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Enterobacteriaceae Associated with Farm Fish and Retailed Ones

[1]Mai F. Elsherief,' [2]Mohamed M. Mousa, [2] Hosam Abd El-Galil, [1]Engy F. El-Bahy.
[1] Department of Food hygiene, animal health research institute, Agriculture research center, Giza, Egypt.
[2] Department of Food hygiene, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Key words

Enterobacteriaceae, Tilapia niloticus, Mugil cephalus.

ABSTRACT:

The present study was carried out for detection and identification of Enterobacteriaceae in retailed and farm fish as Tilapia nilotica and Mugil cephalus and in Kafr El Shiekh Governorate. The obtained results were revealed that 47 Enterobacteriaceae strains were isolated from 50 Tilapia niloticus fish samples with a percentage of 94% (25 from retailed fish and 22 from farm fish). On the other side 46 isolates of Enterobacteriaceae strains were isolated from 50 Mugil cephalus with a percentage of 92% (22 from retailed fish and 24 from farm fish). The most dominants isolated strains were *Citrobacter spp., Enteriobacter spp., Klebsiella spp., Proteus spp., and Serratia spp.* This together with the highly pathogenic Enterobacteriaceae including *Salmonella spp. and E. coli.*

Corresponding Author: Mai Fouad Elsherief (cabtain.nemo@gmail.com)

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. (Hastein et al., 2006).

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). Enterobacteraceae in fish are considered as an indicator to sewage pollution and has been reported as opportunistic pathogen in fish (Rajasekaran, 2008). The pathogenic strains of Enterobacteraceae may cause diarrhea in fish (Shender et al., 2009).
Enterobacteriaceae are widely distributed in nature and found in feces of human, poultry and animals (Wogu and Maduakol, 2010). Enterobacteriaceae are a common water-borne bacterium, which may be present in the tissues of apparently normal fish (Newaj et al., 2008) .Whenever fish are exposed to environmental stress, or injury, it causes serious outbreaks of disease with mortalities. Environmental stresses such as high temperature, poor water quality and high organic content primarily contribute to the onset and severity of Enterobacteriaceae infections in fish (Zheng, et al. 2004 and Sekar et al., 2008).

Some human pathogens such as, *Escherichia, Klebsiella and Salmonella spp.* have been found to survive and multiply in the gut, mucus and tissues of fish and that render fish acting as potential vector of human disease over long periods (Onyango et al., 2009).
The particular isolation of some most pathogenic organisms such as *Salmonella spp., E. coli* and potential pathogenic organisms *as Klebsiella spp., Citrobacter spp. and Proteus spp.*, which when isolated from fish and fish products gives an indication about environmental fecal pollution of fish (Wogu. and Maduakol, 2010).

2. MATERIALS AND METHODS

Collection and Preparation of collected Samples:

A total of 100 random samples of fresh water fish represented by Tilapia nilotica, Mugil cephalus, 50 samples were collected from market and 50 samples were collected from 5 different farms in Kafr El Shiekh Governorate. The samples were placed separately in clean sterile plastic bags and transferred in an insulated icebox to the laboratory under complete aseptic conditions without any delay. All collected samples were subjected to bacteriological examination according to the methods adapted by (ICMSF,1996). All examined

fish samples were apparently normal. The scales and fins were removed; the skin was sterilized by alcohol and flamed. The muscles above the lateral line was cut, from which 5 g were taken under aseptic conditions to sterile homogenizer flask containing 45 ml of sterile peptone water (0.1%). the contents were homogenized at 14000 rpm for 2.5 minutes. The mixture was allowed to stand for 15 minutes at room temperature. 1ml of supernatant was added separately to 9 ml of sterile peptone water 0.1% and thoroughly mixed for preparation of 10^{th}fold serial dilution. 0.1 ml from each of the previously prepared serial dilution was transferred and evenly distributed by a bent glass rod over the surface of previously dried VRBG agar and then overlaid by a thin layer of VRBG agar (ICMSF, 1996). The plates were incubated at 37^{o} C for 48 hours, colonies that showed a purple color surrounded by a purple zone were counted, and the number per gram was calculated and recorded.A loopfull from positive 2% Brilliant Green Bile broth was streaked onto Sorbitol MacConkey agar at 37°C for 24 hours. The positive purple colonies were streaked onto Eosine Methylene Blue agar and incubated at 37°C for 24 hours. Typical colonies (greenish metallic with dark purple center) were picked up and purified for identification according to (Bailey and Scott, 1978). 1ml from supernatant was inoculated into 10 ml of selinite F broth tubes and incubated at 37°C for 24 hours then loopfull from enrichment broth was streaked onto Salmonella-Shigella agar (S.S.) and incubated at 37°C for 24 hours.

Identification of bacterial isolates:-

The isolated organisms were identified biochemichally and serologically according to (Cruickshank et al., 1975, Collins and lyne, 1984, Kauffman, 1974 and Kok et al., 1996) by using rapid diagnostic E.coli antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types of E. coli and by using antigens using Salmonella antisera (DENKA SEIKEN Co., Japan) for Salmonella at Benha University, Faculty of Veterinary Medicine, Department of Food and Control .

3. RESULTS and DISCUSSION

The obtained results were revealed that *Enterobacteriaceae* strains were isolated from 21 retailed Tilapia nilotica fish samples with percentage of 84% and from 25 farm Tilapia nilotica fish samples with percentage of 100%. On the other side the strains were isolated from *2*5 retaild *Mugil cephalus* fish samples with the percentage of 100% and from 23 farms *Mugil cephalus* fish samples with the percentage of 92%.

Fish from catch to consumption are proving to contamination with several types of Micro-organisms. The high perishability of commodity is attributed to intrinsic factors, which favour rapid microbial growth, namely, low, collagen and lipid contents and comparatively high levels of soluble nitrogen compounds in the muscle. The factors which influence microbial contamination include methods of catch, on- board handling fishing vessel sanitation, processing and storage conditions (Sheen et al., 2012)

Table (1). Statistical analytical results of total Enterobacteriaceae count "cfu/g" of examined farm and retailed Tilapia nilotica and Mugil cephalus (n=25)

Type of fish examened	***source of samples***	+ ve samples		Minimum	Maximum	Mean ± SEM
		No	%			
Tilapia nilotica	retailed	21	84	1.8×10^{2}	3.6×10^{3}	$7.64\times10^{2}\pm 1.53\times10^{2}$
	farm	25	100	1×10^{1}	3.4×10^{3}	$4.28\times10^{2}\pm 1.11\times10^{2}$
Mugil cephalus	retailed	25	100	2×10^{1}	2.7×10^{4}	$3.34\times10^{3}\pm 7\times10^{2}$
	farm	23	92	4×10^{1}	1.2×10^{4}	$3.28\times10^{3}\pm 3.6\times10^{2}$

Table (2). Incidence percentage of Enterobacteriaceae isolated from farm and retailed Tilapia nilotica and Mugil cephalus (n=25)

Type of bacteria isolated	Tilapia nilotica				Mugil cephalus			
	Farm		Retailed		Farm		Retailed	
E.coli	2	8%	3	12%	2	8%	1	4%
Salmonella	4	16%	2	8%	1	4%	4	16%
Enterobacter cloacae	2	8%	2	8%	2	8%	2	8%
Enterobacter agglomerans	1	4%	-	-	-	-	-	-
Enterobacter aerogenes	2	8%	3	12%	-	-	1	4%
Enterobacter hafniae	-	-	1	4%	1	4%	-	-
Citrobacter diversus	-	-	1	4%	1	4%	2	8%
Citrobacter freundii	3	12%	-	-	1	4%	2	8%
Proteus mirabilis	2	8%	4	16%	1	4%	2	8%
Proteus vulgaris	1	4%	3	12%	3	12%	2	8%
Provedencia rettgeri	-	-	1	4%	2	8%	-	-
Pseudomonas species	1	4%	-	-	3	12%	2	8%
Serratia marcesens	2	8%	-	-	1	4%	1	4%
Serratia liquefaciens	-	-	2	8%	2	8%	1	4%
Klebsiella pneumonia	-	-	1	4%	3	12%	2	8%
Klebsiella ozaenae	2	8%	2	8%	1	4%	-	-

Table (3): Serological identification of isolated *E.coli* in examined farm and retailed Tilapia nilotica and Mugil cephalus

Tilapia nilotica				**Mugil cephalus**			
Retailed		Farm		Retailed		Farm	
Serodiagnosis	Strain characterization	Serodiagnosis	Strain characterization	Serodiagnosis	Strain characterization	Serodiagnosis	Strain characterization
O86	EPEC	O111 : H4	EHEC	O111 : H4	EHEC	O44 : H18	EPEC
O127 : H6	ETEC						
O114 : H21	EPEC	O125 : H21	ETEC			O127 : H6	ETEC

EPEC: Enteropathogenic *E.coli.*, ETEC: Enterotoxigenic *E.coli.*, EHEC: Enterohaemorrhagic *E.coli.*

Table (4). Serological identification of isolated *Salmonella species* in examined farm and retailed Tilapia nilotica and Mugil cephalus

Tilapia nilotica						**Mugil cephalus**					
Retailed			Farm			Retailed			Farm		
Identified strains	Antigenic structure		Identified strains	Antigenic structure		Identified strains	Antigenic structure		Identified strains	Antigenic structure	
	O	H		O	H		O	H		O	H
Salmonella Enteritidis	1,9,12	g,m : 1,7	*Salmonella Typhimurium*	1,4,5,12	i : 1,2	Salmonella Enteritidis	1,9,12	g,m : 1,7	*Salmonella Typhimurium*	1,4,5,12	i : 1,2
			Salmonella Enteritidis	1,9,12	g,m : 1,7	Salmonella Enteritidis	1,9,12	g,m : 1,7	-	-	-
Salmonella Typhimurium	1,4,5,12	i : 1,2	*Salmonella Typhimurium*	1,4,5,12	i : 1,2	Salmonella Typhimurium	1,4,5,12	i : 1,2	-	-	-
						Salmonella Anatum	3,10,15,34	e,h : 1,6	-	-	-

Bacterial diseases in fish generally do not develop simply as the result of exposing a host to an infectious agent (Wedekind et al., 2010). In most instances, disease occurs as the result of complex interactions between pathogen, fish and environmental stress that affect the susceptibility of the host to disease. Song et al., (2008) recently reviewed the role of stress in the susceptibility of fish to disease. Environmental stresses can affect the homeostatic mechanism of fish, thus reducing their resistance to pathogenic organism (Small et al., 2005).

Fresh water fish are subjected to the risk of contamination with various pathogens from different sources, primary during their presence in aquatic environment and secondary after being harvested through transportation and marketing as well as storage. Such contamination may render these food articles unfit for human consumption or even harmful to consumers.

Results obtained from table (l) revealed that, the total Enterobacteriaceae count cfu/g ranged from 1.8×10^2 to 3.6×10^3 with an average of $7.64\times10^2 \pm 1.53\times10^2$ cfu/g for retailed T.nilotica and 1×10^1 to 3.4×10^3 with an average of $4.28\times10^2 \pm 1.11\times10^2$ cfu/g for farm T.nilotica.

On the other hand, the total enterobacteriaceae count was ranged from 2×10^1 to 2.7×10^4 with an average of $3.34\times10^3 \pm 7\times10^2$ for retailed Mugil cephalus and 4×10^1 to 1.2×10^4 with an average of $3.28\times10^3 \pm 3.6\times10^2$ cfu/g for farm Mugil cephalus.

The incidence percentage of *E.coli* according to microbiological identification showed that the difference between retailed and farm Tilapia (12, 8 %) and Mugil cephalus (4, 8 %) These incidence considered low incidence due to Tilapia and Mugil are mostly bred in area away from sewage (Rashad, 2013) who compared between incidence of *E.coli* in Tilapia niloticus (57.10 %). and Mugil cephalus (91.40%) and incidence of *E.coli* in Claries lazera and Bugeus bayed which was 100% in both of them. Our results are agreed with those reported by (Sanaa O. Yagoub, 2009) who isolated *E.coli* from muscle of raw fish with the incidence of (13%) and disagreed with (Hassan et. al., 2012) who failed to isolate *E.coli* from muscle of Oreochromis niloticus. On the other hand The incidence percentage determination according to microbiological identification of Salmonella showed difference between retailed and farm Tilapia (8, 16 %) and Mugil cephalus (16, 4 %), These variations Could be attributed to the fish species, environments, methods of catch, extend of handling during transportation and distribution as well as marketing (Wang et. al., 1994).

The Presence of different strains of *E.coli* as shown in table (3) such as EPEC, ETEC, EHEC. give an indicator about sewage pollution. Detection of ETEC in fish samples constitute the main causes of food poisoning and hemorrhagic enterocolitis in man due to eating the improperly processed fishmeals (Galal, 2013).

Table (4) Revealed that serotypes of Salmonella isolated from Tilapia nilotica are Salmonella Enteritidis, Salmonella Typhimurium, While in Mugil cephalus Salmonella Enteritidis, Salmonella Typhimurium andSalmonella Anatum.

Salmonella Typhimuium is the most common Salmonellae isolated from cases of food poisoning and represents about 50 - 60 % of such cases WHO (1997). Furthermore, FAO (1993) reported that the cases of food poisoning outbreaks due to Salmonella Typhimurium were 407 cases in Spain (1981), 237 in Poland (1930), 227 in Denmark (1981), 130 in Sweden (1981), 84 in Scotland (1981), 80 in Ireland (1981), 37 in Yugoslavia (1984), 22 in England and 3 cases in Belgium (l981).

Salmonellosis has an epidemiological importance as some of its members are pathogenic and may cause serious infections and food poisoning to man. Moreover, Salmonellosis count can be taken as an indicative of possible enteric contamination in the absence of Coliform bacteria (pogorelova et al., 1993).

The presence of Salmonellae as enteropathogens in farm fish may reflect the unsatisfactory hygienic conditions during catching, handling and marketing of the fish. The presence of considerable numbers of Salmonellosis indicates unsatisfactory hygienic measures during catching and distribution of the fish (Valdivia et. al., 1997).

Table (2) summarized the different enteric bacteria isolated from the examined sample of fishes. The results indicated that the most important enterobacteriaceae isolated from retailed Tilapia nilotica include *Enterobacter cloacae, Enterobacter aerogenes, Enterobacter hafniae ,Citrobacter diversus, Proteus mirabilis, Proteus vulgaris, Provedencia rettgeri, Serratia liquefaciens, Klebsiella pneumonia, Klebsiella ozaenae,* where its incidence 8, 12, 4, 4, 16, 12, 4, 8, 4, 8, % while the most important enterobacteriaceae isolated from farm Tilapia nilotica include *Enterobacter cloacae,*

Enterobacter agglomerans, Enterobacter aerogenes, Citrobacter freundii, Proteus mirabilis, Proteus vulgaris, Pseudomonas species, Serratia marcesens, Klebsiella ozaenae where its incidence 8, 4, 8, 12, 8, 4, 4, 8, 8 %. The results also cleared that the most important enterobacteriaceae isolated from retailed Mugil cephalus *include Enterobacter cloacae, Enterobacter aerogenes, Citrobacter diversus, Citrobacter freundii, Proteus mirabilis, Proteus vulgaris, Pseudomonas species, Serratia marcesens, Serratia liquefaciens, Klebsiella pneumonia* where its incidence 8, 4, 8, 8, 8, 8, 8, 4, 4, 8 % while the most important enterobacteriaceae isolated from farm Mugil cephalus include *Enterobacter cloacae, Enterobacter hafniae, Citrobacter diversus, Citrobacter freundii, Proteus mirabilis, Proteus vulgaris, Provedencia rettgeri, Pseudomonas species, Serratia marcesens, Serratia liquefaciens, Klebsiella pneumonia, Klebsiella ozaenae* where its incidence 8, 4, 4, 4, 4, 12, 8, 12, 4, 8, 12, 4 %. These results are closed to those reported by (Yagoub, 2009) who isolated enterobacteriaceae from muscles of raw fishes in sold in fish market in Khartoum state; she isolated *Klebsiella spp., Citobacter spp., Proteus spp. and Providencia spp*. With incidence 10.9, 8.7, 15.2 and 4.3 %. While differs in incidence of *Enterobacter spp., Serritia spp., Pseudomonas spp*. which failed to be isolated .

Isolation of some potential pathogenic organisms such as *Klebsiella spp., Citrobacter spp., Proteus spp.* may indicates environmental pollution and these supported the findings of (Yagoub and Ahmed 2004) who isolated pathogenic and potential pathogenic organisms from tap water that originated from Nile River. This also confirms the findings of (Herrera et al., 2006) who isolated similar organisms from fish and fish products.

The isolation of Pseudomonas spp from collected fish samples is of highly importance because this organisms plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as spoilage organism. This is in accord with previously mentioned by (Koutsoumanis and Nychas, 2000) and (Jeyasekaran et al., 2006) who identified pseudomonads as a good spoilage index.

This study revealed that the contamination of fish with some enteropathogens may give an indication about bad sanitary conditions under which fish were exposed from catching till reach to the markets, resulting in both public health hazards and economic losses.

4. REFERENCES

Hassan, A.H.M., Noor- El Deen, A.E., Galal, H.M., Sohad, M. Dorgham, Bakry, M.A., Hakim, A.S. 2012. Further Characterization of Enterobacteriaceae isolated from cultured freshwater fish in Kafr El Shiek Governorate: Clinical, biochemical and histopathological study with emphasis on treatment trials. Glob. Vet. 9 (5): 617-629.

Bailey, W.R., Scott, E.G. 1978. Diagnostic Microbiology. A textbook for the isolation and identification of pathogenic microorganisms. 5th Ed., Carroll B. Larson and Marjorie Gould Publisher, Saint Louis, C.V. Mosby Co., Missouri, USA.

Collins, C.H., Lyne, P.M. 1984. Microbiological methods 5th microbiology laboratory manual, British Liberary, Butter Wort Inc., London, UK.

Cruickshank, R., Duguid, J.P., Marmion, B.P., Swain, R.H.A. 1975.Medical Microbiology. 12th ed., Edinburg, London and New York.

Food Agriculture organization "FAO" 1993. Zoonotic diseases in the Near East Region. Regional Office of the United Nation, Cairo.

Galal, H.M. Hakim, A.S.; and Sohad, M. Dorgham 2013. Phenotypic and virulence genes screening of Escherichia coli strains isolated from different sources in delta Egypt. Life Science Journal, 10(2).

Gonzalez-Rodriguez, M.N., Sanz, J.J, Santos, J.A, Otero, A., Garcia-lopez, M.L. 2001. Bacteriological Quality of aquaculture freshwater fish portions in prepackaged trays stored at 3 degrees C. J. Food Prot. 64 (9): 1399 - 1404.

Håstein, T., Hjeltnes, B., Lillehaug, A., Utne Skåre, J., Berntssen, M., Lundebye, A.K. 2006. Food safety hazards that occur during the production stage: challenges for fish farming and the fishing industry. Rev. Sci. Technol. 25(2): 607-625.

Rashad, H. 2013. Enteropathogenes in some fresh water fishes. M.Sc. Thesis, Dept. of food hygiene, Fac. Vet. Med. Alex. Univ., Alexandria, Egypt.

Herrera, F.C., Santos, J.A., Otero, A., Garcia-Lopez, M.L. 2006. Occurrence of Foodborne pathogenic bacteria in retails prepackage portions of marine fish in Spain. J. Appl. Microbiol. 100 (3): 527-36.

ICMSF (International Commission of Microbiological Specification for Foods)1996. microorganisms in foods. I-their significance and methods of enumeration, 3rd Ed. Univ. of Toronto, Canada.

Jeyasekaran, G., Ganesan, P., Anandaraj, R., Jeya Shakila, R., Sukumar, D. 2006. Quantitative and

qualitative studies on the bacteriological quality of Indian white shrimp (Penaeus indicus) stored in dry ice. J. Food Microbiol. 23(6): 526-533.

Kauffman, G. 1974. Kauffmann white scheme. J. Acta. Path. Microbiol. Sci., 61:385.

Kok, T. Worswich, D., Gowans, E.1996. Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill livingstone, UK.

Koutsoumanis K., Nychas, G.J.2000. Application of systemic experimental procedure to develop a microbial model for rapid fish shelf life predictions. Int. J. Food Microbiol. 60 (2-3): 171-184.

Newaj, A., Mutani, A., Ramsubhag , A., Adesiyun, A. 2008. Prevalence of bacterial pathogens and their anti-microbial resistance in Tilapia and their pond water in Trinidad. Zoonoses Public Health, 55(4): 206-213.

Noor El-Deen, A.E., Atta, N.S., Abd El Aziz, M.A. 2010. Oral vaccination of Nile Tilapia (*Orechromis niloticus*) against motile Aeromonas septicemia. Nat. Sci. 8(2): 21-25.

Onyango, M. D., Sarah Wandili, Rose Kakai, Eliud N. W. 2009 Isolation of Salmonella and Shigella from fish harvested from the Winam Gulf of Lake Victoria, Kenya. J Infect Developing Countries, 3(2):99-104.

Pogorelova, N.P., Lartseva, L.v., Boiko, A.V., smirnova, I.E., zhigareva, T.M.; Zhuravleva, L.A. Merkina, E.N. 1993. Microbiological evaluation of water pollution in the Volga delta. Gigiena. Sanitorya, 7: 35 - 38.

Rajasekaran, P. 2008. Enterobacteriaceae group of organisms in sewage-fed fishes. Advanced Biotech., 8:12-14.

Yagoub, S. O., Ahmed T.M. 2004. Pathogenic Microorganisms in fresh water samples collected from Khartoum central market. Sudan J. Vet. Sci. Anim. Husbandry 43(1-2): 32-37.

Yagoub, S. O. 2009. Isolation of Enterobacteriaceae and Pseudomonas spp. from raw fish sold in fish market in Khartoum state, J. Bacteriol. Res.1(7), pp. 085-088.

Sekar, V., T. Santiago, K. Vijayan, S. Alavandi , V. Raj, J. Rajan, M. Sanjuktha and N. Kalaimani 2008. Involvement of Enterobacter cloacae in the mortality of the fish, Mugil cephalus. Lett. Appl. Microbiol., 46(6):667-672.

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.

Shender, L.A., T.R. Spraker 2009. Salmonellosis in a free-ranging population of javelinas (Pecari tajacu) in south central Arizona. J. Wild Dis., 45(4): 941-51.

Small, B., Bilodeau, A 2005. Effects of cortisol and stress on channel catfish (Ictalurus punctatus) pathogen susceptibility and lysozyme activity following exposure to Edwardsiella ictaluri. Gen. Comp. Endocrinol., 142(1-2):256-62.

Song, J., Nakayama, K., Murakami, Y.,Jung, S., Oh, M., Matsuoka, S., Kawakami, H., Kitamura, S. 2008. Does heavy oil pollution induce bacterial diseases in Japanese flounder Paralichthys olivaceus. Mar. Pollut. Bull., 57 (6- 12):889-894.

Valdivia Garvayo, M. de., Ruiz - Lope, M.D., Artacho, Martin - Lagos, R.,Lopez martinez, M.C., Muros-Guadix, P. 1997. Commerical or shelf - life of fresh eviscerated and filleted rainbow trout (Onchrhynchus mykiss). Alimentacion Equipos Technol. 8: 97 - 102.

wang, S.J., chen, J.M., Fan, J.J. 1994. Quality changes in fresh Tilapia and Milkfish during refrigerated (4 degree C) and frozen (-15 degree C) storage J. Food during Analysis, 2(4):311 - 316.

Wedekind, C., Gessner, M., Vazquez, F., Maerkiand, M., Steiner, D. 2010. Elevated resource availability sufficient to turn opportunistic into virulent fish pathogens. Ecol. 91(5): 1251-6.

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.

World Health Organization (WHO). 1997. Microbial aspects of food hygiene, technical Report Series, No. 598, pP.2l - 23. WHO, Geneva, Switzerland.

Zheng, D., K. Mai, S. Liu, C. Limin, Z. Liufu, W. Xu, B. Tan and W. Zhang 2004. Effect of temperature and salinity on virulence of Edwardsiella tarda to Japanese flounder, Paralichthys olivaceus (Temminck et Schlegel). Aqua. Res., 35: 494–500.