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Isolation of and characterization of *E. coli O157/H7* from Common Carp Fish (*Cyprinus carpio*) in Baghdad governorate

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Abstract
This study intended to isolate and characterize *E. coli* O157: H7 strains from Common carp fish (*Cyprinus carpio*) reared in fresh water in Baghdad governorate, in addition, to determine their antibiotic resistant profiles. Fish samples were collected from different local fish markets in Baghdad. The samples were cultured on Macconkey and modified Eosin Methylene Blue (MEMB) media. Additionally, chromogenic media was used for purification of *E. coli* O157/H7. The diagnosis of the bacteria depended on the rate of growth, colonies morphology, biochemical tests, and staining by Gram stain. Latex test was also used to determine the isolated bacteria serotypes depending on the agglutination nature of bacteria. *E. coli* O157: H7 were isolated from 30 (60 %) out of 50 samples. Moreover, all isolated bacteria were positively identified as *E. coli* O157: H7 strains and revealed typical results in biochemical tests and a positive result in latex test. Susceptibility profiles to seven antibiotics were determined, and variations in antibiotic sensitivity gathered. In conclusion, this study revealed the isolation of *E. coli* O157: H7 from Common carp fish. The author recommends to take precaution from *Cyprinus carpio* because it can act as a source of infection for pathogenic *E. coli* O157: H7 strain and can cause dangerous food poisoning in humans, hemorrhagic septicemia in fish and gastro-extraintestinal infection in fish and human.

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Introduction

Escherichia coli belongs to *Enterobacteriaceae.* It is a Gram's negative rods. Human beings and hot-blooded animals harbor *Escherichia coli* as an ordinary microorganism of their intestinal tract. *E. coli* is high prevalence in the bowels; moreover, it is used as chosen index in the estimation of food and water fecal pollution. There are numerous factors differentiate pathogenic from non-Pathogenic *E. coli* strains.

These factors include; its genetic components that enable it to produce toxin and causes severe sickness, attachment and breach of host cells, intrusion with cell metabolism and tissue destruction (Borgattaa *et al.,* 2012). *E. coli O157: H7 (Enterohemorrhagic Escherichia coli)* strains are an important group of foodborne pathogens causing severe sickness in humans globe. *Escherichia coli O157:H7* can be found in the feces and on the skin of meat animals. About 75 % of humans *E. coli O157:H7* outbreaks are associated to bovine-derived products.

When skin has removed during the harvest process, the carcass and subsequent meat products become contaminated (Yilmaz *et al., 2006;* Bosilevac *et al., 2015).* The virulence and carrier status of EHEC in ruminants are still not well understood. There are more than 100 pathogenic genes associated with the settlement of the cattle intestine. These genes are identified by biochemical and genetic analyses *(*Dziva *et al., 2004).* One of these genes is *Escherichia coli O157* type III secretion system that allows the shift of the effector proteins that is necessary for the intestinal colonization, into host cells in cattle *(*Sharma *et al.,* 2012*).*

Ruminants are the common host carriers of *E. coli O157:H7* . It considers as the primary reservoir that transfers *E. coli O157* without clinical symptoms. These carriers animals are passively able to release these organisms in their feces for a long time (Grauke *et al., 2002*). Effective sewage pollution is estimated by measurement of *Escherichia coli (E. coli O157)* in fish. The estimation criteria are used to notify advice for no water contact. There are a diversity types of *E. coli.* Hanson *et al. (2008).* Majorities of the *E. coli* types are a natural nonpathogenic colonist in the intestine. These nonpathogenic E. coli can cause disease if they spread outside the gut. The most characteristic clinical signs of pathogenic *E. coli* is diarrhea that occurs by production and liberation toxins (called enterotoxigenic E. coli or ETEC)(Lee and Marks, 2009).

According to virulence aspects, *Escherichia coli* is categorized to verotoxigenic *E. coli (VTEC)* which produce and release a lethal toxin . This toxin can destroy African green monkey kidney cells (Vero cells) but not for other cell cultured types (Holko *et al.*, 2006). Other *Escherichia coli* is called *enterohemorrhagic E. coli* which are VTEC. These bacteria have the ability to cause hemorrhagic colitis and hemolytic uremic syndrome because they own extra virulence factors (Kobori *et al., 2004).* Long lasting diarrhea (2 weeks or prolong) is occurred due to *Enterotoxigenic E. coli (ETEC)* infection. At the beginning of the infection, ETEC attaches and colonize on the epithelium surface of the small intestine by production the colonization factor antigens. Meanwhile, *enteroinvasive E.coli (EIEC*) invades the epithelial cells of the intestine and distribute from cell to cell. However, enteroaggregative *E. coli (EAGGEC)* strains have the ability to adhere to the cells of tissue culture by the aggregative method and build a distinctive "stacked, brick-like" structure. The milk and water are accused to be the source of EAGGEC outbreaks (DiRita, 2007).

The main reservoir of *VTEC* is ruminants, in particular, beef cattle. Most epidemiological servings approved that bovine is regularly secreting Shiga toxin producing *E. coli (STEC)* in their feces, and this strain is considered as a source of infection. *E. coli* is also the main

cause of acute bovine mastitis which is characterized by local inflammatory changes of the mammary gland and severe systemic clinical signs including fever, rumen stasis, shock, dehydration and even death (Wenz *et al.,* 2001). Calf diarrhea causes more economical, financial loss to cow-calves companies than any other disease-related problem they are facing. The intestine of the diarrheic calf is unable to absorb fluids and secretion into the intestine is increased. Also, dehydration and death are the most important prognosis that depends on the particular serotype of *E. coli (*Tan *et al., 2011*). Naturally, fish is vulnerable to microbial spoilage as it carries high microbial amount on skin, gills and intestine. *Escherichia coli* in fish and water are considered as an index to sewage pollution (Rajasekaran, 2008; Kumar *et al.,* 2001; Rajkhowa *et al.,* 2009). Albeit, different species of bacteria harbor the fish, there are only few studies has been reported regarding the isolation of *E. coli* O157: H7 in Iraq.
Consequently, this study intended to isolate and characterize *E. coli* O157: H7 strains from Common carp fish (*Cyprinus carpio*) reared in fresh water in Baghdad governorate, in addition, to determine their antibiotic resistant features.

Materials and Methods

Samples Collection

Fifty intestinal samples were collected from freshwater fish (Common carp fish /*Cyprinus carpio*). These fish were sold in different local markets in Baghdad governorate. The samples were transferred aseptically by a cool box for bacteriological isolation.

Bacterial culture

The specimens were taken from all samples and inoculated onto different cultures media as following: MacConkey Agar (MA), Modified Eosin Methylene Blue agar (MEMBA) and Sorbitol MacConkey Agar enriched with Cefixime Tellurite supplement (SMA). The MA used for easy identification of lactose fermenting organisms, while, MEMBA prepared by adding 5% of both sucrose and glucose sugars and colonies of *E. coli* reveals particular characteristic green metallic sheen appearance. Moreover, SMA is used as a selective media to distinguish between non-sorbitol-fermenting *E. coli* O157:H7 strains from other *E. coli* strains. Later on, biochemical diagnostic tests were done for all isolates according to Quinn *et al.,* (2002).

Identification of isolated *E. coli*

The bacterial isolates were subjected to characterization tests according to the following procedures:

Morphological characterization

Bacterial Smear prepared from the suspected purified isolated colonies and stained with Gram's stain then examined microscopically (Cruickshank *et al*., 1979). The colony morphology on different cultures media was also reported.

Detection of motility

Isolated bacteria were stabbed into tubes containing semi-soiled nutrient agar medium and then incubated at 37 0C for 24 hrs. Later on, the motility of the inoculated bacteria was reported.

Antibiotic sensitivity test

The isolated E. coli were tested for the antibiotic sensitivity test according to standard procedures method for antimicrobial susceptibility test. Four or five isolated colonies were selected from pure culture and grown in 4 to 5ml of nutrient broth. Consequently, the broth was incubated at 37 0C for 24 hrs. The density of the bacterial inoculum standardized with 0.5 McFarland turbidity standard tubes. The suspension should contain 1x 10 8 CFU/ml. Eventually, 1 ml of bacterial suspension was spread on the surface of Mueller-Hinton agar. The antibiotic discs were applied later on, and the plates were kept at 37 0C overnight. The diameter of the inhibition zones of bacterial growth around the antibiotic disc was measured and recorded. The interpretation was made for each isolate. The absence of inhibition zones around the antibiotic disc indicated the resistance of these isolates for this antibiotics.

Serological identification of *E. coli* O157:H7

Special latex agglutination kit (Figure 1) was used for identified the isolated bacteria serologically. All isolates that were confirmed biochemically as *E. coli* examined for serological identification according to the previous method described by Ewing, (1986). Slid agglutination test was used for testing the *E. coli* somatic (O) antigen.

Figure. 1: Shows the special latex agglutination Kit for *E. coli* O157:H7

Results

In this study, a total of 50 samples were examined. Thirty, *E. coli O157: H7* were isolated from intestinal samples that collected from freshwater fish (Common carp fish /Cyprinus carpio) (Table. 1). The isolated *E. coli* appeared as pinkish colonies on MacConkey agar (Figure .2) and green metallic sheen on EMB (Figure.2). Bacterial smear from isolated culture stained with Gram's stain revealed short Gram-negative bacilli (Figure. 3). All isolates obtained through phonotypical characteristics features (purple colonies on Chromogenic agar) (Figure 4). The results of the biochemical tests for the isolated bacteria were positive for catalase (Production of gas bubbles), indole test (production of indole), and nitrate (Positive reaction (red color) and motility Moreover, all isolates were negative for oxidase, citrate (No growth & no color change), Voges-Proskauer, H2S production and urease (Table 2). All 30 isolates revealed positive clear agglutination reaction in slide agglutination test using with *E. coli O157:H7* latex agglutination (Figure. 5).
The results of the antibiotic sensitivity test revealed that the isolated bacteria expressed sensitivity for Trimethemprim, Amicasine, Erythromysine, Cefetriaxone, Chloramphenicol and Ampicilline (Table 3) (Figure 6) .

Table (1): The results of *E. coli O157/H7* Isolates on culture media.

Animal	Positive results	Negative results	Total number
Fresh water fish	30 60%	20 40%	50 100%

*****the table analyzed by student t-test.

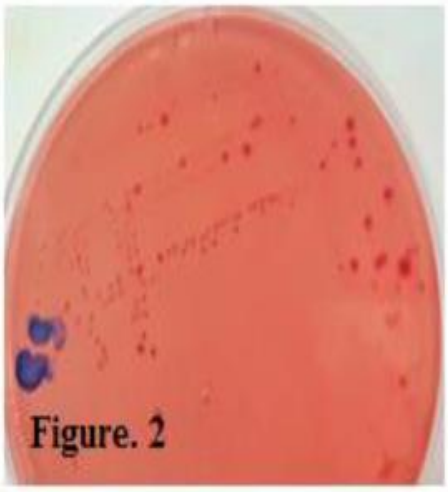

Figure .2: Shows growth of *E. coli* on MacConkey agar
Figure. 3: Shows growth of *E. coli* as green metallic sheen color on EMB

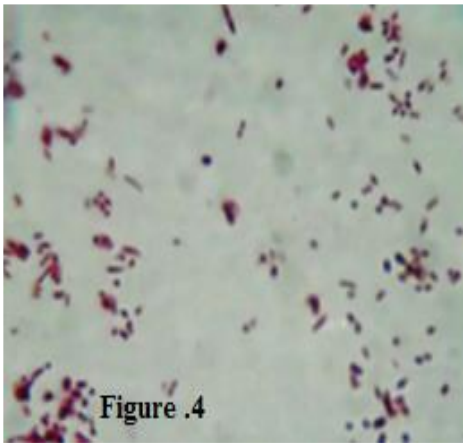

Figure. 4: shows *E. coli* appeared as Gram negative bacilli

Figure. 5 : Shows purple colonies of *E. coli* O157:H7on Chromogenic agar

Table (2): The results of biochemical tests.

Test	Results
Catalase	Production of gas bubbles
Oxidase	Negative reaction
Indole	Production of Indole (red color)
Citrate	No growth & no color change.
Voges-Proskauer (V.P.)	Negative reaction
H2S	No. H2S production
Nitrate	Positive reaction (red color)
Urease	Negative reaction
Motility test	Positive

Table .3: Shows the results of sensitivity test with different types of antibiotic discs with variable concentration

Antibiotic Name	Sensitivity	Interpretation
Trimethemprim	+++	High
Amicasine	++	Medium
Erythromysine	++	Medium
Cefetriaxone	++	Medium
Chloramphenicol	+	Low
Ampicilline	+	Low

Figure. 6

Figure. 6: Shows the sensitivity test on Muller Hinton Agar that shows the isolates more sensitive to Trimethoprim (TMP) antibiotic.

Discussion

E. coli is a common disease of freshwater fish, especially under culture conditions. It plays a significant role in economic losses among the fish industry. Accordingly, the current study intended to isolate and identify different species of *E. Coli* using conventional isolation and characterization methods. The results of this study revealed the high percentage of *E.coli* infection in fish (60%). This result is compatible with previous studies (Tuyet *et al.*, 2006, Hanson *et al.*, 2008, Abeer *et al.*, 2010 and Lee and Mark, 2009). All these studies indicated that the *E. coli* is a bacterium that commonly lives in the intestines of people, animals, and fish. Though, these nonpathogenic *E. coli* able to cause disease when they have an opportunity to distributed outside the intestines

such as the urinary tract (where they cause kidneys infections) or into the blood stream. Some strains of *E. coli* are pathogenic, and they are able to cause disease in the small intestine and colon. Previous studies reported the events that occurred during *E. coli* incidence. These studies revealed that the high organic matter and unionized ammonia (NH3), as well as the severe decrease of dissolved oxygen, can affect the pathogenicity of *E. coli.* The rate of infection differed in the various area depend on environmental factors, and type of food introduced to human, animals and fish and the susceptibility of fish (Tuyet *et al.,* 2006, Canadian Food Inspection 2005,2005 and Kumar *et al.,* 2001).
The results of this study revealed typical biochemical tests for detection Escherichia coli isolates. These results agreed with the previous study reported by Thampuran *et al.,* (2005). Thampuran *et al.,* (2005) isolated *E. coli* and, its indole-methyl red-Voges-Proskauer-citrate (IMVIC) pattern was also determined. The results of the current study approved the serotyping of the isolated *E.coli* in agglutination test against O157: H7 antiserum. All isolated strains showed positive agglutination reaction. This result is compatible with previous studies (Farasata *et al.,* 2012). Farasata *et al.,* (2012) reported the highest concentration of E.coli that determined in the wastewater of food industry. The results of the antibiotic sensitivity in the current study also revealed the sensitivity of isolated *E. coli O157: H7* to different dose and different antibiotics. The most frequent resistance type overall was streptomycin-sulfisoxazole-tetracycline, which accounted for over 70% of the resistant strains. Most isolated *E. coli* were highly sensitive to Trimethemprim. However, these isolates were medium resistance to Amicasince, Erythromycin and Ceftriaxone. Meanwhile, some strain revealed low resistance to Chloramphenicol and Ampicillin. In conclusion, this study approved the isolation of pathogenic *E.coli* from Common Carp Fish (*Cyprinus carpio*) that collected from Baghdad governorate. The author recommends taking precaution from common Carp fish because it can act as a source of infection for pathogenic *E. coli O157: H7* strain and can cause severe food poisoning in humans.

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