level of sewage that, causes decreasing the level of Enterobacteriaceae. While, the *O. niloticus* and *Mugil cephalus* where they bred in fresh water that, have a high level of sewage pollution that causes increasing level of Enterobacteriaceae in this type of fish.

Generally, the presence of *coliform* in fish serves as index of sanitation under which the fish is handled. Thus, their significance is directly associated with the faecal

contamination catching, handling and storage of fish.

Also, the results agreed with those of (Shamshad et al., 1992) concluded that the fish from the coastal waters are generally free from micro-organisms of public health importance and degree of contamination depends on the extend of handling and the hygienic practices employed.

The results in table(6) agreed with those of (Samaha and Hendawy, 2017) where they stated incidence of Salmonella which was (3) 6%, (5) 10%, (4) 8% and (2) 4% of the examined samples of Basa, Barboni, Mackerel and Denise, respectively.

In general, collective enumeration of salmonellosis is recorded as means indicating enteric contamination of food item. Therefore, the salmonellosis count may be used as a broad base in detection of the organisms which may contaminate the fish after catching. Accordingly, the presence of considerable numbers of *salmonellosis* indicates unsatisfactory hygienic measures during catching and distribution of the fish (Valdivia et al., 1997).

5- REFERENCES

Anonymous, 1995. Salmonella data set Communicable Disease Report (CDR). Public Health Laboratory Service, London, UK. Commun. Dis. Rep. Wkly., 5: 29-32.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A, Dvorkin, M, Kulikov, A.S, Lesin, V.M ,Nikolenko, S.I, Pham, S., Prjibelski, A.D, Pyskin, A.V, Sirotkin, A.V, Vyahhi, N., Tesler, G., Alekseyev, M.A, Pevzner, P.A. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J ComputBiol 19:455–477.

Bibi , F., Qaisrani , S .N., Ahmad, A.N., M. Akhtar, M., B. N. Khan,B.N., and Ali , Z. 2015. Occurrence Of Salmonella In Freshwater Fishes, The J. of Animal & Plant Sci., 25(3 Supp. 2) 2015 Special Issue Page: 303-310 ISSN: 1018-7081

Cooper, L. 2014. Using Fish and Shellfish Quality Assessment Methods. Learner Workbook.(www.seafoodacademy.org)

Cowan, S.T. and Steel, K.J. 1974: Manual for identification of medical bacteria. Cambridge Univ. Press, London, New York, Malburne.

Doyle, M.P. and M.C. Ericson, 2006. Closing the door on the faecal coliform assay. .. 1: 162-163.

- Doyle, M.P. 2001. Food Microbiology: Fundamentals and Frontiers. 2nd Edn., ASM Press, Washillgton.
- E.S 3494/2005. Egyptian Standards, chilled fish, part one for whole fish, included different types of imported fish.
- Farag, A.L. 2006. Improvement of the bacteriological quality of fish fillets and peeled prawn available in the Egyptian markets. M. V. Sc. Thesis, Fac Vet Med Alexandria University, Egypt.
- Feldman, D., Ganon, J., Haffman, R. and Simpson, J. 2003. The solution for data analysis and presentation graphics. 2nd Ed., Abacus Lancripts, Inc., Berkeley, USA.
- Harvey, R.W. and Price, T. H. 1981. Comparison of Selenite F, Muller Kauffmann Tetrathionate and Rappaport medium for Salmonella isolation from chicken giblets after pre-enrichment in buffered peptone water. J. Hyg. Camb, 87:219.
- International commission of Microbiological Specification for Foods "ICMSF" 1996. Microorganisms in Food. I. Their Significance and methods of enumeration. 3rd Ed. Univ. of Toronto, Canada
- Kauffmann, G. 1974. Kauffmann white scheme. J. Acta. Path. Microbiol. Sci. 61:385.
- Nagamatsu, K., Hannan, T.J., Guest, R.L., Kostakioti, M., Hadjifrangiskou, M., 2015. Dysregulation of Escherichia coli α -hemolysin expression alters the course of acute and persistent urinary tract infection. Proc Natl Acad Sci USA 112(8): E871-880.
- Noor El-Deen, A.E., Atta, N.S., Abd El Aziz, M.A. 2010. Oral vaccination of Nile Tilapia (*Oreochromis niloticus*) against motile Aeromonas septicemia. Nat. Sci. 8(2): 21-25.
- Rajasekaran, P. 2008. Enterobacteriaceae group of organisms in sewage-fed fishes. Advanced Biotech., 8:12-14.
- Rajkowski, K. T. 2012. Thermal inactivation of Escherichia coli O157:H7 and Salmonella on catfish and tilapia. Food Microbiol. 2012 Jun;30(2):427-31. Epub 2012 Jan 17.
- Rappaport, F., Konforti, N., Navon, B. 1956. New enrichment medium for certain Salmonella. J. Clin. Path., 9: 261.
- Samaha, I.A. and Hendawy, T.A. 2017. Enterobacteriaceae in Some Imported Fish. Dairy and Vet Sci J. 2017; 3(5): 555624.
- Shamshad, S. I.; Zuberi, R. and Qadri, R. B. 1992. Bacteriological status of seafood marketed in Karachi, Pakistan. Quality Assurance in Fish Industry, H. H. Huss et. al. (Eds.), 315-319
- Sheen, S., Huang, L., Sommers, C. 2012. Survival of Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella spp. on Catfish Fillets Exposed to Microwave Heating in a Continuous Mode. J. Food Sci. 77(8):E209-214.
- Valdivia Garvayo, M. de., Ruiz - Lope, M.D., Artacho, Martin - Lagos, R., Lopezmartinez, M.C., Muros-Guadix, P. 1997. Commercial or shelf - life of fresh eviscerated and filleted rainbow trout (*Onchrhynchus mykiss*). Alimentacion E quipos Technol. 8: 97 - 102.

Wogu, M.D., Maduakol M. 2010. Evaluation of microbial spoilage of some aqua cultured fresh fish in Benin City Nigeria. Ethiopian J. Environ. Studies Manag. 3(3): 18-22.