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Isolation and identification of *Escherichia coli* and *Edwardsiella tarda* from fish harvested for human consumption from Zeway Lake, Ethiopia

Adanech Beshada Haile and Temesgen Kassa Getahun*

Ethiopian Institute of Agricultural Research, Holeta Agricultural Research Center. P. O. Box 31, Holeta, Ethiopia.

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A cross sectional study was conducted from November to June 2014/2015 in fish harvested for human consumption at Lake Zeway with the objective of isolating *Edwardsiella tarda* and *Escherichia coli* important pathogens and contaminants its products. Three hundred tissue samples comprising Spleen, Liver and Intestine were collected from 100 fish (12 *Clarias gariepenus* and 88 *Oreochromis niloticus*) originated from lake Zeway. *Escherchia coli* were isolated from spleen, liver, and the intestine of 12 out of 100 (12%) while *E. tarda* was isolated 4 out of 100 (4%) randomly examined fish. The higher percentage of both bacteria was isolated from intestinal samples than those isolated from liver and spleen samples. The *E.tarda* isolates appeared as small scatter pinkish colonies with black center while isolated colonies from *E. coli* were yellowish white on Xylose deoxycholate agar (XLD) after 24 h of incubation at 37°C Both types of isolates were gram negative short to medium rods, motile, catalase positive and oxidase negative. In biochemical test, both of the two types of typical isolates were positive for indole and Methyl red test and negative for Vagos proskaur test and unable to utilize Simmon's citrate test. In addition to this, *E. tarda* ferments sodium thiosulfate and result its byproduct of H_2S production (blackening) on TSI test which were absent in *E.coli*. The distribution of *E.tarda* and *E.coli* infection among the three organs examined indicate both bacteria were isolated most frequently from intestine followed by spleen and liver with significance difference (P <0.05) in the rate of isolation among organs. The occurrence of *E.tarda* and *E.coli* infection with respect to sex were not significant (P>0.05) indicating that sex has no role in influencing the exposure these bacteria. The isolation of *E.tarda* and *Escherchia coli* from wild fish population of Lake Zeway destined for human consumption in the current study indicated that *E. tarda* and *Escherchia coli* are a potential threat for fish and public health.

Key word: Catfish*, Escherchia coli, Edwardsiella tarda,* intestine, liver, spleen tilapia, zeway.

INTRODUCTION

Fish has become an increasingly important source of protein and other element necessary for the maintenance

*Corresponding author. E-mail: aridian181@gmail.com.

of health. Many species of fish normally live in fresh water lakes and rivers. Fish is one of the most highly perishable food products in which quality deterioration occurring rapidly during handling and storage that limits the shelf life of the product (Sallam, 2007).

The advantage of fish as food is as a result of its easy digestibility and high nutritional value. However fish are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease and are considered by some to be saprophytic in nature (Petronillah et al., 2013). Apart from being a primary fish pathogen, some Microbes present a potential hazard to public health through contamination of fish products. The safety of fish products for human consumption is therefore a prime concern from the point of view of managing of the fish culture systems, as well as ensuring public health. Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. The actual role of these micro-organisms may vary from that of a primary pathogen to that of an opportunist invader of a host with compromised immune system due to some other diseases (Abowei and Briyai, 2011). Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients' contact with fish and related environment, dietary habits and the immune status of the exposed individual.There are bacterial species facultativelly pathogenic for both fish and humans which may be isolated from fish without apparent symptoms of disease. The infection source may be fish kept either for food or those kept in aquaria (Acha and Szyfres, 2003).

Therefore, determination of bacterial micro biota in fish destined for human consumption is an indicator of the quality of fresh fish (Costa, 2013).

Of a number of fish pathogenic bacteria species that cause food borne zoonoses *Escherchia coli* and *Edwardsiella tarda* constitute only a couple of them associated with either infection of fish or contamination of fish products destined for human consumption. *E. coli* is a bacterium that commonly lives in the intestine of people, animal and fish. Most of the *E. coli* that are normal inhabitants in the small intestine and colon are non-pathogenic. Nevertheless; these non-pathogenic *E.coli* can cause disease if they spread outside from the intestine to other organs. The pathogenic strains of *E.coli* may cause diarrhea by producing and releasing toxins (called enterotoxigenic *E. coli* or ETEC) and may be the cause of food spoilage in fish (Soliman et al., 2010).

Edwardsiella tarda, a Gram-negative bacteria belonging to *Enterobacteriaceae*, is the etiological agent for edwardsiellosis, a devastating fish disease prevailing worldwide (Mohanty and Sahoo, 2007). Infected fish processed for human consumption may cause gastroenteritis, meningitis, cholecystitis, endocarditis, liver abscess and osteomyelitis (Srinivas et al., 2001).

In Ethiopia, the prevailing habit of consuming raw or lightly cooked fish meal coupled with the poor facilities for slaughter, filleting, transport and storage of fish undoubtedly make fish meal the potential source of food borne zoonoses. However, there were no detailed studies so far to investigate the microbiological safety of fish products particularly of raw fish carcass, This study is therefore intended to investigate a couple of bacterial pathogens which compromise the safety of fish products. With the following objectives

1. To isolate *E.coli* and *E.tarda* from fish harvested from Lake Zeway
2. To elucidate the safety of fish product with respect to *E.coli* and *E. tarda* contamination.

MATERIALS AND METHODS

Study area

The study was conducted from November 2014 to June 2015 in Oromia regional state East Shoa zone at Lake Zeway, which is located some 165km south of Addis Ababa. The area is located between 7° 51' N to 8° 7' N and 38°43' E to 38° 57' E, at an altitude of 180m above sea level. The lake is 25km long and 20km wide. It covers an area of 434 $km^2$and its average depth is 2.4m (LFDP, 1995). Lake Zeway is one of the Ethiopian lakes with an indigenous fishing population. The major fish species in the lake includes Nile tilapia, Tilapia zilli, Catfish, Barbus and Carp species (Yimer, 2000).

Study animals

Fifty African catfish (*Clarias gariepenus*) and fifty Nile tilapia (*Tilapia niloticus*) harvested from Lake Zeway for human consumption were used for bacterial isolation and identification.

Sample collection and transportation

A total of 100 fish harvested from Lake Zeway were randomly selected for specimen collection. Tissue samples from liver, intestine, and spleen were collected aseptically from each fish and placed in sterile sampling bottle containing commercially preapared normal saline. The bottles containing the sample were then immediately kept in ice box containing ice pack all the way to College of Veterinary Medicine and Agriculture at microbiology laboratory and are processed for further bacterial isolation and identification.

Presumptive isolation and identification of *E.tarda* and *Escherchia coli*

Hundred specimens from liver, Hundred specimens from Intestine and Hundred specimens from spleen from hundred fish were treated separately and samples were processed in microbiology laboratory for culturing and isolation. The inoculums were taken from each sample of liver, intestine and spleen and cultured on xylose lysine deoxycholate (XLD) agar media at 37°C for 24 h for isolation and colonial morphology. The typical growing isolated colonies were picked up and sub cultured on nutrient and MacConkey agars and then incubated at 37°C for 24 h. The

Table 1. Distribution of *E. coli* and *E. tarda* isolates by organ.

Organ	*E. coli*			*E. tarda*		
	Negative	**Positive**	**Total**	**Negative**	**Positive**	**Total**
Spleen	98	2	100	99	1	100
Liver	99	1	100	99	1	100
Intestine	91	9	100	98	2	100
Total	288	12	300	296	4	300
X^2			0.005.			

Table 2. Frequency of occurrence of *E. coli* and *E. tarda* by sex.

Sex	*E. coli*			*E. tarda*		
	Negative	**Positive**	**Total**	**Negative**	**Positive**	**Total**
Male	34	8	42	40	2	42
Female	54	4	58	56	2	58
Total	88	12	100	96	4	100
Male	34	8	42	40	2	42
X^2		3.424			0.109	

resulting pure culture isolates were used as stocks for further morphological or biochemical identifications.

Primary identification of isolates

From the resulting pure culture gram stain, oxidase test and catalase test, indole and methylen red test were conducted. Those colonies which were gram negative, short rods, oxidase negative and catalase positive were considered for further tests as these characteristics confirm to be *E. tarda* and *E.coli.* Motility test wereconducted using SIM media by inoculating fresh colony into the medium and by incubation at 37°C for 24 h. Turbidity of the medium or out growths from the line of inoculation indicates motility (Yan et al., 2014).

Secondary identification of isolates

Edwardtela tarda was presumptively identified based on formation of red slant, yellow butt with H_2S production while *Escherchia coli* shows both slant and butt yellow without H_2S production on TSI agar, production of indole on SIM medium after addition of 0.2ml kovac's reagent and citrate negative.

Data analysis

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

RESULTS

Of the total of three hundred tissue sample comprising spleen, liver and intestine collected from 100 fish, only 12(4%) tissue samples was positive for *E.tarda* (1 % from liver, 1% from spleen, and 2% for intestine) while 36(12%) tissue samples *p*ositive for *Escherchia coli*, (9%) from intestine, (1%) from liver, and (2%) from spleen. *E.tarda* isolated appeared as small scatter pinkish colonies with black center while *E. coli* isolates were yellowish white on Xylose deoxycholate agar (XLD) after 24 hrs of incubation at 37°C. Both types of isolates were gram negative short to medium rod, motile, catalase positive and oxidase negative. In biochemical test, these typical isolates were positive for indole and Methyl red test and negative for Vagos proskaur test and unable to utilize Simmon's citrate test. In addition to this *E. tarda,* utilizes sodium thiosulfate resulting H_2S as a by product observed as blackening of TSI medium which was absent in case of isolates identified as *E.coli.* The frequency of isolation of *E.tarda* and *E.coli* from the three organ specimens showed significant variation ($P<0.05$) with both species being most frequently isolated from intestine followed by spleen and liver (Table 1).

Although *E.coli* was isolated more frequently from male fish than female, the difference in the occurrence of *E.coli* infection with respect to sex was not significant (P-value=0.196) indicating both sexs are equally susceptible. The same scenario was also observed in case of *E.tarda* isolation with respect to sex (Table 2)

There were no statistical difference among fish species in which in the occurrence of *E.coli* and *E.tarda* indicating that both species are equally exposed or susceptible to

Table 3. Frequency of occurrence of *E. coli* and *E. tarda* by species.

Species	*E. coli*			*E. tarda*		
	Negative	**Positive**	**Total**	**Negative**	**Positive**	**Total**
Cat fish	10	2	12	10	2	12
Tilapia	78	10	88	86	2	88
Total	88	12	100	96	4	100
X^2		0.783			1.083	

the infection.

DISCUSSION

Fishery products are important not only from nutritional point of view, but also as an item of international trade and foreign exchange for a number of countries. However, they can also function as carriers of several microbial and other health hazards. The greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products (Baya and White, 1997). *E.coli* and *E.tarda* are one of the bacterial pathogens of freshwater fish especially under cultured conditions causing considerable losses (Baya and White, 1997). These pathogens also play significant role being one of fish related food borne illness (Yagoub**,** 2009).

The occurrence of *E.coli* (12%) and *E.tarda* (4%) recorded in the present study from apparently healthy fish was in agreement with previous reports of Nuru et al. (2012) who reported 2% of *E.tarda* from Lake Tana and 15% of *E. coli* from Bagdad by Maysoon, 2014.

The prevalence of *E.coli* in the present studies was higher than the previous studies, which were conducted by El-olemy et al. (2014) were 2% by taking skin swab in market fish in Qalyoubia. But, the higher prevalence were reported by Yagoub (2009) which were 23.2% while *E.tarda* in the present study were lower than the previous study which were reported by Maysoon, 2014 wich was 12% in Baghdad.

The finding of *Escherchia coli* and *E. tarda* most frequently from intestine than in spleen and liver of the studies fish may be attributed to the existence of both bacteria as part of the normal intestinal micro flora of fish in some instances (Wyatt et al., 1979; Van Damme and Van depitte 1980; Kanai et al., 1988, Hanson et al., 2008). Such intestinal micro flora may result in clinical infection if they get spread to other organs or may be associated with product contamination during processing of fish which has important implication from the point of view of both fish and public health.

The characteristics of the micro-environment of the various sections of the alimentary tract of each fish species is known to influence the species composition and the relative abundance of bacterial species (Horsely 1997). Magnadóttir (2006), also indicated that differences in the intestinal micro biota can exist with fish species owing to the differences in digestive systems.

These factors may contribute for differences in the chance of isolating commensal enteric bacteria from different species of fish. The absence of significant differences in the frequency of isolating *E. coli* and *E.tarda* from catfish and Nile tilapia is however contrary to the above facts (Table 3). This may be due to the small sample size used in the current study which may not be sufficiently enough to show such differences.

The absence of significant difference in the occurrence of *E.coli* and *E.tarda* between male and female indicate that both sex are equally susceptible to the bacterium ,this is an agreement with several work were both sex are equal chance of being infected *E.tarda* (Savan et al., 2005; Yu et al., 2004). The isolation of E. coli and E. tarda, important zoonotic bacteria, from apparently healthy fish harvested for human consumption may indicate that the fish product presents potential risk to public health if consumed raw or improperly cooked.

Conclusion and recommendations

E.coli and *E. tarda* are some of the important bacterial pathogens causing severe economic loss and hindrance in fishering. Apart from veterinary health importance, both pathogenshave also public health significance in people engaged in fishery industry, those depend on fish products for their annual income and consumers. The isolation of *E.coli* and *E.tarda* from wild fish population of Lake Zeway destined for human consumption, therefore, indicates that *E.coli* and *E. tarda* are a potential threat of both the fishery subsector and the general public.

Owing to the above conclusion the following points require future attention

1. Further study on and *E. coli* and *E. tarda* should be conducted in different species of fish residing in the different aquatic environments in the country to have a national picture of their distribution and importance.
2. Fish processing need to be carried out under hygienic condition to avoid product contamination from intestinal contents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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