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MOLECULAR IDENTIFICATION OF E. COLI AND SALMONELLA SPECIES ISOLATED FROM FRESH WATER FISH BY POLYMERASE CHAIN REACTIONS (PCR)

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ABSTRACT

*Bacteriological examination of 400 fish samples taken from **Oreochromis niloticus (O. niloticus)**, **Clarias gariepinus**, **Cyprinus carpio**.l **(Common carp)** and **Mugil cephalus** (100 from each) revealed isolation of (317) bacterial isolates, which were identified as 171 **E.coli** isolates and 146 **Salmonella sp** isolates with an incidence of (53.94% and 46.06 %) respectively. The incidence of **E.coli** and **Salmonella spp**. differ among different species of fish under investigation, the higher percentage of bacterial isolates observed in Clarias garipinas (53.31%), followed by (29.97 %), **Cyprinus carpio**.l (11.99 %), and the lower incidence was observed in Mugil cephalus (4.73%). Frequency distribution of isolated bacteria from the surface lesions and internal organs of examined fish and the antibacterial susceptibility to different chemotherapeutic agents were discussed. PCR based technique was used in identification of **E. coli** and **Salmonella sp**., the results showed that E. coli O26 & O111 were positive strains for stx1, stx2 and eaeA genes, E. coli O55 was positive strain for stx2 and eaeA genes, **E. coli** O91 & O103 were positive strains for stx1 and stx2 genes, **E. coli** O119 was positive strain for stx2 gene, **E. coli** O124 was positive strain for eaeA gene and E. coli O128 was positive strain for stx1 gene. In case of **Salmonella sp, S. Typhimurium** strain was positive for stn, invA and hilA genes, **S. Enteritidis** strains was positive for stn, invA and hilA genes, **S. Virchow** strain was positive for invA gene, **S. Typhi** strain was positive for invA and hilA genes and **S. Infantis** strain was positive for invA genes.*

INTRODUCTION

Fish has become an important protein resource in Egypt to meet the gap between low production and a rapidly expanding human population **(Areej., 2014) .** Fish is susceptible to wide variety of bacterial pathogens which are responsible for many diseases and higher mortality rate **(Yooyen et al., 2006)**.

Soliman et al., (2010) showed that, ***E. coli*** strains O55, O148, O157 and O125 were isolated from ***Oreochromis niloticus*, *Clarias gariepinus*, *common carp*** and ***Mugil cephalus*** fish samples.

Fatin et al., (2014) declared that 12 isolates of ***E.coli*** were isolated from examined fried seafood represented as 38.6% from the ***M. cephalus*** with serotypes O55:H7 2.9%, O125:H18, 2.9% & untypable 2.9%, 25.7% Saurus with serotype O55:H7 only, 411.4% Sepia with serotypes O55:H7 5.7% & O125:H18 5.7%, 38.6% from Shrimp with serotypes O55:H7 5.7% & untypable 2.9%.

Mai et al., (2014) isolated 46 ***Enterobacteriaceae*** strains from 50 ***Mugil cephalus*** with a percentage of 92% the most dominants isolated strains were ***Salmonella sp.*** and ***E. coli.***

The emergence of antibiotic resistance in bacteria that may be encouraged by extra use of antibiotics in fish and animal. The problem concerns bacteria carrying different resistance mechanisms can directly cause an undistinguished carrier state and may cause and implicate accretion to infections that are not recognized **(Ajamaluddin et al., 2000)**. **Soliman et al., (2010)** indicated that, the sensitivity of ***E. coli*** isolated from fish samples were highly to Enrofloxacine, Oxanilic acid and spectinomycine and low sensitive to Erythromycin, Chloramphenicol. (**Efuntoye, et al., 2012**) recorded that ***E. coli*** strains were highly resistant to ampicillin, chloramphenicol and oxytetracycline (82.4%) and sensitive to ciprofloxacin, novobiocin and ofloxacin. Concerning with ***Salmonella spp.*** antimicrobial resistance is a global public health concern and the propagation of antimicrobial resistance between food-borne pathogens incline within recent decades (**Akbar and Anal, 2014**). (**Efuntoye, et al., 2012**) concluded that most of the ***Salmonella sp***. were not sensitive to erythromycin (85.7%), gentamycin (71.4%), amoxicillin (57.1%), chloramphenicol (57.1%) and sulphamethoxazole (57.1%), and highly sensitive to ciprofloxacin, novobiocin and ofloxacin.

The present study was aimed to isolation and identification of ***E. coli*****,** ***Salmonella sp.*** from fresh water fish in Egyptian cultured fish, identification of ***E. coli*** and ***Salmonella sp.*** by Polymerase Chain Reaction (PCR) and carrying out antibiotic sensitivity test to evaluate the most effective antibiotic against isolated bacteria.

MATERIALS AND METHODS

- **Samples**

A total number of 400 samples taken from *Oreochromis niloticus*, *Clarias garipinas, Common carp and Mugil cephalus* fish. (100 for each) were collected alive and examined freshly from different Delta governorates in Egypt. Fish samples were transported to the bacteriology laboratory as soon as possible under complete aseptic condition. Samples from skin, internal organs (liver, kidneys and spleen) and muscles were inoculated into nutrient broth, tryptic soy broth and tetrathionate broth for propagation of bacteria and incubated at 25°C - 27°C for 24-48 hours, then streaked on specific media.

Isolation and Identification:- Isolation and Identification of ***E. coli*** and ***Salmonella sp.*** was carried out according to **Cruickshank et al., (1975**) and **Collee, et al., (1996).**

Serodiagnosis of *E.coli*: According to **Kok, et al. (1996).**By using rapid diagnostics *E.coli* antisera sets **(DENKA SEIKEN Co., Japan)** for detection of the Enteropathogenic types.

Serological identification of Salmonellae: According to Kauffman – White scheme **(Kauffman, 1974)**: The identification of Somatic (O) antigens was done using Salmonella antiserum **(DENKA SEIKEN Co., Japan).**

Antimicrobial susceptibility testing: It was performed by disk diffusion method after **NCCLS 2001** using Mueller-Hinton (MH) agar and Mueller-Hinton broth (Oxoid, Basingstoke, U.K.).

Polymerase Chain Reaction (PCR) for identification of *E. coli* and ***Salmonella sp*.**

Reagents used for agrarose gel electrophoresis: Agarose powder,

Biotechnology grade (Bioshop^R, Canda inc. lot No: OE16323). It prepared in concentration 2% in 1× TAE buffer.

Tris acetate EDTA (TAE) electrophoresis buffer (50 × liquid concentration) Bioshop^R , Canda inc. lot No: 9E11854. The solution diluted to 1× by adding 1 ml stock solution to 49 ml dist. Water to be used in the preparation of the gel or as a running buffer. **Ethedium bromide solution (stock solution) biotechnology grade (Bioshop ® Canda Inc, Lot No: 0A14667):** The stock solution was diluted by 25µl /200ml double distilled water and stored covered at 4ºC. It was used for staining of PCR products that electrophoreses on agarose gel to be visualized by UV light.

Gel loading buffer (6×stock solution) (Fermentas, lot No: ooo56239). The components were dissolved in sterile double distilled water and stored covered with aluminum foil at room temperature.

DNA ladder (molecular marker) 100 bp **(Fermentas, lot No: 00052518).**

5X Taq master (Fermentas): Containing polymerase enzyme, Magnesium chloride (Mg Cl_2), Deoxy nucleotide triphosphate (dNTP) and PCR grade water.

Sequences of *E.coli* primers used for PCR identification: by using the following primers (Pharmacia Biotech) as shown in Table (1).

DNA Extraction using QIA amplification kit (Shah et al., 2009):

After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100ºC for 20 minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and the following steps were carried out: Equal volume from the lysate (50-200 µl) was added, addition of 20-50µl of proteinase K, then incubation at 56 ºC for 20-30 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The solution was added to the column and centrifuged at 8000 rpm for 1 min. then the filtrate was discarded. The sediment was washed using AW1 buffer (200 µl), the column was centrifuged at 8000 rpm / 1 min, and the filtrate was discarded. Washing was applied by using the AW2 buffer (200µl), the column was centrifuged at 8000 rpm / 1 min. and the filtrate was discarded. The column was placed in a new clean tube then, 25-50 µl from the Elution buffer was added, centrifuged at 8000 rpm/1min. Then the column was discarded. The filtrate was put in clean tube containing the pure genomic DNA. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Amplification reaction of *E. coli* (Fagan et al., 1999):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out in 1 ml of nucleic acid template prepared by using reference EHEC isolates (approximately 30 ng of DNA), 10 mM Tris-HCl (pH 8.4), 2 mM concentrations of each primer, 0.2 mM concentrations of each 29-deoxynucleoside 59-triphosphate, and 4 U of AmpliTaq DNA polymerase (Perkin-Elmer). Amplification conditions consisted of an initial 95°C denaturation step for 3 min followed by 35 cycles of 95°C for 20 sec, 58°C for 40 s, and 72°C for 90 sec. Final cycle was followed by 72°C incubation for 5 min. The reference strains were *E. coli* O157:H7 Sakai (positive for stx1, stx2, eaeA and hylA) and *E. coli* K12DH5α (a nonpathogenic negative control strain) that does not possess any virulence gene (kindly from Prof. Mohamed Hassan, Fac. Vet. Med., Benha Univ.). Amplified DNA

fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

Primer sequences of Salmonella species used for PCR identification system:

The demonstration of virulence factors including invasion A (invA), Enterotoxin (stn) and hyper-invasive locus genes of the isolates.

DNA Extraction using QIA amplification kit (Menghistu, 2010) :

After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water. Suspension was heated at 100ºC for 20 minutes, 50-200 µl of the culture were placed in Eppendorf tube and stored at -20°C till use.

DNA amplification for the selected virulent genes (Singh et al., 2013):

The reaction mix consiste of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase (it contain 1.5 mM MgCl2, 2 µl of 10mM dNTP mix), 1 µl each of forward and reverse primer (10 pmol), 1.25 U of Taq DNA polymerase and up to 50 µl nuclease free water. The PCR cycling protocol was applied as following: An initial denaturation at 94°C for 60 sec, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 64°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 7 min. Finally, 5 µl of each amplicon was electrophoresed in 1.5 % agrose gel (Sigma – USA, stained with ethidium bromide and visualized and captured on UV transilluminator. A 100 bp DNA ladder was used as a marker for PCR products.

Table (1) : Primer used for *E.coli* identification by PCR.

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	**5′** ACACTGGATGATCTCAGTGG **′3**	614	**Dhanashree and Mallya (2008)**
Stx1 (R)	**5′** CTGAATCCCCCTCCATTATG **′3**		
Stx2 (F)	**5′** CCATGACAACGGACAGCAGTT **′3**	779	**Dhanashree and Mallya (2008)**
Stx2 (R)	**5′** CCTGTCAACTGAGCAGCACTTTG **′3**		
eaeA (F)	**5′** GTGGCGAATACTGGCGAGACT **′3**	890	**Mazaheri et al. (2014)**
eaeA (R)	**5′** CCCCATTCTTTTTCACCGTCG **′3**		

Table (2) Salmonella species was carried out using the following primers:

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
invA (F)	**5′** GTGAAATTATCGCCACGTTCGGGCA **′3**	284	**Shanmugasamy et al. (2011)**
invA (R)	**5′** TCATCGCACCGTCAAAGGAACC **′3**		
Stn (F)	**5′** CTTTGGTCGTAAAATAAGGCG **′3**	260	**Makino et al. (1999)**
stn (R)	**5′** TGCCCAAAGCAGAGAGATTC **′3**		
hilA (F)	**5′** CTGCCGCAGTGTTAAGGATA **′3**	497	**Guo et al. (2000)**
hilA (R)	**5′** CTGTCGCCTTAATCGCATGT **′3**		

Table (3): Incidences of of *E.coli* isolates and *Salmonella spp* among different fish species.

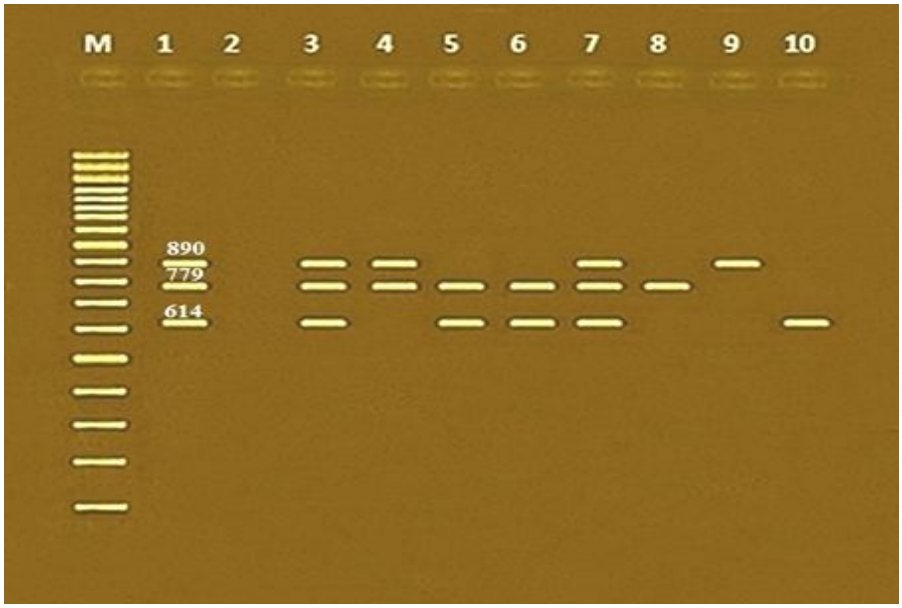
Bacterial isolates / Fish species	N	*E. coli*	*Salmonella spp.*	Total	%
O. Niloticus	100	57	38	95	29.97
Clarias garipinas	100	91	78	169	53.31
Common carp	100	18	20	38	11.99
Mugil cephalus	100	5	10	15	4.73
Total	400	171	146	317	100

Table (4): Frequency distribution of *E. coli* and *Salmonella spp.* isolated from surface lesions and internal organs among different fish species.

Bacterial isolates / Fish species	*E. coli* (171)		*Salmonella spp.*(146)	
	No		No	
	Ex	In	Ex	In
O. Niloticus	7	50	0	38
Clarias garipinas	23	68	21	57
Common carp	3	15	0	20
Mugil cephalus	0	5	3	7
Total	33	138	24	122
%	19.30	80.70	16.44	83.56

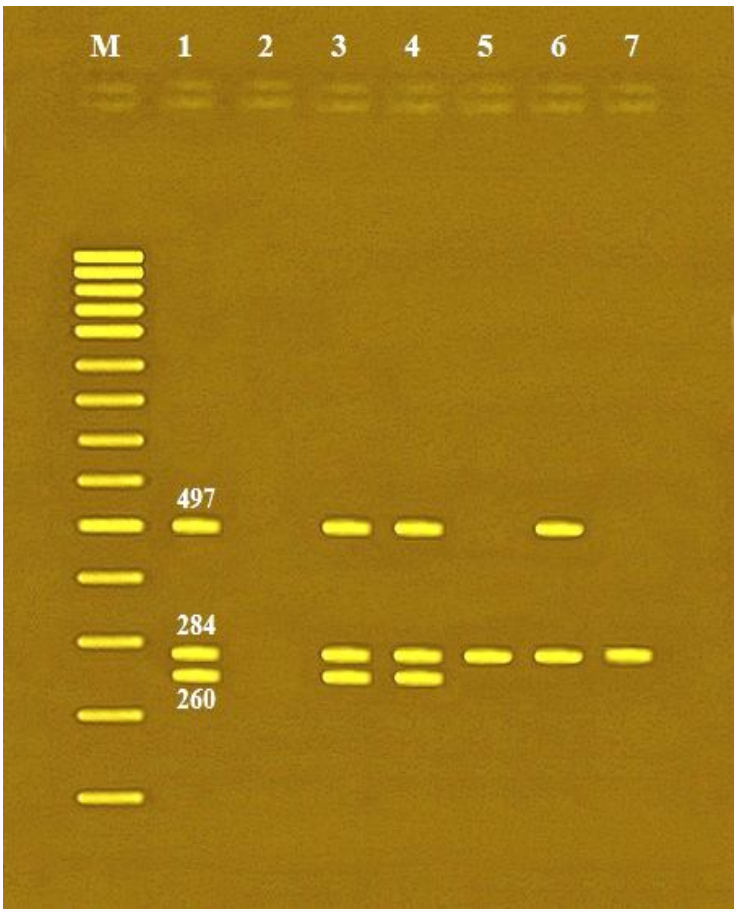
Table (5): Percentages of Antimicrobial susceptibility of *E. coli* and *Salmonella* strains isolated from the examined samples of fish.

Chemotherapeutic agents	*E.coli* (n=8)						*Salmonella spp.*(n=5)					
	S		I		R		S		I		R	
	No	%	No	%	No	%	No	%	No	%	No	%
Penicillin (P)	-	-	-	-	8	100	-	-	1	20	4	80
Erythromycin (E)	-	-	-	-	8	100	-	-	1	20	4	80
Amoxicillin(AMX)	-	-	1	12.5	7	87.5	-	-	1	20	4	80
Streptomycin (S)	-	-	2	25.0	6	75.0	-	-	-	-	5	100
Ampicillin (AM)	1	12.5	1	12.5	6	75.0	1	20	-	-	4	80
Sulphamethoxazol(SXT)	-	-	3	37.5	5	62.5	1	20	1	20	3	60
Nalidixic acid (NA)	1	12.5	2	25.0	5	62.5	1	20	1	20	3	60
Norfloxacin (NOR)	2	25.0	1	12.5	5	62.5	2	40	1	20	2	40
Oxytetracycline (T)	3	37.5	1	12.5	4	50.0	2	40	-	-	3	60
Ciprofloxacin (CP)	2	25.0	3	37.5	3	37.5	4	80	-	-	1	20
Neomycin (N)	4	50.0	1	12.5	3	37.5	2	40	-	-	3	60
Chloramphenicol (C)	4	50.0	2	25.0	2	25.0	1	20	2	40	2	40
Kanamycin (K)	5	62.5	2	25.0	1	12.5	-	-	3	60	2	40
Gentamicin (G)	6	75.0	-	-	1	12.5	3	60	1	20	1	20



Photograph (1): Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp) and eaeA (890 bp) genes for characterization of *Enteropathogenic E. coli.*

Lane M: 100 bp ladder as molecular size DNA marker.
Lane 1: Control positive *E. coli* for stx1, stx2 and eaeA genes.
Lane 2: Control negative.
Lanes 3 & 7 (*E. coli* O26 & O111): Positive strains for stx1, stx2 and eaeA genes.
Lane 4 (*E. coli* O55): Positive strain for stx2 and eaeA genes.
Lanes 5 & 6 (*E. coli* O91 & O103): Positive strains for stx1 and stx2 genes.
Lane 8 (*E. coli* O119): Positive strain for stx2 gene.
Lane 9 (*E. coli* O124): Positive strain for eaeA gene.
Lane 10 (*E. coli* O128): Positive strain for stx1 gene.



Photograph (2): Agarose gel electrophoresis of multiplex PCR of stn(260 bp), invA (284 bp) and hilA (497 bp) virulent genes for characterization of Salmonella strains isolated from Nile Delta fish species.

Lane M: 100 bp ladder as molecular size DNA marker.

Lane 1: Control positive *S. Typhimurium* for stn, invA and hilA genes.

Lane 2: Control negative.

Lane 3: Positive *S. Typhimurium* strain for stn, invA and hilA genes.

Lanes 4: Positive *S. Enteritidis* strains for stn, invA and hilA genes.

Lane 5: Positive *S. Virchow* strain for invA gene.

Lane 6: Positive *S. Typhi* strain for invA and hilA genes.

Lane 6: Positive *S. Infantis* strain for invA genes.

RESULTS & DISCUSSION

Clinical signs and postmortem findings. The most common clinical signs and post-mortum lesion were scale loss, darkness coloration of the skin, ascites, exophthalmia and tail rot, hemorrhagic patches all over the body, while the postmortem findings revealed ascetic fluid in the abdominal cavitie, liver was congested and friable, spleen and kidneys were enlarged, similar findings was recorded by, **(Enany et al,. (2011), Aya (2013) and Ismail, et al.,(2013).**

bacteriological examination of 400 samples obtained from organs (kidney ,liver and spleen) and muscle of *O. niloticus* , *Clarias garipinas*, *Common carp* and *Mugil cephalus* fish reveled isolation of (317) bacterial isolates which were identified as 171 ***E.coli*** isolates and 146 ***Salmonella spp*** isolates with an incidence of (53.94% and 46.06 %) respectively**,** Soliman et al., (2010) isolated *E.coli* from (42,86%) of (***O. niloticus***, ***Clarias gariepinus***, *Common carp* and *Mugil cephalus*) fish samples. In case of *Salmonella Spp.* (24.95 %), **Elhadi (2014)** isolated *Salmonella Spp.* from (***O. niloticus and Clarias gariepinus***) at rates of (66.7% and 60.0%) respectively, but **Sebnem et al., (2011)** isolated *Salmonella Spp.* from (3%) of carp fish also **Akinyemi and Ajisafe (2011)** isolated *Salmonella Spp.* from (11%) of *Mugil cephalus.*

The incidences of *E.coli* isolates and *Salmonella spp* among different fish species under examination table (3) cleared that the higher percentage of bacterial isolates observed in *Clarias garipinas* (53.31%), followed by *O. niloticus* (29.97 %), *Common carp* (11.99 %), and the lower one was observed in *Mugil cephalus* (4.73%), isolation of members of family Enterobacteriaceae are considered as means indicating enteric contamination of fish and water. **(Valdivia et al., 1997) and Petronillah et al., (2014)**.

Ward and Baj, (1988), Wang et al., (1994) Rajkowski, (2012) and Sheen et al., (2012) concluded that freshwater fish have a very different microbiological characters due to environment, methods of catching on board, handling, fishing vessels, sanitation and processing, the farms which depends on the use of the manure in fish farms usually increases the bacterial number causing zoonotic problems.

According to the site of examination (table 4) *E. coli* incidence reached (7 %) in the external surface samples and reached (50 %) in the internal organs samples, **El –Olemy et al.,(2014)** isolated *E. coli* from the surface of (1%) ***O. niloticus*** fish samples but no isolates were obtained from internal organs, while *Salmonella Spp.* incidence in the external surface was (0 %) in the examined samples and reached to (38%) from the internal organs**. El –Olemy et al.,(2014)** recoded that *Salmonella spp.* were isolated from the external surface and internal organs at the same percentage (4%). **Mai et al.,(2014)** recorded that *E. coli* incidence in the external surface samples and internal organs were (2% and 1%) respectively while **Udeze et al., (2012)** recorded that *E. coli* was isolated from the intestine of ***Clarias garipinas*** but not isolated from the surface. The incidences of *Salmonella Spp.* in external surface reached (21 %) while from the internal organs samples reached (57 %), **Mai et al.,(2014)** recorded it (8% and 7%). The incidence of *E. coli* incidence isolated from ***Common carp*** fish samples reached (3 %) in the external surface samples and reached (15 %) in the internal organs samples, **Eman (2008)** recorded that *E. coli* was isolated from external surface and internal organs at percentage of (0% and 13.33

%) respectively. while, ***Salmonella spp.*** incidence was (0 %) in the external samples surface and (20 %) from the internal organs samples.

The incidences of *E. coli* and *Salmonella spp* isolated from the external surface of *Mugil cephalus* were (0 % and 3%) and from the internal organs were (5 % and 7%), respectively, **Mai et al., (2014)** isolated *E. coli* and *Salmonella spp.* from muscles of *Mugil cephalus* at a rate of (8% and 4%) respectively.

Results of P C R achieved in photo. (1) which illustrated that STEC isolated from examined Nile Delta fish species prove to have virulence genes. The use of Multiplex PCR with specific primers for Stx1, Stx2 and eae genes revealed the presence or absence of such genes in the tested isolates. The obtained results showed that *E. coli* serovars O26 and O111 (EHEC) had the 3 virulence genes, however, O91 and O111 (EHEC) both Stx1 and Stx2 genes. *E. coli* O55 proved to have Stx2 and eae genes while, *E. coli* O119 had only Stx2 and O128 had only Stx1. In contrast, *E. coli* O124 had no any of such virulence genes. PCR was more sensitive, more accurate and rapid for bacterial isolation in freshly isolated bacteria as subculturing of slopes for different times may lead to missing of virulence genes on bacterial plasmid lead to false negative result in PCR **(Moalic et al., 1997).** Shiga toxin producing *E. coli* (STEC) of different serovars have been isolated from human, fish and apparently healthy domestic animals. Many of those isolates were typical STEC belonging to serovars O26, O111 and O157 **(Karamali, 1989)**.

Occurrence of virulence genes of ***Salmonella*** strains isolated from the from the examined Nile Delta fish species is shown in photo. (2). Accurately, PCR indicated that both *S. Enteritidis* and *S. Typhimurium* had all virulence genes represented by stn, invA and hilA. *Salmonella Typhi* had invA and hilA, however, *S. Infantis* had stn and invA genes. While, *S. Virchow* had only invA virulent gene. In other words, invA gene was detected in all investigated Salmonella strains.

Salmonella serovars differed in the virulence among having been attributed to the variable evolvement acquisition of the virulence genes **(Falkow, 1996)**. The genes identified from the ***Salmonella*** specific virulence genes which toke an important role in the pathogenicity of it have been identified. On a shorter scale 80 different virulence genes have been detected in ***Typhimurium*** serovar. Some genes are responsible for being involved in adhesion and invasion as hilA **(Guo, et al., 2000)**, invA **(Shanmugasamy et al., 2011)** and other similar genes like stn were associated with toxin production **(Makino et al., 1999)**.

Out of (146) ***Salmonella*** isolates, 5 isolates were serologically identified and the results showed that ***S.typhimurium***, ***S. enteritidies, S. virshow, S. typhi*** and S.infantis were detected. The five strains were examined for enterotoxin genes by PCR and the results was cleared in photo (8).

Concerning the percentages of antimicrobial susceptibility of *E. coli* and ***Salmonella*** strains, tables (5), rise of antimicrobial resistance in *E. coli* to multiple antibiotics is a major concern both in developed and developing countries. This finding may be due to *E. coli* was Gram-negative bacteria that produced B -lactamase enzyme that breakdown the B- lactam ring of penicillins **(Chandran et al., 2008)**. Concerning with ***Salmonella*** strains, tables (5) indicated that all the isolated Salmonella serotypes were resistant to Streptomycin (100%) followed by Amoxicillin, Penicillin and Erythromycin (80% of each). However, Ciprofloxacin, Gentamicin and Norfloxacin

had great effect on the isolated strains of Salmonella by percentages of 80%, 60%, and 40%, respectively. Also, the MAR index of the isolated Salmonella strains was 0.586.

Antimicrobial resistance is a global public health concern and the propagation of antimicrobial resistance between food-borne pathogens incline within recent decades (**Akbar and Anal, 2014)**.

P.C.R. results and discussion:-

Out of (171) ***E.coli*** isolates, 8 isolates were serologically identified and the results showed that O26, O111, O91, O103, O119, O124 and O128 were detected. The eight strains, were examined for enterotoxin genes by PCR and the results was cleared in photo (1).

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الملخص العربي

التصنيف الجزيئي لميكروب الإيشرشيا كولاي والسالمونيلا المعزولة من أسماك المياه العذبة باستخدام تفاعلات البلمرة المتسلسل

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عند اجراء الفحص البكتيريولوجي لعدد (٤٠٠) من انواع الاسماك (اسماك البلطي و اسماك القرموط و المبروك والبوري) ١٠٠ عينة من كل نوع . تم عزل ٣١٧ عزله بكنيريه منها ١٧١الميكروب القولوني و١٤٦ لميكروبات السالمونيلا بنسبة ٥٣،٩٤ و٤٦،٠٦ % على التوالى واختلفت نسبة العزل من انواع الأسماك حيث كانت اعلى نسبة عزل لتلك االبكتريا من اسماك القرموط (٥٣،٣١%) يتبعها البلطي (٢٩،٩٧) ثم اسماك المبروك بنسبة (١١،٩٩) وكان اقلهم اسماك البوري بنسبة (٤،٧٣%).تم مناقشة نسبة عزل و تواجد تلك الباكتريا على السطح الخارجى وداخليا وكذالك مقاومة الميكروبات المعزوله للعديد من المعالجات الدوائيه .وعند اجراء اختبار تفاعل البلمرة المتسلسل لتصنيف كل من الميكروب القولوني و ميكروبات السلمونيلا وجد ان عترات الميكروب القولوني المصنف سيرولوجيا o111 , o26 كان كل منهم يحتوي علي stx2 ,stx1 و eaeA في حين كانت عترات الميكروب القولوني سلالة O55 تحتوي علي جينات ، stx2 و eaeA وكانت عترات الميكروب القولوني سلالة O91, O 103 تحتوي علي جينات stx2 ,stx1 و كانت سلالة O119 تحتوي علي جين stx2 و كانت السلالة O124 موجبه لجين ال eaeA و كانت سلالة الميكروب القولوني O128 تحتوي علي جين stx1، فقط. وبتصنيف ميكروبات السالمونيلا كانت ميكروبات سالمونيلا تيفينيوريا و سلالات السلمونيلا انترتيتز تحتوي علي جينات ال invA , stn ، و hilA و كانت ميكروبات السلمونيلا فيرشو تحتوي علي جين invA في حين كانت ميكروبات السلمونيلا تيفي تحتوي علي جينات invA و hilA و كانت ميكروبات السلمونيلا انفانتس تحتوي علي جين invA .