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## FOOD SCIENCE & TECHNOLOGY | RETRACTION

# RETRACTED ARTICLE: Screening for the presence and prevalence of *Edwardsiella tarda* infection in fish harvested from Lakes Zeway and Langano, Southern Oromia, Ethiopia

Teshome Habtamu<sup>1</sup> and Bedaso Kebede<sup>1\*</sup>

**Abstract:** A study was carried out from October 2009 to April 2010 with the objective of isolating *Edwardsiella tarda* an important fish pathogen from fish harvested for human consumption from Lake Zeway and Langano. A total of 372 tissue samples (three from each fish) comprising liver, intestine and kidney were collected from 124 fish (*Clarias gariepinus* and *Oreochromis niloticus*). Distribution of *E. tarda* infection among these three organs examined indicated that *E. tarda* was isolated most frequently from liver (6.5%) followed by intestine (2.4%) and kidney (0.8%) with significant difference. Statistical significant differences ( $p < 0.05$ ) were found in *E. tarda* infection with respect to tissue samples. Fish from Lake Zeway was prevalently infected by *E. tarda* than Lake Langano. Male fish were more frequently harbor *E. tarda* than female fish and were not statistically significant ( $p > 0.05$ ). The current study is signified that *E. tarda* infection is a potential threat to the fishery sector and public health. Therefore, awareness should be created on the hazardousness of *E. tarda* on public health significantly and hence further studies have to be conducted in other lakes of Ethiopia that harbor fishes.

### ABOUT THE AUTHORS

Dr Teshome Habtamu and Dr Bedaso Kebede graduated from Addis Ababa University Faculty of Veterinary Medicine since July, 2010. Their research interests are focused on the animal diseases and public health. This paper focused on the impact of *Edwardsiella tarda* on fish sector and public health.

### PUBLIC INTEREST STATEMENT

*Edwardsiellosis* is the most important bacterial diseases causing severe economic losses in fish farms of many countries. *E. tarda* is a health threat to other animals and humans apart from threat to fish that means it has a zoonotic significance. The predisposing risk factors were exposure to aquatic environment, pre-existing liver diseases, iron overload and raw sea food ingestion. *Edwardsiellosis* in humans usually cause diarrhea, gastroenteritis, wound infection and even death. There are reports of extra intestinal infection of the diseases with the clinical pictures including a typhoid like illness, peritonitis with sepsis and cellulites with occasionally liver abscess and meningitis. The practice of consuming partially cooked fish meals, manual handling of fish and unhygienic practice during filleting would expose the public to the higher risk of contracting the disease. Therefore, the disease deserves attention due to its impact on the fishery sector and its potential threat to public health.

**Subjects: Food Science & Technology; Food Additives & Ingredients; Food Laws & Regulations**

**Keywords: catfish; *E. tarda*; intestine; kidney; Langanoo; liver; Nile Tilapia; Zeway**

### 1. Introduction

Aquaculture is growing rapidly worldwide with fish being the primary source of animal protein in many countries. The fishery sector plays a significant role in food security through supplementation of food for developing countries. As a whole fish currently makes up about 19% of the total protein consumption or just over the 5% of proteins from both plants and animals origin (Dugenci & Candan, 2003).

The major problems hampering production, development and expansion of the aquaculture industry is due to the fact that fish are possibly susceptible to microbial diseases. Microbial diseases are a global problem affecting fresh water, marine water, cultured, sport and ornamental fish. The problem is extremely important when fish are subjected to intensive culture practices (Trust, 1986).

The control of fish diseases is particularly difficult because fish are often farmed in system where production is dependent on natural environmental conditions. Changes or deterioration in the aquatic environment cause the occurrence of most fish diseases and also environmental effects play a great role in influencing the health status of fish. Therefore, understanding characteristics of potential pathogenic microorganisms of fish, aspects of the biology of fish as well as a better understanding of the environmental factors affecting such cultures will allow the application of adequate measures to prevent and control the diseases limiting fish production (Toranzo, Magarinos, & Romalde, 2005; WHO, 1999).

*Edwardsiellosis* is among the most important bacterial diseases causing severe economic losses in fish farms of many countries. The disease is caused by *Edwardsiella tarda* which is a gram negative, motile, facultative anaerobic, short rod shaped bacterium (1 µm in diameter and 2–3 µm long) pathogenic to a wide range of fish hosts such as Channel catfish (*Ictaluri punctatus*), Striped bass (*Morone saxatili*), eel (*Anguilla anguilla*), Nile Tilapia (*Oreochromis niloticus*), carp (*Cyprinus cyprio*) and Flounder (*Paralichthys olivaceus*) (Plumb, 1999). These organisms found frequently in organically polluted and poor quality water which predispose fish to the disease by resulting stress in them (Novotony, Dvornik, Loremcova, Beran, & Pavil, 2004; Wei & Musa, 2008). Media used to isolate *E. tarda* were Edwardsiella Isolation Media (EIM), Brain Heart Infusion (BHI), Tryptic Soya Agar (TSA), Xylose Lysine Deoxycholate (XLD) and MacConkey agar. The culture of such bacteria is characterized as small, circular, raised whitish colonies with black center on XLD as well as appearing as pale colonies on MacConkey agar. Several factors hinders culture or grows of *E. tarda* on the medium are temperature ranges 25–37°C, pH ranges 7.0–8.0 and 0.5% NaCl concentration (Wei & Musa, 2008). Biochemical characteristics of *E. tarda* are catalase positive, cytochrome oxidase negative, glucose fermentative, indole positive, citrate negative, lysine positive, mannitol, dulcitol, sorbitol, inositol, xylose, rhamnose negative, produce hydrogen sulfide, alkaline slant and acid butt on Triple sugar iron Agar (Carter, 1984).

*E. tarda* is a health threat to other animals and humans apart from threat to fish that means it has a zoonotic significance (Clarridge, Musher, Fainstein, & Wallace, 1980). The predisposing risk factors were exposure to aquatic environment, pre-existing liver diseases, iron overload and raw sea food ingestion (Wang, Liu, Cheng, & Kao, 2008). *Edwardsiellosis* in humans usually cause diarrhea, gastroenteritis, wound infection and even death (Plumb, 1999; Vandamme & Vandepitte, 1980). There are reports of extra intestinal infection of the diseases with the clinical pictures including a typhoid like illness, peritonitis with sepsis and cellulites with occasionally liver abscess (Zighelboim, Williams, Bradshaw, & Harris, 1992) and meningitis (Plumb, 1999). The infection is more severe in immunocompromised individuals. The practice of consuming partially cooked fish meals, manual handling of fish and unhygienic practice during filleting would expose the public to the higher risk of contracting the disease. Therefore, the disease deserves attention due to its impact on the fishery sector and its

potential threat to public health (FAO, 1995; WHO, 1999). In Ethiopia, the bacterium has been isolated from apparently healthy fish of Lake Zeway and Tana (Nuru, 2007; Yimer, 2000). However, there is no further work done in covering the different fish species and aquatic environments. Therefore, this study was conducted with the aim of screening for the presence and prevalence of *E. tarda* infection in Fish harvested from Lakes Zeway and Langanoo, Southern Oromia, Ethiopia.

## 2. Materials and methods

### 2.1. Study animals, sample size and design

Study animals used in this study included African catfish (*Clarias gariepinus*,  $N = 30$ ) and Nile Tilapia (*O. niloticus*;  $N = 94$ ) which were harvested from Lake Zeway and Langanoo for human consumption. In this study area Nile Tilapia was more abundant than other species. Those harvested fish were kept under cold chain until reach necropsy room and further assessment take place. The fish were physically examined for any external lesions before necropsy and collecting tissue samples. The necropsy was undertaken at the necropsy room of Zeway Fishery Resource and Research Center, Batu via fish dissected in ventral approach to expose organs of study. The fish sample was cut along the midline of the abdomen starting from the anus up to the mouth using sterile dissecting scissor followed by another dissection from the anus to the lateral line and further along the lateral line up to the gills cover to remove the lateral side of the abdominal wall and expose the internal organs. Internal organs were examined for any gross pathology and the findings recorded. Tissue samples were then taken from intestine ( $N = 124$ ), liver ( $N = 124$ ) and kidney ( $N = 124$ ) aseptically using sterile scalpel blade and forceps kept in sterile universal bottles of 100 ml capacities. The bottles containing the samples were then kept in ice box containing ice packs and processed for bacterial isolation and identification in Microbiology laboratory of College of Veterinary Medicine and Agriculture and National Veterinary Institute, Bishoftu.

### 2.2. Study site

#### 2.2.1. Lake Zeway

It is located on the Eastern side of Batu town, 163 km Southeast of Addis Ababa it lies in northern part of the wet valley between  $7^{\circ}51' N$  to  $8^{\circ}7' N$  and  $38^{\circ}43' E$  to  $38^{\circ}57' E$  with an open water area of  $422 \text{ km}^2$  and shoreline length of 137 km. The lake is fed by two major rivers Ketar and Meki Rivers and has one outflow in the South Bulbula river which flow into Abiyata (LFDP, 1993). Five bigger islands are situated in the lake Viz Tulu Gudo ( $4.8 \text{ km}^2$ ), Tsedecha ( $2.1 \text{ km}^2$ ), Funduro ( $0.4 \text{ km}^2$ ), Gebrenna ( $0.3 \text{ km}^2$ ) and Galila ( $0.2 \text{ km}^2$ ). While the latter two have few inhabitants, the three bigger ones are populated with several hundreds of people (Anonymous, 1999). A fish habitat in the lake consist almost Nile Tilapia (*Oeochromis niloticus*). Since recent years, however, Catfish (*Carias gariepinus*) and crucian carp (*Carcasius gracius*) have appeared in small amounts of the total catch (LFDP, 1994). There are a number of landing points around the lake from where fish is collected either by boat or trucks and brought to the major landing points adjoining Batu town.

#### 2.2.2. Lake Langanoo

It is located 200 km South of Addis Ababa lying between  $7^{\circ}36' N$ ;  $38^{\circ}45' E$ . It is 18 km long and 16 km wide with an open water area of  $230 \text{ km}^2$ , 7.5 km shoreline and  $1,600 \text{ km}^2$  catchments area (LFDP, 1993). The main fish species in the lake include *Barbus* species, *Clarias* species and *Oreochromis niloticus* (FAO, 1995) with the total annual catch of 1,000 tones.

### 2.3. Laboratory examination

#### 2.3.1. Isolation of *E. tarda*

Tissue samples from kidney, liver and intestine of Catfish and Nile Tilapia were homogenized in physiological saline. The homogenate was then taken by sterile loop and streaked on XLD agar plate (Titan Biotech) and then incubated at  $37^{\circ}C$  for 24 h. Colonies showing or resembling with morphological characteristics of *E. tarda* were further subcultured on MacConkey agar plates and incubated

at 37°C for 24 h. All lactose non-fermented colonies were further subcultured on TSA containing 0.5% NaCl and incubated at 37°C for 24 h. Presumptive identification of the resulting isolates (colonies) was done employing different tests which included primary bacteria identification techniques and biochemical identification tests (Baron, Peterson, & Fine Gold, 1994; Quinn, Carter, Markey, & Carter, 1999; Rowland, Walsh, Teel, & Carnahan, 1994; Woodland, 2006).

### 2.4. Data analysis

Descriptive statistics such as proportions and frequency were employed in summarizing the data. Chi-square test of independence was employed in comparing the prevalence/occurrence of *E. tarda* infection with respect to site, sex, fish species and isolated organ. A confidence interval of 95% was used to interpret the statistical association and significance was considered when *p*-value is less than 0.05 (Agrawa, 1996).

### 3. Results

A total of 372 tissue samples comprising kidney, liver and intestine collected from 124 fish. *E. tarda* was isolated from 12 tissue samples (8 from liver, 3 from intestine and 1 from kidney). The isolates appeared as small punctuate grayish white colonies on XLD agar after 24 h of incubation at 37°C. Except few of the isolates, most showed typical characteristics of *E. tarda*. In biochemical tests, these typical isolates were positive for indole, H<sub>2</sub>S production, and lysine decarboxylase and unable to utilize Simmon's citrate and the different sugars used in the study (Table 1). However, some of the isolates showed variation from the typical characteristics. One isolate was negative for indole and able to utilize Simmon's citrate while the remaining was able to ferment mannitol, rhaminose, xylose and inositol (Table 1).

Distribution of *E. tarda* infection among the three organs examined indicated that *E. tarda* was isolated most frequently from liver (6.5%) followed by intestine (2.4%) and kidney (0.8%) with statistical significant difference (*p* < 0.05) among organs (Table 2).

Statistical significant difference (*p* < 0.05) were found in *E. tarda* infection with respect to site although the bacterium was isolated from fish originating from both lake Zeway and Langanoo with *E. tarda* being more prevalent in fish sampled from lake Zeway. *E. tarda* was isolated more frequently from male fish, the differences in the occurrence of *E. tarda* infection with respect to sex were not significant (*p* > 0.05) indicating that both sexes are equally susceptible (Table 3).

**Table 1. Cultural, morphological and biochemical characteristics of *E. tarda* strains**

Parameters	Results	Remark
Cultural characteristics on XLD agar	Small, circular, grayish white colonies	
Morphological characteristics	Gram negative, motile short rods	Two isolates, non-motile
Biochemical characteristics		
Indole production	+	One isolate, indole -ve
H <sub>2</sub> S production	+	
Oxidase	-	
Catalase	-	
Citrate	-	One isolate, citrate +ve
Lysine	+	
Mannitol	-	Four isolates, mannitol +ve
Dulcitol	-	
Inositol	-	Four isolates, inositol +ve
Sorbitol	-	
Xylose	-	Four isolates, xylose +ve
Rhaminose	-	Four isolates, rhaminose +ve

**Table 2. Distribution of *E. tarda* isolates among the organs**

Organ	Results				Total
	Positive		Negative		
	Observed	Expected	Observed	Expected	
Intestine	3	(4)	121	(120)	124
Liver	8	(4)	116	(120)	124
Kidney	1	(4)	123	(120)	124
Total	12	(12)	360	(360)	372

Notes:  $\chi^2 = 6.5$ ,  $df = 2$ ,  $p < 0.05$ .

**Table 3. Occurrence of *E. tarda* isolates with respect to site and sex of fish**

Parameters		Positive		Negative		Total	$\chi^2$ value	p-value
		Observed	Expected	Observed	Expected			
Site	Zeway	7	(3.3)	27	(30.7)	34	6.38	0.012
	Langanoo	5	(8.7)	85	(81.3)			
Total		12	12	112	112	124		
Sex	Female	3	(2.91)	27	(27.07)	30	0.005	0.945
	Male	9	(9.09)	85	(84.9)			
Total		12	12	112	112	124		

**Table 4. Occurrence and distribution of *E. tarda* with respect to fish species**

Species	Result		Total
	Positive	Negative	
Catfish	1	29	30
Nile Tilapia	11	83	94
Total	12	112	124

Notes:  $\chi^2 = 0.59$ ,  $df = 1$ ,  $p > 0.05$ .

There was no statistical significant difference ( $p > 0.05$ ) in isolation of *E. tarda* from Catfish (*C. g. g. g.*) and Nile Tilapia (*O. niloticus*) indicating that both fish species are susceptible to the infection (Table 4).

#### 4. Discussion

The genus *Edwardsiella* consists of two species *E. tarda* and *E. ictaluri*. *E. tarda* infects fish and other animals including human, while *E. ictaluri* is a pathogen of fish only (Woo & Bruno, 1999). In this study, *E. tarda* were isolated from intestine, kidney and liver of fish to screen presence and prevalence *E. tarda* which is a potential threat to aquaculture and public health importance. The organism has been previously isolated from different samples such as intestine of fish and humans stool with sporadic cases of diarrhea (Vandamme & Vandepitte, 1980) and dressed fish (Noga, 1990; Wyatt, Nickelson, & Vanderzant, 1979).

In current study morphological and biochemical characteristics of *E. tarda* isolates were consistent with those reported previously (Ling, Wang, Lim, & Leung, 2001; Roberts, 1989; Stoskopf, 1993). However, one isolate was negative for indole production and positive for Simmons citrate test which indicates an atypical strain as previously reported in early similar studies (Acharya, Maiti, Mohanty, Mishra, & Samanta, 2007; Kumar et al., 2007; Wei & Musa, 2008) and indole production (Ewing, Mcwhorter, Escobar, & Lubin, 1965).



The present study showed two isolates were found non motile and this fact is similar to report of Okuda et al. (2007). Most of the phenotypic characteristics of the isolates were similar as claimed by Holt (1997) and variation in some of the biochemical tests particularly in the utilization of sugars which included mannitol, rhamnose, xylose and inositol. Such variation contradicts the study of Baya et al. (1997) where no variation was observed with respect to these biochemical tests among forty-four *E. tarda* isolates studied. Hence, Variation among *E. tarda* isolates was reported with respect to utilization of rhamnose (Wei & Musa, 2008), mannitol (Stock & Wiedemann, 2001). The occurrence of variation in phenotypic characteristics among *E. tarda* isolates may be due to the presence or absence of plasmid that control metabolic activities. Generally, the significance for the prevalence of *E. tarda* in catfish and Nile Tilapia could not be substantiated by references to the phenotypic characteristics of *E. tarda* alone (Acharya et al., 2007). Other means of identification may prove necessary for clarifying such aspect.

Nowadays, molecular based identification techniques such as nucleic acid probes and the polymerase chain reaction (PCR) have been engaged unlike the traditional phenotypic identification of *E. tarda* (Horenstein, Smolowitz, Uhlinger, & Roberts, 2004; Savan, Kono, Itami, & Sakai, 2005). In addition a fluorescence *in situ* hybridization (FISH) technique using twenty four mer oligonucleotide probe (Ootsubo et al., 2002) and higher performance capillary electrophoresis (HPCE) (Yu, Yuan, Feng, & Li, 2004) used identify *E. tarda* in fish and traceable bacteria.

The absence of significant differences in the occurrence of *E. tarda* between males and females indicates that both sexes are equally susceptible to the bacterium. This is in agreement with the works of Savan et al. (2005) and Yu et al. (2004). The significant differences in the rate of isolation of *E. tarda* between the study lakes may be attributed to differences in the nutritional status of the fish, the environmental condition and water quality changes in temperature, pH and fluctuation in dissolved oxygen which affect the occurrence of *E. tarda* infection (Cahill, 1990; Nuru, 2007; Ringoa, Olsenb, Mayhew, & Myclebustel, 2003). In current study *E. tarda* affect intestine, liver and kidney of catfish and tilapia but a higher percentage of the pathogen was isolated from liver this could be due to the metabolic activities of the organs (Cahill, 1990).

### 5. Conclusion and recommendations

*Edwardsiella* is the most important bacterial disease causing severe economic loss and hindrance in aquaculture. Apart from veterinary health importance, *E. tarda* has also public health significance in people engaged in fishery industry and those depend on fish products for their annual income. The isolation of *E. tarda* from wild fish population of Lakes Zeway and Langanoo destined for human consumption indicates that *E. tarda* fish pathogen may prove to be a serious threat to the fishery sector and public health. The finding of certain isolates that divert in their biochemical characteristics warrants further investigation using more advanced methods of bacteria characterization. Therefore, assessments on factors such as environmental condition, management strategies for controlling fish pathogen as well as other stress factors that could enhance the prevalence, distribution and severity *E. tarda* infestation, are crucial in designing effective disease control and prevention. Since the current state of knowledge on *E. tarda* infection in fish and humans in Ethiopia is relatively unknown, further studies into the epidemiology of such pathogen in different hosts and environments as well as comprehensive profiling of *E. tarda* strains for controlling measures and maintaining overall public health, merits scientific pertinence.

## List of abbreviations

µm	micrometer
BHI	brain heart infusion agar
CHO	carbohydrate
EIM	<i>Edwardsiella</i> isolation media
<i>E. tarda</i>	<i>Edwardsiella tarda</i>
FAO	Food and Agricultural Organization
FISH	fluorescence <i>in situ</i> hybridization
g/l	gram per liter
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
H <sub>2</sub> S	hydrogen sulfide
HPCE	higher performance capillary electrophoresis
LAMP	loop-mediated isothermal amplification
LFDP	lake fisheries development working paper
ml	milliliter
PCR	polymerase chain reaction
SIM	sulfur, indole and motility test media
TSA	tryptic soya agar
TSIA	triple sugar iron agar
USA	United States of America
V/V	volume by volume
WHO	World Health Organization
XLD	xylose lysine desoxycholate

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## Competing Interests

The authors declare no competing interest.

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