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Investigation and PCR diagnosis of *Escherichia coli* infection (Zoonosis) in fishin Khartoum state, Sudan

Mohammed Abass Albashire Mohammedali[1*], Siham Elias Suliman[2], Mohammed[2], AbdeLSalam Abdalla[2] and Mohamed Ismail Fangama[3]

[1]*West Kordofan University*
[2]*College of Veterinary Medicine (SUST)*
[3]*Ministry of Public Health, Qatar*

**Corresponding author*

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A B S T R A C T

Fish known as source of protein and the number of pathogenic bacteria that cause food borne zoonoses such as *Escherichia coli* (*E. coli*) as an important contaminants to fish and its products, also investigate some risk factors associated with infection. One hundred and fifty tissue samples (gill, liver and kidney) were taken from 50 different breed of fish (*Synodontis schall*, *Tetraodon lineatus*, *Oreochromis niloticus*, *Bagrus bayad* and *Lates niloticus*) for bacteriological diagnosis and conventional PCR was applied confirmation. The risk factors recorded were age, sex, breed, body weight and body condition. The results revealed that the common lesions were local haemorrhage at gill cover and lower jaw with abdominal swelling. Bacteriologically 44(29.3%) samples were positive to the organism. The distribution of *E.coli* in the gill was 13(29.5%), liver 14(31.8%) and kidney 17(38.7%) organism. When using PCR obious clear single band was appeared for isolated *E.coli*. The following risk factors showed association with *E.coli* infection in the univariate analysis under significant level (P-value ≤ 0.25) were age (0.00), breed(0.041) and body weight (0.004).When using multivariate analysis the age of fish was confined to the infection. In conclusion fish contamination from the surrounding and generally young fish are more susceptible to infection by *E.coli*. PCR is rapid and realistic tool for diagnosis of the infection in fish.

Introduction

Fish is a food of excellent nutritional value, providing high-quality protein rich in essential amino acids, and a wide variety of minerals, including phosphorus, magnesium, iron, zinc, and iodine in fish. Many species of fish normally live in fresh water lakes and rivers (Haile and Getahun, 2018). Fish are generally considered as major vehicles for several bacterial disease transmissions (Saad *et al.*, 2018). Biological contaminants such as bacteria constitute the major cause of food-borne diseases (Teklu *et al.*, 2019). The bacterium *Escherichia coli* is the major sea food bacteria are coliforms belonging to *Enterobacteriaceae* and widely used as indicator of the bacteriological

condition of food and environments due to its almost exclusively fecal origin (Chakravarty *et al.,* 2015). The presence of *E. coli* in fresh-marketed seafood indicates recent contamination and is usually attributed to infected handlers or storage on contaminated ice (Rocha *et al.,* 2014) and the occurrence of *E. coli* is related to water contamination and/or unhygienic conditions during the handling process (Costa, 2013). Food safety has become an important issue in fresh produce production worldwide due to many factors such as importation of fresh produce from various countries, potential sources of bacterial pathogens from the growing environment, and inappropriate domestic food preparation (Wang *et al.,* 2020). The poor unhygienic conditions of the landing centers, storage and domestic retail markets exacerbate the problem of poor hygiene and consumer safety of fish (Kumar *et al.,* 2005). Contaminated fish, when handled, can play a role in the transmission of these agents to consumers, as well as contaminate food and surfaces (Barbosa *et al.,* 2014).

The aim of this study was to isolate and identify *E.coli* in fish, PCR for confirmation and some risk factors associated with infection. The potential of mucus to serve as a substrate for *E. coli* contamination is also investigated (Suhalim, 2003).

Materials and Methods

Study area

This study was conducted in Khartoum state is a capital of Sudan and composed of Omdurman, Khartoum and Bahri. Omdurman district contains three localities and these are; Omdurman, Karrary and Ombeda (Annual report, 2017). Elmorda market is a big market for sealing of fish in Omdurman locality.

Sample collection

A total of 50 fish for deferent breed were Nile tilapia (*Oreochromis niloticus*), Synodontis (*Synodontis schall*), Nile puffer (*Tetraodon lineatus*), Bagrus catfishes (*Bagrus bayad*) and Nile Perch (*Lates niloticus*). Subjected to postmortem examination and 150 samples were taken from gill, liver and kidney from each fish for bacteriological diagnosis of *E.coli* infection) (Barrow and Felthman, 2003; Oxoid, 2006; Austin 2011).

Form risk factors associated with *E. coli* infection were recorded were age, sex, breed, body weight and body condition. For confirmation of the organism PCR was used (ICMSF, 1996).

DNA extracted of highest count of colonies from fish tissues using Luria Bertoni broth and this broth was further used for PCR amplification. Phenol, chloroform and isoamyl alcohol were used for extraction (Alkagrover, 2012). DNA samples were screen for the organism using 16 rRNA gene of the bacteria. To amplify 528 base pair (BP) (Tsen *et al.,* 1998).

Extraction of DNA

E. coli isolated showed the highest count in the kidney and liver of the freshwater fish. Pathogens from kidney, liver and gill were isolated and inoculated into Luria Bertoni broth. This broth was used for bacterial DNA isolation which was further used for PCR amplification. Alkagrover *et al.,* (2012) isolated DNA using phenol, chloroform and isoamyl alcohol method. Thus, the same protocol was followed in this study for bacterial DNA isolation. Then, agarose gel electrophoresis was performed.

Meiyarasi1 *et al.,* (2017) used direct colony PCR for diagnosing bacterial fish diseases, with decreased cost and time compared with the classical methods used in Brazil, such as isolation, biochemical tests, and conventional PCR.

Agarose gel electrophoresis

0.9% agarose was used for agarose gel preparation. DNA sample was injected into the wells of agarose gel and electrophoresis was performed for 2 hours.

As a result of agarose gel electrophoresis, clear bands were obtained for the DNA isolated from *E. coli.*

PCR amplification

Joshi *et al.*, (2010) used the specific primers for amplification of Gram- negative bacteria. Since *E. coli* is a gram negative bacteria, the same *E. coli* protocol was used in this study for PCR amplification. Agarose gel electrophoresis was performed for PCR amplified gene using markers.

Statistical Analysis

Statistical analysis of data was performed using SPSS (Statistical Package for the Social Sciences) version 20.0 (SPSS inc. Chicago, USA) by using frequencies.

Results and Discussion

The common lesions observed in fish infected by *E.coli* were focal haemorrhage at gill cover (operculum) and lower jaw and Swelling of the abdomen at left side (figure 2, 3).

From 150 samples obtained only 44 samples were positive to *E.coli*, and the overall prevalence was 14 % (Table 1). The distribution of the organism in the gill was 13(29.5%) lower than liver 14(31.8%) and kidney 17(38.7%).

The results of this study showed the distribution of 150 fish samples examined for *E.coli* infection according to age. Total number of young fish was 69(46.0%), medium age was 66(44.0%) and old fish was 15(10.0%). The rate of infection within young fish was 11(15.9%), medium age was 21(31.8%) and old fish was 12(80.0%). The Chi-square test showed that there was significant association between *E.coli* infection and age of fish (p-value =.000), (Table 2). But the sex of fish revealed no significant association between the disease and sex of fish (p-value =.290). Also the rate of infection with in fish breed were *Bagrus bagrus* 4(33.3%), *Lates niloticus* 7(38.8%), *Oreochromis niloticus* 17(29.8%), *Synodontis batensoda* 10(18.5%) and *Tetraodon lineatus* 6(66.7%). Whereas chi square results was significant association between *E.coli* infection and breed of fish (p-value =.041). The high rate of infection in fish was70-79 gm B.W of fish 19(22.6%), but the lower rate 100 gm-above 3(14.3%) with a significant association between the weight of fish and the disease (p-value =.004). While rate of infection of good body conditions was nearly to poor condition, but there was no significant association between the weight of fish and the disease (p-value =.004) (Table 2).

As shown in table 3 the age was more associated with a disease specially in young and medium ages.

Diagnosis of *E.coli* using PCR revealed obvious clear single band for isolated organism in agrose gel electrophoresis (Fig 3).

The prevalence of *E.coli* in various sea foods may be due to fecal contamination and improper handling. Also contamination can be occurred by water quality, fishing method and storage (Chakravarty *et al.*, 2015). The isolation and identification of *E.coli* by bacteriological techniques are useful for giving valuable results (Austin, 2011). Also PCR performed to identify *E.coli* as common disease causing pathogen in fish under cultured condition leading to economic losses in fish and fish products (Baya and White, 1997). In this study obvious clear single band was running in agrose gel electrophoresis (Fig 3), this tool is rapid and reliable for diagnosis of the organism (Meiyaras *et al.*, 2017). The common lesions observed in this study in fish infected by *E.coli* was swelling of the abdomen and hemorrhage at operculum and lower jaw agree with (Wamala *et al.*, 2018). The normal habitat of *E.coli* inside small and large intestines and can cause the infection when spreads outside intestines to other organs by its toxins and also spoilage the fish its products (Soliman *et al.*, 2010; Hail and Getahun, 2018).

Table.1 Programming of thermal cycler for detection of *E.coli* by conventional PCR

No of cycle	Temp(°C)	Time	Target
1 cycle	94	5 mins	Initial denaturation
35 cycles	95	30 seconds	Denaturation
35 cycles	56	30 seconds	Annealing
35 cycles	72	30seconds	Extention
	72	5 mins	Final extention
			Preservation

The PCR thermal cycler was adjusted as shown in Table (1).

Table.2 Distribution of *E.coli* infection among fish examined in Khartoum state

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-ve	106	70.7	70.7	70.7
	+ve	44	29.3	29.3	100.0
	Total	150	100.0	100.0	

Table.3 Univariate analysis for potential risk factors of *E.coli* infection in fish (in=150) in Khartoum state-Sudan

Risk factors	Number tested	Number positive (%)	df	X^2	P.Value
Age					
1-2 months	69	11(15.9%)	2	24.742[a]	.000
3-5 months	66	21(31.8%)			
>6 months	15	12(80.0%)			
Sex					
Male	102	28(27.5%)	1	.545[a]	.290
Female	48	16(33.3%)			
Breed					
Bagrusbagrus	12	04(33.3%)	4	9.990[a]	.041
Latesniloticus	18	07(38.9)			
Oreochromis niloticus	57	17(29.8%%)			
Synodontisbatensoda	54	10(18.5%)			
Tetraodon lineatus	09	06(66.7%)			
Body weight					
70-79 gm	84	19(22.6%)	3	13.280[a]	.004
80-89 gm	30	16(53.3%)			
90-99 gm	15	06(40%)			
>100 gm	21	03(14.3%)			
Body condition					
Good	111	33(29.7%)	1	.032[a]	.515
Poor	39	11(28.2%)			

Table.4 Multivariate analysis of potential risk factors of of *E.coli* infection in fish (in=150) in Khartoum state-Sudan

Risk factors	df	P-value	Exp(B)	95.0%C.I for Exp(B)	
				Low	Upper
Age **1-2 months** **3-5 months** **>6 months**	2	.000	12.862 5.053	3.061 1.258	54.038 20.294

P.value 0.05

Fig.1 PCR setup for detection of *E.coli* by conventional PCR

Fig.2 Tilapia(*Oreochromis niloticus*): haemorrhage at gillcover (operculum)and lower jaw (see arrow)

Fig.3 Bagrus catfishes (*Bagrus bagrus*): Swelling of the abdomen at left side (see arrow).

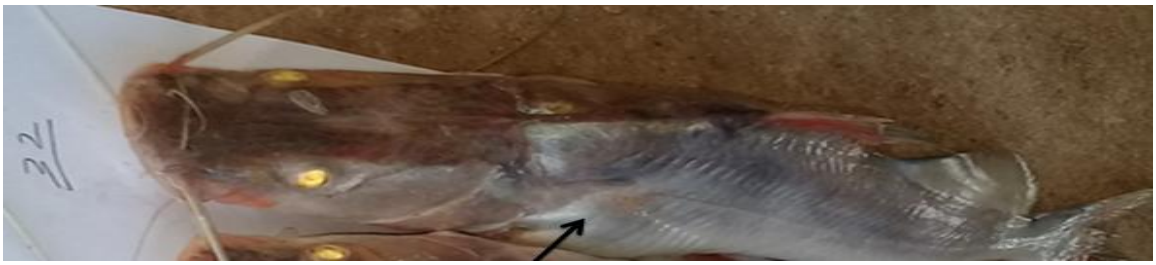
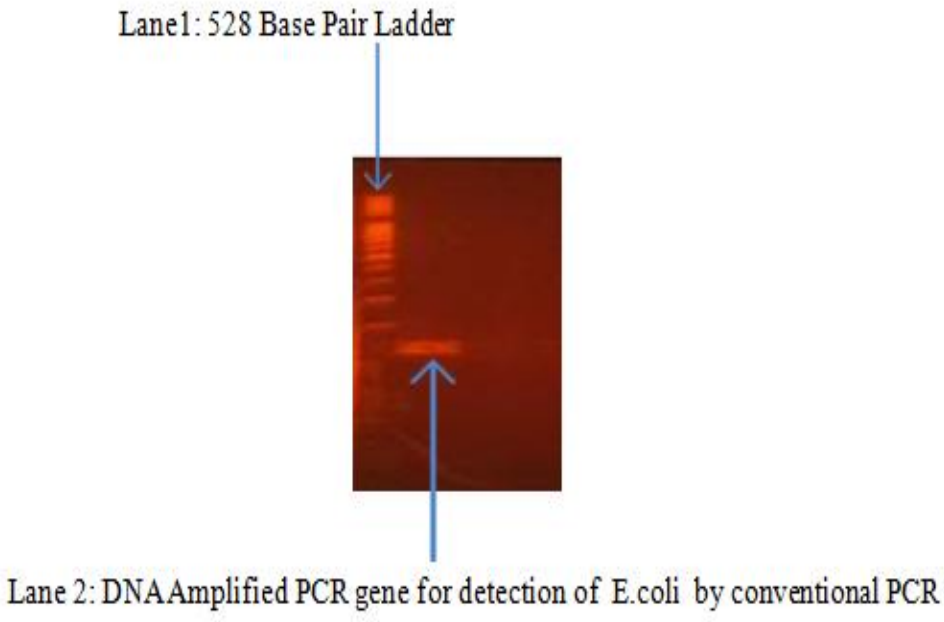


Fig.4 Lane1: 528 Base Pair Ladder , Lane 2: DNA Amplified PCR gene for detection of *E.coli* by conventional PCR



The prevalence of *E.coli* in the present results is 29.3% and the presence of the organism in the gill (29.5%), liver (31.8%) and the kidney (38.7%) may be attributed to the existing of the organism in digestive system of fish and known to influence the species composition and the relative abundance species and also intestinal microbiota which exist with fish species (Horsely, 1997; Magnadottir, 2006). The age, breed and body weight of fish revealed association with the infection (table2). In this results (P value ≤ 0.025). But the age specially young fish showed high statistical significant difference to *E.coli* infection (table 3) by multivariate analysis (P value ≤ 0.05), this may be due to immunological defence of the fish species. There was no significant association between the sex of the fish and the disease, this finding in agreement with finding of Yu *et al.*, (2004); Savan *et al.*, (2005) and Haile and Getahun, (2018) who recorded that sex are equally susceptible to the bacterium, but they contact with finding of the breed or species which showed significant association with infection (table 1). The disease has not associated with body of the fish that indicated that fish in good or poor body condition can be infected. *E.coli* infection diagnosed by PCR which realistic tool for confirmation. Also this disease can be infected all ages of fish which consume by human and this represents potential risk factor to public health as zoonotic disease.

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