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Isolation and Identification of *E. coli* from Cultured Freshwater Fish

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ABSTRACT

The present study carried out to isolate different species of ***E. coli*** and identifying it by different methods and investigate the clinical signs, postmortem findings. The higher isolates of ***E. coli*** obtained from intestine and liver. The results obtained during the course of ***E. coli*** incidence cleared that the high organic matter and un-ionized ammonia (NH_3) can affect the incidence and pathogenicity of ***E. coli*** . The Antibiogram test indicated that, the sensitivity of isolated ***E. coli*** to different antibiotics that, the antibiotics of high effect on ***E. coli*** were Enrofloxacine, Oxanilic acid and spectinomycine and the least effect include Erythromycin, Chloramphenicol. Also, our results indicated that, the most important ***E. coli*** isolates, that isolated from +ve fish samples to ***E. coli*** were O55, O148, O157 and O125.

Keywords:

INTRODUCTION

Escherichia coli (*E. coli*) in fish are considered as an indicator of potential sewage pollution. Levels of it are used to determine whether local beaches should be posted with "no water contact" advisories. There are a variety of types of *E. coli.* Hanson et al (2008).

E. coli is a bacterium that commonly lives in the intestine of people, animal and fish. There are many strains (types) of *E. coli*. Most of the *E. coli* are normal inhabitants in the small intestine and colon and are non-pathogenic, meaning they do not cause disease in the intestine. Nevertheless, these non-pathogenic *E. coli* can cause disease if they spread outside the intestine. The pathogenic strains of *E. coli* may cause diarrhea by producing and releasing toxins (called enterotoxigenic *E. coli* or ETEC) and cause of food in fish *(Lee and Marks, 2009)*.

- The aim of this study was :

1. Determination of the prevalence rate and virulence character associated with *E. coli* isolates in cultured freshwater fish.

2. Isolation and characterization of *E. coli* isolates from cultured freshwater fish via Biochemical tests and serotyping.
3. Characterization of *E. coli* isolates using pathogenicity test and calculation of LD_{50} and recording clinical signs, P.M lesions with testing of Antibiogram of different antibiotics .

MATERIALS AND METHODS

Materials

1- Naturally collected fish for isolation of E. coli

We collect about 350 samples of fish species summarized as; *Clarias garipinas* (*Catfish*) (95 fish), *Oreochromis niloticus* (73 fish), (common carp 45 fish and silver carp 30 fish), *Mugil cephalus* (50 fish), *Mugil Capito* (57 fish). These fish were obtained from different farms from different governorates, El-Behera (50), Kafr El-Sheikh (100) and Damiette (200) at different seasons. The fish were exposed to bacteriological isolation and other many diagnostics media . Nutrient agar, Trypticase Soya broth and trypticase soya agar and MacConkey's agar.

2- Fish for experimentally infection

A total number of 560 apparently healthy fish *Oreochromis niloticus*, were obtained from private fish farm. Fish were transported a live to the laboratory Departement of poultry and fish disease of Faculty of veterinary medicine, Alexandria Univeristy in plastic bags containing water enriched by air (2/3).Average body weight of fish about (50 ± 5 gm).

3- Fish pathogens

a. Bacteria used for preparation of bacterin and challenge.

b. *Candida albicans* used for phagocytes was kindely provided by Poultry and fish diseases Dep. Fac. Vet. Med. Alex. Univ.

4- Water samples

Water samples were collected from each farm during fish samples collection . the water samples were transferred to the laboratory of microbiology, Fac. Vet. Med. Alex. Univ. and examined physically and chemically.

5- Media

A. Media used for isolation of bacteria

Nutrient medium (Buinn ***et al.,*** 1994) : It used for incubation of sample before their inculation into solid media.

MacConkey's agar (Oxoid, 1987) : It used as selective medium for isolation of *E. coli* as a members of family Enterobacteriaceae.

Methods

1) Bacterial culturing of samples

The samples collected were examined externally for any lesion and post mortoum was done . The inoculi were taken from intestine, kidney and spleen and cultured on agar media and Eosin methylene blue agar media (EMB) for purification and colonical morophology .

The typical growing isolated colonies were picked up and subcultured on slobe agar slants and incubated at 25 ^{o}C for 24 hrs. The growing isolates were used as stocks for further identifications morphologically and biochemically and random isolates were further identified serologically.

2) Identification of E. coli isolates

The bacterial isolates were subjected to characterization by studying there morphology . Cultural and biochemical characteristics as well as their motility as follows :

A. Morphologiacl characterization

Films were prepared from the suspected purified isolates and stained with Gram's stain then examined microscopically (Cruickshank,et al., 1979).

B. Culture characterization

The colonial morphology into MacConkey's agar and eosin methyle blue agar media were studied .

C. Haemolysis assay

E. coli isolated were streaked on blood agar medium supplemented with 5% washed sheep erythrocytes blood agar plates then incubated at 37 ^{o}C for 24 hrs. and colonies producing clear zones of haemolysis was then recorded as haemolysis positive (Heller and Drabkin; 1979) .

D. Detection of motility

Isolates were stabbed into tubes containing semi-soild nutrient agar medium and then incubated at 37 ^{o}C for 24 hrs. The inocubated tubes were examined for detecting motility of inoculated isolates . Then preserved the refrigerator at 4 ^{o}C .

3) Biochemical characterization

Bacteria were identified according to Quinn *et al.,* (2002) .

4) Antibiotic sensitivity test

The isolated ***E. coli*** were subjected to antibiotic . Test using 8 different method recommended by the national committee for clinical laboratory standard, (N.C.C.L.), subcommittee on antimicrobial suspectability testing (1979) .

With a sterile wire loops, the tops of four or five isolated colonies of

a similar morphologie type were transferred to a tube containing 4 to 5ml of nutrient broth medium . The broth was incubated at 37 ^{o}C for 24 hrs.

A Mueller Hinton agar was inoculated wih 10^{8} organisms/ml equivalent to a Mac for land 05 turbidity standard of each tested ***E. coli***, on the surface of plate .

The plates were allowed to dry before applying the antibiotic discs were distributed on the surface of plates the inoculated plates were incubated at 37 ^{o}C for 24hrs.

Plates were examined for presence of inhibition zones of bacterial growth around the antibiotic disc, indicated the susceplability of isolated strains to these antibiotic discs and diameter of inhibition zones were measured and interpreted .

Absence of inhibition zones around after antibiotic disc indicated resistance of the isolated organisms to these antibiotics .

5) Serological identification of E.coli isolates

Eight isolates, from eighty six isolates that were confirmed biochemically to be *E.coli,* were subjected to serological identification according to Ewing (1986*)* using slide agglutination test using somatic (O) antiserum with heat inactivated bacteria .

6) Factor of pathogenicity

a. Haemolytic activity

Each isolate of *E. coli* was tested for haemolysis by culturing on 5% sheep blood agar for 24 hrs. at 37 ^{o}C .

b. Invasiveness assay

The ability of *E. coli* isolates to invade epithelial cells was tested . The rabbit eye model (Sereny test) "Boiley and Scolt (1990)" . A suspension of tested organism was dropped within the conjungtival sac of a white NewZealand rabbit.

c. Aerobic cultivation of E. coli

Inoculation of *E. coli* in nutrient broth as enriched media and incubated at 37C for overnight after incubation a *loopfule* from each incubated tube was inoculated on solid media, the inoculated plates was incubated at 37C for 24-48hrs. aerobically, at the end of incubation the plates were examined for the typical characteristic colonies of *E. coli*, which were taken and exposed for purification and studying of culture characteristics.

7) Examination of water collected sample

were analyzed using the salicylate method and diazolization method, respectively, using the Hach

DREL 2400 portable water quality laboratory (Rowland; 1995).

RESULTS

1. Results of naturally infected fish :

Table (1): *Showed the locality of sampling:*

Fish farms	Number of samples
Domitte	200
El-Behera	50
Kafr El-Sheikh	100

2. Type of fish :

Table (2): *Showed the different species of examined fish* :

Type of fish	No. of samples	Apparently healthy	Diseased
Catfish	**95**	**35**	**60**
Nile Tilapia	**73**	**28**	**45**
Common Carp	**75**	**24**	**51**
Mugil Cephalus	**107**	**29**	**78**
Total	**350**	**116**	**234**

From the Table (3) they observed that the 40, 30, 40 and 40 isolates of *E. coli* were isolated from *Catfish, Nile Tilapia, Carp species and Mugil Cephalus* respectively.

From Table (4) they illustrated that the organ distribution for isolated ***E. coli*** and non ***E. coli*** from different fish species . They found that the intestine is the most predilection site for isolate of ***E. coli*** and non ***E. coli*** in most affected fish species where 15, 10, 13 and 15 isolates of ***E. coli*** isolated from intestine of both apparently healthy and diseased catfish, ***Nile tilapia***, ***carp*** and ***Mugil Cephalus*** respectively. One hundred and fifty isolates of ***E. coli*** were observed in all exposed organs to examination in all species of fish, while organs to examination in all species of fish, while 200 isolates of non ***E. coli*** were isolated.

Table (3) : *Showed the incidence of bacterial isolates according to species affected:*

Type of fish	No. of E. coli			No. of non E. coli		
	Apparently healthy	Diseased	Percentage	Apparently healthy	Diseased	Percentage
Catfish	**15**	**25**	**26.7**	**20**	**35**	**27.5**
Nile Tilapia	**10**	**20**	**20.1**	**18**	**25**	**21.5**
Common Carp	**10**	**30**	**26.6**	**14**	**21**	**17.5**
Mugil Cephalus	**12**	**28**	**26.6**	**17**	**50**	**33.5**
Total	**150**		**100**	**200**		**100**

Table (4) : *Showed the organ distribution of total number of **E. coli** and non **E. coli** isolates from different organs :*

Type of fish	Organ	No. of E. Coli			No. of non E. coli		
		Apparently healthy	Diseased	Total isolates	Apparently healthy	Diseased	Total isolates
Catfish	Intestine	**5**	**10**	**15**	**7**	**12**	**19**
	Spleen	**4**	**6**	**10**	**6**	**11**	**17**
	Liver	**3**	**5**	**8**	**6**	**7**	**13**
	Kidneys	**3**	**4**	**7**	**1**	**5**	**6**
Nile Tilapia	Intestine	**4**	**6**	**10**	**6**	**9**	**15**
	Spleen	**2**	**5**	**7**	**6**	**6**	**12**
	Liver	**2**	**5**	**7**	**3**	**5**	**8**
	Kidneys	**2**	**4**	**6**	**3**	**5**	**8**
CommonCarp	Intestine	**4**	**9**	**13**	**5**	**7**	**12**
	Spleen	**3**	**8**	**11**	**3**	**6**	**9**
	Liver	**2**	**7**	**9**	**3**	**5**	**8**
	Kidneys	**1**	**6**	**7**	**3**	**4**	**7**
Mugil Cephalus	Intestine	**4**	**11**	**15**	**5**	**14**	**19**
	Spleen	**3**	**8**	**11**	**5**	**13**	**18**
	Liver	**3**	**6**	**9**	**4**	**12**	**16**
	Kidneys	**2**	**3**	**5**	**2**	**11**	**13**
Total		**47**	**103**	**150**	**68**	**132**	**200**

From table (5) the data revealed that the intestine is the most harbored organ for the bacterial isolates followed by the spleen , liver and kidneys .

The total number of ***E. coli*** and non ***E. coli*** in the intestine was higher than that of spleen then liver and kidneys.

3) Water Quality monitoring during the E. coli isolates

The results obtained during the course of ***E. coli*** incidence in Table (6) cleared that the high organic matter and un-ionized ammonia (NH_3) as well as severe decrease of dissolved oxygen .The data noted that the range of organic matter (O.M.) can be reached to 2 mg/L, NH_3 0.9 mg/L , D.O. 1.8 mg/L and water temperature 22C .

4) Anti-biogram test

The results of antibiotic sensitivity test for the all isolates of ***E.coli*** after pooling showed that the Spectinomycin, Enrofloxacine and Oxanilic acid were the most susceptable antibiotics for ***E.coli*** strains.

Table (5) : ***Showed the percentage of E. coli and non E. coli isolates from different isolates :***

Type of samples	No. of samples	E. coli		Non E. coli	
		No.	%	No.	%
Intestine	350	53	35.3	65	32.5
Spleen	350	39	26	56	28
Liver	350	33	22	45	22.5
Kidneys	350	25	16.7	34	17
total	1400	150	100	200	100

Table (6) : ***The water quality parameters in the different locality during this study .***

Water quality parameters	Domitta	Kafr El-Sheikh	El-Bohera	Permiss limits
Odour	Some what offensive	Normal	Normal	Clear with algae
Odour	Slight brown	Slight green	Green to brownish	Intermediate color
Turbidity	25-30 cm	40 cm	45 cm	60 cm
Organic matter	2 mg/L	1.8 mg/L	1.6 mg/L	0.5 mg/L
Salinity	Zero	Zero	Zero	Fish water
PH	8.8	8.3	8.1	8.5
Temperature	32 ^{o}C	32 ^{o}C	31 ^{o}C	26-30 ^{o}C
Total ammonia	0.9 mg/L	0.35 mg/L	0.21 mg/L	0.1 mg/L
Uiionized ammonia	0.07 mg/L	0.04 mg/L	0.04 mg/L	0.01 mg/L

Table (7): ***Different types of antibiotic disces with variable concentration***

Antibiotic name	sensetivity	Decesion
Lincospectin	++	Medium = s
Neomycin	++	Medium = s
Enrofloxacin	+++	High = S
Ampicilin	++	Medium = s
Oxanilic acid	+++	High = S
Erythromycin	++	Medium = s
Spectinomycin	+++	High = R
Chloramphenicol	+	Low = R

Table (8): *Showed biochemical characters of* ***Escherichia coli :***

Tests	Reactions
Catalase	Production of gas bubbles
Oxidase	Negative reaction
Indole	Production of indole (red color)
Citrate	No growth and no color change
Methyl red	Positive reaction (bright red)
Voges Proskauer (V.P.)	No ring during two hours
H_2S	No H_2S
Urease	Negative reaction (yellow color)
TSI	Acid butt and acid slant
Nitrate	Positive reaction (red color)
Gelatin liquefaction	Negative reaction
Sugar fermentation :	
- Glucose	+
- Lactose	+
- Maltose	+
- Sucrose	D
- Mannitol	+
- Dulictol	D
- Xylose	+
- Mannose	+
- Salicin	D

+ = positive ***D = Differe***

5) Results of Clinical and postmortem lesions in naturally infected fish

The naturally infected fish species (*O. niloticus, Common carp, M. cephalus and C. garipenus*) showed the following clinical signs :

Scaleloss, ascites, darkness coloration of the body, hemorrhagic patches all over the body, eye cataract, exophthalmia and tail rot.

While the most postmortem lesions were:- congestion of all internal organs especially kidney, liver and spleen.

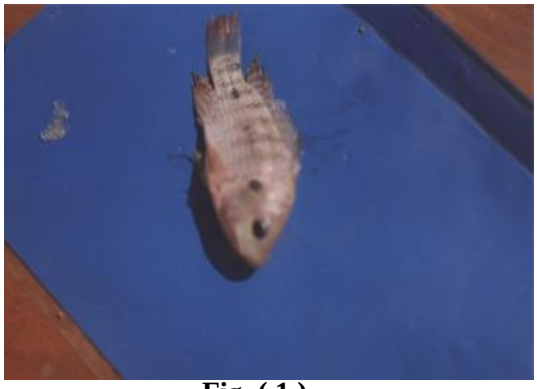

Fig. (1)

O. niloticus naturally infected with E. coli strain showing scale loss and darkness.

Fig. (2)

Mugil cephalus naturally infected with E. coli showing scale loss

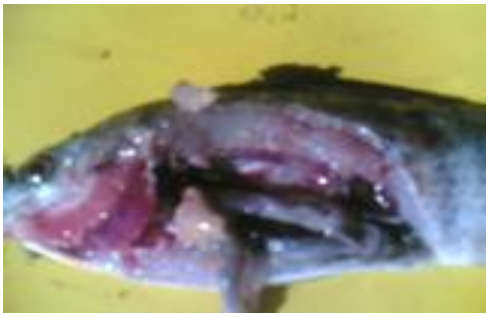

Fig. (5)

O. niloticus naturally infected with E. coli (O148) whole bacteria post infection showing congestion of gills and liver .

Fig. (6)

Mugil cephalus ***naturally infected with*** *E. coli* ***showing haemorrhagic patches all over the body***

Fig. (7)

O. niloticus naturally infected with E. coli showing darkness coloration of the body and eye cataract

Fig. (8)

Catfish ***naturally infected with E. coli showing darkness coloration of the body .***

Fig. (9)

Common carp naturally infected with ***E. coli*** showing scale loss and ascites .

DISCUSSION

E. coli is a common disease of freshwater fish especially under cultured conditions (Baya and White 1997), and play a big role in economic losses among fish industry.

Therefore, the present study was carried out to isolate different species of *E. Coli* and identifying it by different methods .

For study the incidence of *E. coli* we must collected the samples from different localities to gave a good chance for isolation of different serotypes of *E. coli* and for determination of the most serotype of greater incidence in fish farms in Egypt. Germani *et al.* (1997) noted that, *E. coli* incidence differ from area to another area according to the demographic and socioeconomic characteristics, environmental factors, and type of food introduced to human, animals and fish and susceptibility of fish species. Meanwhile, the results of Tuyet *et al.* (2006) indicated that the serotype of *E. coli* and the type of the food introduced to fish controlled the incidence of the fish in certain locality than the other locality.

We also put in our considerations to collect samples from different fish species which include (Catfish, Tilapia, Carp and Mugil) for determination of the most important fish susceptible to infection with *E. coli*. this results agreed with those of *(Hansen et al., 2008)* where they reported that, the *E. coli* incidence differe from species to another according to species susceptability to infection.

The sample during its collection include both apparently healthy and diseased fish for determination of the most serotype of *E. coli* of higher incidence and causes sever pathogenicity among fish.

The higher isolates of *E. coli* obtained from intestine and liver . Meanwhile, the the kidney and spleen constituted the lower level of isolates. This results agreed with those of *(Hanson et al., 2008)* who reported that, the *E. coli* firstly discovered in the intestine of wild fish. Also, *Lee and* Marks (2009) indicated that, the *E. coli* is a bacterium that commonly lives in the intestines of people, animals and fish. Nevertheless, these non-pathogenic *E. coli* can cause disease if they spread outside of the intestines, for example, into the urinary tract (where they cause kidneys infections) or into the blood stream . Some strains of *E. coli* are pathogenic, meaning they can cause disease in the small intestine and colon

The results obtained during the course of *E. coli* incidence cleared that the high organic matter and un-ionized ammonia (NH3) as well as severe decrease of dissolved oxygen can affect the incidence and pathogenicity of *E. coli* . This results nearly agreed with those of Zaky (2009) who arranged the antibiotic according its effect on *E. coli* isolated from fish, the least to high were chloramphenicol (30 mcg), ampicillin (10 mcg), penicillin G (10 mcg), streptomycin (10 mcg) and Gentamycin (10 mcg).

The results of biochemical tests for detection of biochemical characters of isolated Escherichia coli were cleared that , the biochemical characters of *E. coli*, produce gas bubbles inb catalase test, produce indole (red colour) in indole test, no growth and no clour in citrate test, positive reaction (bright red) in methyl red test, no ring formation in Voges proskaure (V.P) test, no H2S formation in H2s test, negative reaction (yellow colour) in urease test, acid butt and acid slant in TSI test, positive reaction (red colour) in nitrate test., the glelatin liquefaction test gave negative reaction, the sugar fermentation tests (glucose, lactose, maltose, mannitol, xylose and mannose) gave positive reaction +ve reaction , meanwhile, the sucrose, dulictol and salicin gave defferent results.

This results agreed with those of Thampuran et al. (2005) observed that, from a total of 484 presumptive *E. coli* were isolated, and their indole-methyl red-Voges-Proskauer-citrate (IMVIC) pattern was determined. These strains were also tested for labile toxin production by a reverse passive latex agglutination method and checked for *E. coli* serotype O157 by latex agglutination with O157 – specific antisera. Certain biochemical marker tests, such as methylumelliferyl-beta-glucuronide (MUG), sorbitol fermentation, decarboxylase reactions, and hemolysis, which are useful for screening pathogenic *E. coli*, were also carried-out. Results showed that 81.4 % of the *E. coli* isolates were sorbitol positive.

Also, our results indicated that, the most important *E. coli* isolates, that isolated from +ve fish samples to *E. coli* were O55, O148, O157 and O125.

The clinical signs & P.M. lesions mainly due to septacemic infection of *E. coli* infection and its endotoxin which affecting body of fish , these observation were partially similar to those reported by (Saad 2006).

El-saka (2006) reported that there was a high mortality percentage among the experimentally infected fish under different stress conditions. These conditions were overcrowdness, external parasitism and transportation with mortality rates of 83 %, 70% and 60 % in *O. niloticus* , respectively, and all of these stress conditions facilitated the infection with *E. coli*.

Also, Baird *et al.* (2003) reported that, the total losses attributed to the severe mortality among affected fish especially at marketable fish due to *E. Coli* infection.

Finally, *E. coli* is an important cause as secondary invador that help in

haemorrhgic septicemia in fish and also gastro-extraintestinal infection in fish and human.

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عزل وتصنيف ميكروب الايشريشيا القولونية المعزولة من أسماك المياه العذبة المستزرعة

مجدى خليل سليمان ـ رياض حسن خليل ـ طلعت طلعت سعد ـ محمد حسام الدين لطفى الجمل ـ علاء عيد جبريل

قسم أمراض الدواجن والاسماك كلية الطب البيطرى ـ جامعة الاسكندرية

٠وجود الايشريشيا القولونية فى الاسماك دليل هام على تلوث المياه ، كما أنه دليل على تلوث الشواطئ بمياه الصرف كما أنه يحدد مدى إستخدام المياه للاستهلاك الآدمى كما أنها تسبب نسبة نفوق عالية لاسماك المياه العذبة .

ـ استهدفت هذه الدراسة :

١. تحديد نسبة ومعدل حدوث الايشريشيا القولونية فى الاسماك المستزرعة ، وتحديد الخصائص الميكروبيولوجية للايشريشا القولونية المعزولة من الأسماك .

٢. تحديد خصائص الايشريشيا القولونية المعزولة من الاسماك عن طريق الاختبارات المعملية .

٣. أيضا تحديد خصائص الايشريشيا القولونية المعزولة من الاسماك بإستخدام تفاعل البلمره المتسلسل .

تم تجميع الاسماك من ثلاثة مناطق مختلفة متمثلة فى محافظة البحيرة (لعدد ٥٠ مزرعة) ، كفر الشيخ (لعدد ١٠٠ مزرعة) ، دمياط (١٠٠ مزرعة) تم عزل الايشريشيا القولونية على عدد من الاوساط المختلفة .

تم زراعة العينات على الاجار الطبيعى ، محلول الصويا و أجار الصويا ثم تم العزل على وسط مخصص لعزل الايشريشيا القولونية مث الثيوسلفات سيتريت أجار والمضاد الحيوى الفوفوبيوسين .

ـ تصنيف الايشريشيا القولونية تم عن طريق :

١. -عزل البكتيريا على أوساط معملية خاصة بالايشريشيا القولونية.

٢. تصنيف البكتيريا عن طريق الاختبارات البيوكيميائية المعملية.

ـ كانت أهم النتائج التى تم الحصول عليها من تلك الدراسة والتى تمثلت فى:

١. عدد المعزولات البكتيرية إختلفت معنويا من عضو على اخر حيث كان أكثر الاعضاء التى تم عزل منها من الامعاء ثم الطحال وكانت أهم الأعضاء الأقل عزل منها هى الكبد والكلية.

٢. تم عزل ٦١ معزولا من الايشريشيا القولونية ، حيث كانت أكثر المعزولا ت من من الامعاء ، والكبد. بينما الكلية والطحال كانت أقل الاعضاء التى تم عزل الايشريشيا القولونية منها.

بينما كان عدد البكتيريا الغير الايشريشيا القولونية هى ٩٠ معزولا وان أكثر الاعضاء التى تم العزل منها للبكتيريا الغير الايشريشية هى الامعاء بينما أكثر الاعضاء عزلا هى الكبد ، الطحال و الكلية .

٣. أوضحت الدراسة أن العوامل البيئية مثل إرتفاع تركيز المواد العضوية فى المياه ، وإرتفاع نسبة وتركيز الأمونيا مع نقص فى نسبة الاكسجين الذائب كلها عوامل تؤدى إلى سرعة إنتشار الايشريشا القولونية وإصابتها للاسماك.

٤. أوضحت الدراسة أن أكثر المضادات الحيوية تأثيرا على الايشريشيا القولونية هى الانروفلوكساسين ، حمض الاوكسانيلك، و الاسبكتينومايسين و أقل المضادات الحيوية تأثيرا على الايشريشيا القولونية المعزولة من الاسماك هى الاريثرومايسين ، الكلورامفينيكول بينما بقية المضادات الحيوية مثل اللينكوسباكتين ، والنيومايسين ، السبروفلوكساسين ، الامبيسيلين و الاوكسى تتراسيكلين كلها مضادات حيوية ذات تأثير متوسط على الايشريشيا القولونية المعزولة من الاسماك.

٥. أوضحت الاختبارات البيوكيميائية أن المعزولا ت للايشريشيا القولونية أنها تفرز غازات مع **Catalase test** ، ووجود لون أحمر مع الـ **Indole test** ، كما لوحظ عدم نمو للبكتيرا المعزولة وعدم تغير اللون مع الـ **Citrate test** كما لوحظ أن هناك تفاعل موجب (تكون لون أحمر قاتم) مع **Methyl red** ، عدم تكون حلقة موجبة فى الـ **Voges proskauer test** ، عدم تكون الـ **H2S**7 إعطاء نتيجة سالبة (عدم تكون اللون الاصفر) فى إختبار اليوريا ، إختبار النيتريت أعطى نتيجة إيجابية (تكون اللون الاحمر) ، إختبار السيولة للجيلاتين أعطى نتيجة سالبة إختبار تخمر السكريات (الجلوكوز ، اللاكتوز ، المالتوز ، المانيتول الزيلوزو المانانوز) أعطت نتائج إيجابية بينما تخمر السكروز و الديلكتول أعطت نتائج متغيرة ومختلفة.

كما أوضحت النتائج أن أهم المعزولات التى تم عزلها من الايشريشيا القولونية التى تم عزلها من العينات الموجبة للايشريشيا القولونية هى O55, O148 , O157 , O125