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Antibiotic Resistance and Virulence Genes of *E. Coli* Isolated From Fresh Nile Tilapia (*Oreochromis Niloticus*) in El-Behera Governorate, Egypt

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ABSTRACT

Two hundred and eighteen (218) fish samples collected from gills and muscles of Nile tilapia sold in two market regions in El-Behera governorate; Damanhour and Abou-humus, were examined for the presence of *E. coli*. Forty *E. coli* isolates (29 from gills and 11 from muscles) were identified by culture, biochemical analysis and PCR. Further, the isolates were studied for their antimicrobial susceptibility patterns using 9 antibiotics commonly used in the veterinary and medical fields. The highest resistances were by *E. coli* isolates from the gills against ampicillin, streptomycin, sulfamethoxazole-trimethobrim and tetracycline with 97.5%, 65%, 57% and 50%, respectively. While sensitivities were detected to Amikacin (97.5%), Ciprofloxacin (90%), Cefotaxime (67.5%) and Gentamycin (60%). Multiple antibiotic resistances were detected in 95% (38/40) of the isolates. Higher MAR index was detected from *E.coli* isolates from the gills and 24 different resistance phenotypes were detected. PCR for the detection of the virulence genes; *eaeA* and *stx2* and antimicrobial resistance genes; bla_{TEM}, *tetA(A)*, *sul1* and *aadA2* was performed. All tested isolates were 100% positive for the bla_{TEM} gene. The *eaeA*, *tetA(A)* and *Sul1* genes were detected in 83.3% (5/6) of the tested isolates, while the *stx2* and *aadA2* genes were detected in 50% only. These results collectively indicate that Nile tilapia in market can harbor pathogenic *E. coli* and act as a reservoir for multi-resistance *E. coli* and facilitate its transmission and dissemination.

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1. INTRODUCTION

Nile tilapia is highly consumed in Egypt due to its economical price and palatability. Further, it is cultivated in many regions in Egypt owing to its high productivity and adaptation to different culture conditions (Elsaidy *et al.*, 2015). However, contamination is one of the challenging factors either in the ponds or during harvesting or selling in the market and can be a source of foodborne pathogens and may be a potential source of infection to consumers (Kromhout *et al.*, 1985). The contamination of water or other surroundings can be checked using the prevalence of *Escherichia coli* (*E. coli*) as an indicator (Elsaidy *et al.*, 2015). Thus, detection of *E.coli* in fresh fish in the market is usually accredited to contamination from different sources such as unsanitary handling, storage or use of contaminated materials (Lateef *et al.*, 2004, Rocha *et al.*, 2014). The challenging factors behind such sources resides in the type of pathogenic organism detected in fish, mainly the Shiga toxin (stx)-producing *E.coli* (STEC), that constitutes a main cause of foodborne infections in man (Galal *et al.*, 2013).

Using antimicrobial drugs to control infectious diseases has increased in recent years consequently increasing the bacterial antimicrobial resistance (Van den Bogaard and Stobberingh, 1999, Schroeder *et al.*, 2002). The resistant microbes may act as a potential source for transfer of antimicrobial resistance to human beings. Shiga-toxin producing *E. coli* (STEC) is defined by the production of one or more types of Shiga toxin (Stx1, Stx2) through the possession of *stx1* and *stx2* genes. Stx2-producing strains are linked to increased risk of human diseases, especially Hemorrhagic colitis (HC) and Haemolytic-uremic syndrome (HUS) than stx1, especially when the former is associated with the intimin gene; *eae*, which aggravates the pathogenicity (Adu-Bobie *et al.*, 1998, Paton and Paton., 1998, Oswald *et al.*, 2000, Paton and Paton. 2002). Little is reported on the antibiotic resistance of *E. coli* as well as STEC prevalence in Nile tilapia in Egypt. The aim of the present study was to spotlight on the prevalence of *E. coli* in Nile tilapia,

estimate its antimicrobial resistance and detection of virulence genes that may result in posing threat to human health.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 218 samples of Nile tilapia (*Oreochromis niloticus*) were collected from retail market at Abou-humus (118 fish samples) & Damanhour (100 fish samples). Fish samples were put in ice box at 4°c and were transported to laboratory for examination (Rocha *et al*, 2014). Swabs were taken from gills and muscle (Alonso *et al.*, 2012).

2.2. *E. coli* Isolation

Swabs were taken from gills and muscle were put in nutrient broth and incubated at 37°C for 24 hrs. For isolation of *E. coli* in pure culture, Loopful from enrichment media were taken and streaked on MacConkey agar and Eosin Methylene Blue (EMB) agar media and incubated aerobically for 24 hrs at 37°C (Gupta *et al.*, 2013). Colonies from each sample were subjected to Gram staining and biochemical testing (indole production, methyl red, citrate utilization, sugar fermentation and H_2S production) (MacFaddin, 1985, Alexander *et al.*, 2010).

2.3. Antimicrobial susceptibility testing of *E. coli*

The Antimicrobial susceptibility testing by using disc diffusion method was carried out according to the Clinical laboratory standards Institute (CLSI, 2012). The following antibiotics were used: Tetracycline (TE; 30 µg), Amikacin (AK; 30 µg), Ampicillin (AML; 30 µg), Ciprofloxacin (CIP; 5 µg), Streptomycin (S; 25 µg), Gentamycin (GM; 10 µg), Cefotaxime (CTX; 30 µg), sulfametoxazole-trimetoprim (SXT; 1.25/23.75 µg) and Amoxicillin/clavulanate (AMC; 20 µg/10 µg) (Rocha *et al.*, 2014).

2.4. Polymerase Chain Reaction (PCR) for detection of *E.coli* virulence and antibiotic resistance genes

From pure cultures, DNA was extracted by phenol-chloroform method according to Shambrook *et al.*, (1989). PCR (Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit) was prepared by adding 12.5 µl of Emerald Amp GT PCR master mix (2x premix), 4.5 µl PCR grade water, 1 µl forward primer (20 pmol), 1 µl reverse primer (20 pmol) and 6 µl template DNA to a total volume of 25 µl. Primers used for the detection of the different genes and cycling conditions are listed in Table (1).

3. RESULTS

3.1. Detection of *E. coli* in samples from Nile tilapia

A total of 218 samples were collected from Damanhour (100) and Abou-humus (118) from gills and muscles of Nile tilapia collected from retail markets. *E. coli* were isolated from 40 out of 218 samples (18.3%) as shown in **Table (2)**. Isolates were 29 from the gills (26.6%) as compared to 11 (10%) from the muscles. On EMB, colonies gave the characteristic greenish-black metallic sheen and pink colonies on MacConky agar. Gram's staining revealed gram-negative rods. *E. coli* isolates were indole, Methyl red, sugar fermentation and gas production positive, Citrate utilization, urease and H_2S production negative. There was no significant differences between Damanhour and Abou-humus for the percentage of *E. coli* isolation as they were 18% and 18.6%, respectively.

Table (1): Oligonucleotide primers sequences

Gene	Primer Sequence 5'-3'	Amplified product	Reference
tetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576 bp	Randall *et al.* (2004)
eaeA	GACCCGGCACAAGCATAAGC CCACCTGCAGCAACAAGAGG	384 bp	Wen-jie *et al.*, 2008
aadA2	TGTTGGTTACTGTGGCCGTA GATCTCGCCTTTCACAAAGC	622 bp	Walker *et al.* (2001)
bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516 bp	Colom *et al.*, 2003
Sul1	CGG CGT GGG CTA CCT GAA CG GCC GAT CGC GTG AAG TTC CG	433 bp	Ibekwe *et al.*, 2011
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779 bp	Dipineto *et al.* (2006)

Table (2). Incidence of *E.coli* isolation from Nile tilapia in retail market in Damanhour and Abou-humus

Samples	Damanhour			Abou-humus			Total +ve
	No.	*E. coli* +ve	%	No.	*E. coli* +ve	%	No. (%)
Gills	50	9	18	59	20	33.9	29 (26.6)
Muscles	50	9	18	59	2	3.39	11 (10)
Total	100	18	18	118	22	18.6	40 (18.3)

3.2. Antimicrobial susceptibility of *E. coli* isolates

Antimicrobial susceptibility was observed in *E.coli* isolates from all samples; from gills; *n* = 29 and muscles; *n* = 11. As shown in Table (3), the highest percentage of resistance was to the ampicillin, streptomycin, sulfamethoxazole-trimethobrim and tetracycline with 97.5%, 65%, 57% and 50%, respectively. The resistant isolates were more from the gills than the muscles. While sensitivities of the isolates were detected to amikacin (97.5%), ciprofloxacin (90%), cefotaxime (67.5%) and gentamycin (60%). Multiple antibiotic resistances (MAR) were detected in 38 out of 40 isolates; 95% (gills; 28/29 (96.5%), muscles; 10/11 (90%)). Higher MAR index was detected from *E.coli* isolates from the gills as shown in Table (4). In addition, 24 different phenotypes of antimicrobial resistances were displayed by the isolates as shown in Table (5).

3.3. Detection of the *E. coli* virulence and antimicrobial resistance genes by PCR

Some of *E. coli* positive samples, as shown in Table (6) and Figure (1) were subjected to PCR for the detection of *E. coli* virulence factors as *eaeA* (intimin) and *stx2* (shiga toxin 2) genes as well as the antimicrobial resistance genes; *tetA(A)*, bla_{TEM}, *aadA2* and *Sul1* genes. All isolates were positive for the bla_{TEM} gene. The *eaeA*, *tetA(A)* and *Sul1* genes were detected in 83.3% (5/6) of the tested isolates, while the *stx2* and *aadA2* genes were detected in 50% only.

4. DISCUSSION

Nile tilapia (*Oreochromis niloticus*) acts as an important source of food for humans because of its high digestible protein, vitamins, minerals and essential nutrients required for man (Abdullahi *et al.*, 2001). *E. coli* may be present in Nile tilapia due to contamination of cultured water by excreta of human and animal and using contaminated water in preparation of fish (James *et al.*, 2001).

In the present study, 218 fish samples were collected from retail market at Damanhur and Abou-humus from gills and muscle of Nile tilapia (*Oreochromis niloticus*) and were examined for the presence of *E. coli*. Forty *E. coli* isolates (29 from gills and 11 from muscle) were obtained with prevalence of 18% and 18.6% from Damanhour and Abu-humus, respectively. Azza *et al.*, (2012) reported that the incidence of *E. coli* isolation from 40 tilapia samples was 27%, which is higher than our total isolation rate; 18.3%.

Table (3). Antibiotic sensitivity of *E.coli* isolated from Nile tilapia in retail market in Damanhour and Abou-humus.

Antibiotic	Resistant*	Intermediate*	Sensitive*
Ampicillin	39 (97.5%)	1 (2.5%)	0 (0%)
Sulph/trim	23 (57%)	6 (15%)	11 (27.5%)
Streptomycin	26 (65%)	11 (27.5%)	3 (7.5%)
Tetracycline	20 (50%)	8 (20%)	12 (30%)
Amoxi/clav	8 (20%)	18 (45%)	14 (35%)
Gentamycin	7 (17.5)	9 (22.5%)	24 (60%)
Ciprofloxacin	2 (5%)	2 (5%)	36 (90%)
Cefotaxime	12 (30%)	1 (2.5%)	27 (67.5%)
Amikacin	0 (0%)	1 (2.5%)	39 (97.5%)

*Number of the isolates is displayed and the percentage in between brackets.

Table (4). Multiple antibiotic resistance index of *E.coli* isolated from Nile tilapia in retail market in Damanhour and Abou-humus

MAR index	Number of isolates		Total
	Gills	Muscles	
0.1	1	1	2
0.2	7	3	10
0.3	8	5	13
0.4	4	0	4
0.55	4	2	6
0.66	5	0	5

Table (5). Resistance phenotypes of *E. coli* isolates from Nile tilapia in retail market in Damanhour and Abou-humus

Samples		Resistance phenotype
Gills	muscles	
1	0	AML,SXT,TE,GM
2	0	AML,SXT,TE,S
1	0	AML,SXT,S,CIP
1	1	AML,S,AMC
1	0	AML,AMC,CTX
1	1	AML,S,SXT
3	0	AML,SXT,TE
1	0	AML,SXT,CTX
1	0	AML,S,CTX
0	1	AML,S,TE
0	1	AML,SXT,CTX
0	1	AML,SXT,GM
3	2	AML,S
1	0	S,TE
1	0	AML,AMC
2	1	AML,TE
1	1	AML
2	0	S,TE,AMC,CTX,SXT,AML
2	0	S,TE,GM,CTX,SXT,AML
1	0	S,TE,GM,AMC,SXT,AML
1	0	S,AML,SXT,AMC,CTX
2	0	S,AML,SXT,GM,TE
1	1	S,AML,SXT,TE,CTX
0	1	S, AML, SXT, CTX, CIP

Tetracycline (TE), Amikacin (AK), Ampicillin (AML), Ciprofloxacin (CIP), Streptomycin (S), Gentamycin (GM), Cefotaxime (CTX), sulfametoxazole-trimetoprim (SXT) and Amoxicillin/clavulanate (AMC)

Table (6): Detection of *E.coli* virulence factors and antimicrobial resistance genes

Samples		Genes						MAR index
		virulence		antimicrobial resistance				
		eaeA	*stx2*	*tetA(A)*	bla_{TEM}	*aadA2*	*Sul1*	
Muscles	1	+	+	+	+	-	+	0.55
	2	+	-	+	+	+	+	0.3
Gills	3	+	+	+	+	-	+	0.2
	4	+	-	+	+	+	+	0.4
	5	-	+	-	+	+	-	0.55
	6	+	-	+	+	-	+	0.55

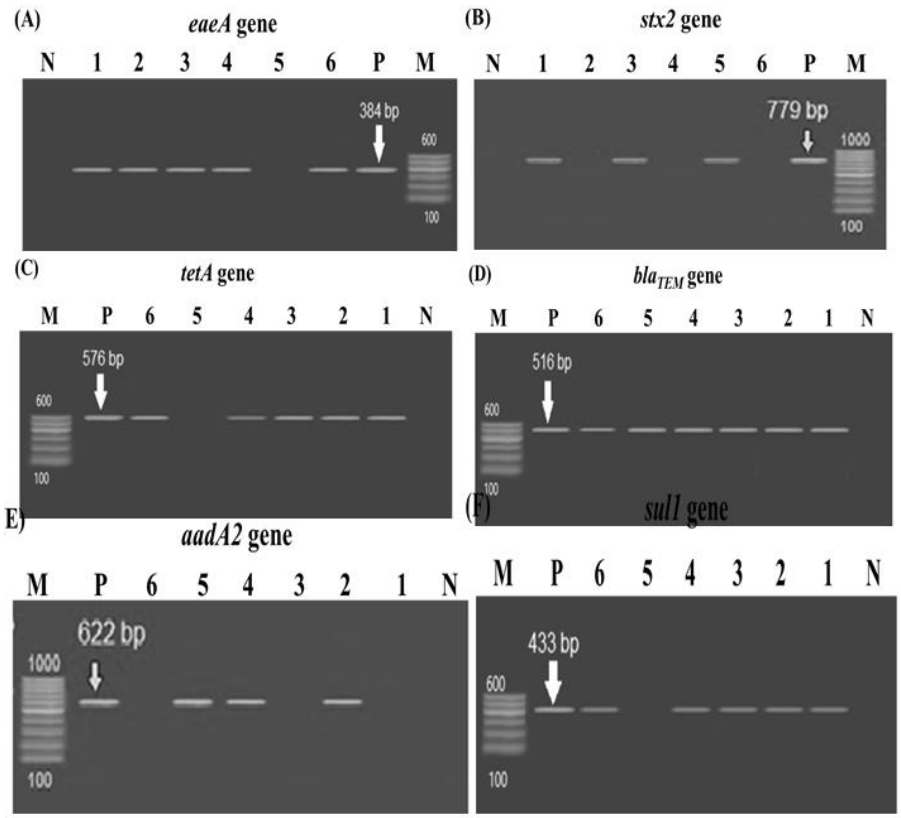

Figure 1. Agarose gel electrophoresis of amplified DNA showing the specificity of the single reactions for the detection of the different genes. **(A)** *eaeA* gene **(B)** *stx2* gene **(C)** *tetA*(A) gene **(D)** bla_{TEM} gene **(E)** *aadA2* gene **(F)** *sul1* gene. P; positive control, N; negative control, M; 100 bp DNA ladder. 1, 2, 3, 4, 5; 6; *E.coli* isolated from Nile tilapia.

Further, Galal *et al.*, (2013) reported that *E. coli* was isolated with a 36% from *Oreochromis niloticus*. Also, Rocha *et al.*, (2014) characterized 44 *E. coli* isolates from specimens of Nile tilapia (*Oreochromis niloticus*) that were collected from twelve supermarkets in Fortaleza (Cear´a, Brazil); 25 (56.82%) were isolated from gills, 4 (9.09%) from muscles and 15 (34.09%) from body surfaces. This agrees with our results that the isolation rate from the gills is higher than the muscles, however, our percentage was much lower for the gills (18%) and almost equal for the muscles (10%). In addition, Elsherief *et al.*, (2014) also showed only 12% *E. coli* isolation from retail market in Kafr EL Sheikh Governorate. This may indicate that different environmental conditions controls the contamination load with *E. coli* in samples derived from different geographical locations and possibly sanitary level of handling, storage and display procedures. Further, the isolation rate from the gills is higher than that from the muscles owing to that the gills are exposed to the external environment in contrast to the muscles were the detection of *E. coli* could be attributed to the fish intestinal contents (Musefiu *et al.*, 2011).

Antimicrobial susceptibility was performed with *E. coli* isolates from all samples; from gills and muscles. As shown in Table (3), the highest percentage of resistance was to the ampicillin, streptomycin, sulfamethoxazole-trimethobrim and tetracycline with 97.5%, 65%, 57% and 50%, respectively. This agrees with Jiao et al., (2007) who reported a 38% and 12% resistance against tetracycline and sulfamethoxazole-trimethobrim, respectively in *E. coli* isolates from Nile tilapia. Only 2% of their samples were resistant to ampicillin. However, their samples were derived from the intestine not from the gills or the muscles. Our results also agree with Wang *et al.*, (2011) who found high indexes of resistance, however less than ours, to tetracycline (30.7%), streptomycin (12.8%) and ampicillin (6.7%) in strains of *E. coli* isolated from fish and seafood collected from wholesale and retail markets in Seoul, Korea. Tiamiyu *et al.*, (2015) showed 66.67% and 100% resistance to tetracycline

by *E. coli* isolates from wild and cultured Nile tilapia, respectively, in Nigeria, however, they used very low number of isolates. Collectively, the resistant isolates in our study were more from the gills than the muscles possibly because the gills are exposed to the external environment and much more in contact with the handling, storage and selling, while the muscles can be exposed only in case of injury during bad harvesting and/or handling.

Multiple antibiotic resistances (MAR) were detected in 38 out of 40 isolates; 95% (gills; 28/29 (96.5%), muscles; 10/11 (90%)) and agrees with Rocha *et al.*, (2014) and Jiao *et al.*, (2007) as well. However, the number of MAR isolates reported by them are much lower in contrast to our isolates. This could greatly affect the antibiotic choice for treatment and calls for alternatives to control fish diseases. Higher MAR index was detected from *E.coli* isolates from the gills as shown in Table (4) and this may indicate a high risk assessment of the used antibiotics as well as possible environmental contamination. Rocha *et al.*, (2014) showed a very low MAR index; 0.026 from the gills, however, this could be attributed for using less sample number and antibiotics as compared to our sample number and number of antibiotics used. In addition, 24 different phenotypes of antimicrobial resistances were displayed by the isolates as shown in Table (5).

Antimicrobial sensitivities of the isolates were detected to amikacin (97.5%), ciprofloxacin (90%), cefotaxime (67.5%) and gentamycin (60%) indicating that these antibiotics could be of choice to be used in control of infectious diseases in fish. This is in agreement with Hatha *et al.*, (1999), Harish *et al.*, (2003), Jiao *et al.*, (2007) and Jeyasanta *et al.*, (2012) who reported that amikacin and ciprofloxacin proved to be the best antibiotics to treat *E. coli* infection since they were highly effective, while Tiamiyu *et al.*, (2015) showed that only gentamycin was effective against *E. coli*. The difference in the results from those reported by others may be due to the use of different antibiotics in different settings and purposes as well as by humans. In addition to different behavioral and hygienic conditions applied.

Samples, which were representative for the variable antimicrobial resistances, were selected for the detection of *E. coli* antimicrobial resistance genes; tetracycline resistance; *tetA(A)*, beta-lactam resistance; bla_{TEM}, aminoglycoside resistance; *aadA2* and sulfamethoxazole-trimethobrim resistance; *sul1* genes by PCR. All isolates were positive for the bla_{TEM} gene. The *tetA(A)* and *sul1* genes were detected in 83.3% (5/6) of the tested isolates, while the *aadA2* gene was detected in 50% only. This is in contrast to Ryu *et al.*, (2012) who reported that 70 out of 179 isolates from marine products, which were resistant to one or more drugs had bla_{TEM} gene in 15 (21.4%) isolates, while *aadA* gene was found in 18 (25.7%) isolates. Further, sample 1 as shown in Table (6) was negative for the *aadA2* gene but was resistant to streptomycin. Also, sample 5 was negative for the *tetA(A)* and *sul1* genes but resistant to tetracycline and sulfamethoxazole-trimethobrim and sample 6 was negative for the *aadA2* gene but resistant to streptomycin and gentamycin. This may indicate that there could be other mechanisms adopted by *E. coli* for resistance other than the specific genes detected in our work. And further necessitate the analysis of not only the resistant isolates but also the susceptible ones.

Shiga toxin (stx)-producing *E.coli* (STEC) are important pathogens that can cause severe human diseases such as hemorrhagic colitis (HC) and haemorrhagic uremic syndrome (HUS) (Paton and Paton, 1998a, Karch *et al.*, 2005). Several studies have associated the *eae* gene with the capacity of STEC strains to cause severe human disease, especially HC and HUS (Adu-Bobie *et al.*, 1998, Paton and Paton, 1998b, Oswald *et al.*, 2000, Paton and Paton, 2002). Stx2-producing strains are more related with HUS than stx1-producing strains. Further, the co-existence of *stx2* and *eae* in STEC increases the pathogenecity of the diseases caused (Bielaszewska *et al.*, 2005, Karch *et al.*, 2005, Mellmann *et al.*, 2005, Sonntag *et al.*, 2004, Zhang *et al.*, 2007).

Representative *E. coli* positive samples, as shown in Table (6) were subjected to PCR for the detection of *E. coli* virulence genes as *eaeA* (intimin) and *stx2* (shiga toxin 2). The *eaeA* gene was detected in 83.3% (5/6) of the tested isolates, while the *stx2* gene was detected in 50% only. The detection of both genes in the same sample was 33.3% (2/6) of the tested isolates. Galal *et al.*, (2013) reported the detection of the *stx2* gene in few samples from Nile tilapia, however they all were negative for the *eaeA* gene. Kumar *et al.*, (2001) and Kumar *et al.*, (2004) also detected the STEC in fish and seafood samples in India. Information regarding the detection of STEC in Nile tilapia are scarce, but those from seafood or shellfish are available. In contrast, Bennani *et al.*, (2011) found 0.48% prevalence of *stx2* in the seafood samples from Morocco and they were *eaeA* negative.

Also Kumar *et al.*, (2004) had all of their samples *eaeA* negative.

The detection of STEC, especially those with multiple antimicrobial resistances, in Nile tilapia in retails could indicate a possible cross contamination from infected workers or during harvesting or from contaminated ice or grass during marketing of the fish. All together represent a human health threat and calls for application of more hygienic practices during all stages of fish production and processing for selling. Further, it requires a wide microbiological surveillance and strict governing of the uncontrolled use of antimicrobials either for treatment or as growth promoters, not only in fish production but also in other livestock production systems. Further, the use of chicken manure and cow dung as fertilizers in fish ponds should be assessed considering that chicken and large animals are a reservoir of different types of *E. coli*, especially STEC.

5. REFERENCES

Abdullahi, S.A., Abolude D.S., Ega, R.A. 2001. Nutrient quality of four oven dried freshwater cat fish species in Northern Nigeria. J. Trop. Biosci. 1:70-76.

AduBobie, J., Frankel, G., Bain, C., Goncalves, A.G., Trabulsi, L.R., Douce,G., knutton, S., Dougan, G. 1998. Detection of intimin derivatives expressed by attaching and effacing microbial pathogens. J. Clin. Microbiol. 36: 662-668.

Alexander, T. W., Inglis, G. D., Yanke, L. J., Topp, E., Read, R. R., Reuter, T., Mcallister, T. A. 2010. Farm-to-fork characterization of *Escherichia coli* associated with feedlot cattle with a known history of antimicrobial use. Int. J. Food Microbiol. 137: 40-48.

Alonso, M.Z., Lucchesi, P.M.A., Rodriguez, E.M., Parma, A.E., Padola, N.L. 2012. Enteropathogenic and shigatoxigenic *E. coli* in broiler chickens and derived products at different retail stores. J. Food Cont. 23: 351-355.

Azza, H.M., Hassan Noor- El Deen, A.E., Galal, H.M., Sohad, M., Dorgham, B. 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. Vete. 9 (5): 617-62.

Bennani, M., Badri, S., Baibai, T., Oubrim, N., Hassar, M., Cohen, N., Amarouch, H. 2011. First Detection of Shiga Toxin-Producing *Escherichia coli* in Shellfish and Coastal Environments of Morocco. Appl. Biochem. Biotechnol. 165:290–299.

Bielaszewska, M., Zhang, W., Tarr, P.I., Sonntag, A.K., Karch, H. 2005. Molecular profiling and phenotype analysis of *E.coli* O26: H11 and O26: NM: secular and geographic consistency of enteropathogenic and enterohemorrhagic isolates. J. Clin. Microbiol. 43: 4225-4228.

Clinical and Laboratory Standards Institute (CLSI). 2012. Performance standards for antimicrobial susceptibility testing; 23th informational supplement. M100-S23. 33 (1).

Colom, K., Pèrez, J., Alonso, R., Fernández-Aranguiz, A., Lariño,E., Cisterna, R., 2003. Simple and reliable multiplex PCR assay for detection of blaTEM, blaSHV and blaOXA-1 genes in Enterobacteriaceae. FEMS Microbiol. Letters 223 (2): 147-151.

Dipineto, L., Santaniello, A., Fontanella, M., Lagos, K., Fioretti, A., Menna, L.F., 2006. Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens. Letters in Applied Microbiol. 43: 293–295.

Elsaidy, N., Abouelenien, F., Kirrella, G.A.K. 2015. Impact of using raw or fermented manure as fish feed on microbial quality of water and fish. Egypt. J. Aqua. Res. 41: 93–100.

Elsherief, M.F., Mousa, M.M., Abd El-Galil, H., El-Bahy, E.F. 2014. *Enterobacteriaceae* associated with farm fish and retailed ones. Alex. J. Vet. Sci. 42: 99-104.

Galal, H.M., Hakim, A.S., Dorgham, S.M. 2013. Phenotypic and virulence genes screening of *Escherichia coli* strains isolated from different sources in delta Egypt. Life Sci. J. 10 (2): 352-361.

Gupta B, Ghatak, S., Gill, J.P.S. 2013. Incidence and virulence properties of *E. coli* isolated from fresh fish and ready-to-eat fish products. Vet. World 6(1): 5-9.

Harish, R., Sumitha C.M., Hatha, A.A.M. 2003. Prevalence and antibiotic sensitivity of *E.coli* in extensive brakishwater aqua culture ponds. Fish Technol. 40: 8-12.

Hatha A.M., Dhanalakhsmi, M., Smith K. P., Lakshmi P., George, L. 1999. Antibiotic resistance of *E.coli* strains isolated from river water. Poll. Res. 18: 519.

Ibekwe, A.M., Murinda, S.E., Graves, A.K. 2011. Genetic Diversity and Antimicrobial Resistance of *Escherichia coli* from Human and Animal Sources Uncovers Multiple Resistances from Human Sources. PLoS ONE 6 (6): 201-210.

James, C. E., Stanley, K. N., Allison, H. E., Flint, H. J., Stewart, C. S., Sharp, R. J., Saunders, J. R., McCarthy, A. J. 2001. Lytic and lysogenic infection of diverse *Escherichia coli* and Shigella strains with a verotoxigenic bacteriophage. Appl. Environ. Microbiol. 67: 4335–4337.

Jeyasanta, K.I., Aiyamperumal, V., Patterson, J. 2012. Prevalence of Antibiotic Resistant *Escherichia coli* in Sea Foods of Tuticorin Coast, Southeastern India. Adv. Biol. Res. 6 (2): 70-77.

Jiao, S.C., Fami, R.M.L., Pedernal, V.A.D., Cabrera, E.C. 2007. Prevalence of Multiple Drug-Resistant *Escherichia coli* from Chicken, Pig and Nile Tilapia (*Oreochromis nilotica*) Intestines Sold in Wet Markets in Manila and the Conjugative Transferability of the Resistance Antimicrobial Resistant Escherichia coli from Livestock. The Philipp. Agri. Sci. 90 (1) 64-70.

Karch, H., Tarr, P.I., Bielaszewska, M. 2005. Enterohaemorrhagic *E.coli* in Human medicine. Int. J. Med. Microbial. 295: 405-418.

Kromhout, D., Bosschieter, E.B., de Lezenne, C.C. 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med. 312: 1205–1209.

Kumar, H.S., Otta, S.K., Karunasagar, I., Karunasagar, I. 2001. Detection of Shiga-toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR. Lett. Appl. Microbiol. 33: 334-338.

Kumar, H.S., Karunasagar, I., Karunasagar, I., Teizou, T., Shima, K., Yamasaki, S. 2004. Characterization of Shiga toxin-producing Escherichia coli (STEC) isolated from seafood and beef. FEMS Microbiol. Lett. 233: 173–178.

Lateef, A., Oloke, J.K., GueguimKana, E.B., Pacheco, E. 2004. The microbiological quality of ice used to cool drinks and foods in Ogbomoso Metropolis, Southwest, Nigeria. Int. J. Food Safety 8: 39–43.

MacFaddin, J. F. 1985. Media for isolation, cultivation, identification, maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Mellmann, A., Bielaszewska, M., Zimmerhackl, L.B., Prager, R., Harmsen, D., Tschape, H., Karch, H. 2005. Enterohamorrhagic *E.coli* in human infection: in vivo evolution of a bacterial pathogen. Clin. Infect. Dis. 41:785-792.

Musefiu, T. A., Obuko, E. B., Bolarinwa, A.O. 2011. Isolation and identification of aerobic bacteria flora of the skin and stomach of wild and cultured Clarias Gariepinus and *Oreochromis niloticus* from Ibadan, Southwest Nigeria. J. Appl. Sci. Res. 7(7): 1047–1051.

Oswald, E., Schmidt, H., Morabito, S., Karch, H., Marches, O., Caprioli, A. 2000. Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *E.coli*: characterization of a new Intiman variant. Infect. immune. 68: 64-71.

Paton, J.C., Paton, A.W., 1998. Pathogenisis and diagnosis of shiga toxin producing *E.coli* infections. Clin. Microbiol. Rev. 11: 450-479.

Paton, A.W., Paton, J.C. 1998. Detection and characterizations of shigella toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1, stx2, eaeA*, enterohemorrhagic *E.coli hlyA*, rfbO111 and rfbO157. J. Clin. Microbiol. 36: 598- 602.

Paton, A.W., Paton, J.C. 2002. Direct detection and characterization of shiga toxigenic *E.coli* by multiplex PCR for *stx1, stx2, eae, ehxa* and *saa*. J. Clin. Microbiol. 40: 271-274.

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J.V., Woodward, M.J. 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of Salmonella enterica isolated from humans and animals in the UK. J. Antimicrob. Chemotherap. 53: 208–216.

Rocha, R., Leite,L., Sousa,O., Vieira,R. 2014. Antimicrobial Susceptibility of *Escherichia coli* Isolated from Fresh-Marketed Nile Tilapia (*Oreochromis niloticus*). Brazil. J. Pathol. 1-5.

Ryu, S.H., Park, S.G., Choi, S.M., Hwang, Y.O., Ham, H.J., Kim, S.U., Lee, Y.K., Kim, M.S., Park, G.Y., Kim, K.S.,Chae, Y.Z., 2012. Antimicrobial resistance and resistance genes in *Escherichia coli* strains isolated from commercial fish and seafood. Inter. J. Food Microbiol. 152: 14-18.

Shambrook, J., Fritscgh, E.F., Mentiates. 1989. Molecular coloning. A laboratory manual. Vol.1 Cold spring Harbor Laboratotry press, New York.

Schroeder, C.M., Meng J., Zhao, S. 2002. Antimicrobial resistance of *Escherichia coli* O26, O103, O111, O128 and O145 from animals and humans. Emerg. Infect. Dis. 8: 1409-1414.

Sonntag, A.K., Prager, R., Bielaszewska, M., Zhang,W., Fruth, A., Tschape, H., Karch, H. 2004. Phenotypic and genotypic analysis of enterohamorrhagic *E.coli* O145 strains from patients in Germany. J. Clin. Microbial. 42: 954-962.

Tiamiyu, A.M., Soladoye, M.O., Adegboyega, T.T., Adetona, M.O. 2015. Occurrence and Antibiotic Sensitivity of Bacterial Strains Isolated from Nile Tilapia, Oreochromis niloticus Obtained in Ibadan, Southwest Nigeria. J. Biosci. Med. 3: 19-26.

Van den Bogaard, A.E., Stobberingh, E.S. 1999. Antibiotic usage in animals: impact on bacteria resistance and public health. Drugs 58: 589-609.

Walker, R. A., Lindsay, E., Woodward, M. J., Ward, L.R., Threlfall, E.J. 2001. Variation in clonality and antibiotic-resistance genes among multi-resistant Salmonella enterica serotype Typhimurium phage-type U302 (MR U302) from humans, animals, and foods. Microbial Drug Resist.7 (1):13–21.

Wang, F., Jiang, L., Yang, Q., Han, F., Chen, S., Pu, S., Vance, A., De, B. 2011. Prevalence and antimicrobial susceptibility of major foodborne pathogens in imported seafood. J. Food Prot. 74: 1451- 1461.

Wen-jie, J., Zhi-ming, Z., Yong-zhi, Z., Ai-jian, Q., Hong-xia, S., Yue-long, L., Jiao, W., Qian-qian, W., 2008. Distribution of Virulence-Associated Genes of Avian Pathogenic *Escherichiacoli* Isolates in China. Agricultural Sciences in China 7(12):1511-1515.

Zhang,W., Mellmann,A., Sonntag,A.K., Bielaszewska, M., Tschape,H., Karch, H., Wieler, L.H., Friedrich, A.W. 2007. Structural and functional difference between disease- associated genes of enterohaemorrhagic *E.coli* O111. Int. J. Med. Microbial. 297: 17-26.