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Detection of ESBL *E.coli* That Carried STX1 and STX2 Form Common Carp (*Cyprinus carpio*) in Salhaldeen Province

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E.coli is the most important bacteria that contaminates of fish farms and leads to Pollution and corruption of fish, which causes a threat to public health, the current study aimed to find the distribution of ESBL *E.coli* that carried STX1 and STX2 from common carp (*Cyprinus carpio*) in Salhaldeen province, for this purpose 100 sample were collected from fish, traditional and genetic methods were used. The results of the current study reveal to that Out of 100 fish sample, *E. coli* isolated from 48 in rate of 48% , and 19 isolates out of 48 were diagnosed as ESBL *E.coli* in rate of 39.5% , according to PCR test Stx1 gene detection on 31 isolates out of 48 isolates in rate of 64.5% while Stx2 gene detection on 39 isolates in the rate of 81.2%. We can conclude the high contamination rate of fish and its farms with *E.coli* in Salhaldeen province, most isolates are ESBL, Stx2 gen is more frequent than Stx1 gene.

Keywords: *E. coli*, *Cyprinus carpio*, ESBL, shigatoxi

Introduction

Because of the different diets and health cultures, people's consumption of fish has increased, This is because it has a large amount of vitamins, minerals, and fatty acids, fish is considered as source of about 30% of animal proteins, Despite all these benefits, it can be a carrier of many pathogens that affect humans [1]. *E.coli* is considered as one of the most important bacterial contaminants that may be considered as an indicator of contamination and spoilage of meat [2, 3, 4]. *E.coli* are Gram-negative bacteria, spherical to ciliary-shaped with rounded ends, non-spores forming, move by peritrichous flagella, arranged singly or in pairs. facultative aerobic or anaerobic, possesses both fermentative and oxidative pathways of metabolism, the optimum temperature for its growth is 37 C. It many carbohydrates such as lactose, glucose, mannitol, maltose, and arabinose, producing acid and gas, oxidase test , citrate consumption test, hydrogen sulfide production, and it can analyze urea, , and it is positive for catalase, methyl red, and nitrate reduction tests [5]. Due the use of antibiotics, several antibiotics resistant bacteria strains were isolated, and the most important of these antibiotics are the beta-lactam group. Bacteria have several mechanisms for antibiotic resistance, these bacterial strains and their resistance mechanism may be transmission to humans by taking of contamination food and water or due to environmental pollution with these bacteria [6, 7, 8]. Shiga toxin one of most important virulence factors of *E.coli*, it takes his name due to its similarity with Shigella toxin except one amino acid [9, 10]. *E.coli* producing Shiga toxin is one of most important causes of enteric disease, shiga tosxin play important role in pathogenicity of *E.coli* that causes food poisoning [11, 12].

Material and Methods

Samples: 100 swabs from fish skin gills and intestine were collected from the local market in Salhaldeen province.

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Swabs cultured on trypton soya broth (Himedia-India) and cultivated at 37C for 24h then loop full subcultured on macConkey agar (Himedia- India) and cultivated at 37C for 24h. suspected colony were sub cultured on Eosin methylene blue (Himedia-India) and cultivated at 37C for 24h, then Gram stain and group of biochemical tests were done according to [13].

Detection of ESBL *E.coli* : single purified *E.coli* coloy eultured on chromagar (CHROMagar™ ESBL- Chromogenic media Pioneer – UK).

Detection of shiga toxin producing done by following strategies:

a. DNA extraction: were done by use of (DNA Preparation Kit PP-206-Jena Biosciences, Jena, German) and according to Manufacturer's instructions.

b.Primer:stx1: F:TCTTGCGGTACTCTAGTAG

R:AGAACGCCCACTGAGATCATC (give out product in size of 180bp).

c.Stx2: F: AGAACGCCCACTGAGATCATC, R: TCGCCAGTTATCTGACATTCTG (give out product in size of 255bp). [14].

Results and Discussions

Out of 100 fish samples, *E.coli* was isolated from 48 at rate of 48%, all isolate appeared as gram negative bacteria, fermented to lactose sugar which appear as pink colony on MacConkey agar(Fig. 1) while the colony appear as give green metallic colony on EMB agar figure (2). All *E.coli* isolates give positive to catalase, MR test and Indol test while negative results on the oxidase test, VP, and citrate test.

The isolation rate is less than the results recorded by Alttai *et al*. [15] in the local market in Mousal , Nineveh governorate, Iraq, In the study of Taha and Yassin's [16] isolated *E.coli* in rate of 39% from *Cyprinus carpio* fish in Dohuk province, Iraq. The isolation of *E.coli* from fish refers to water pollution from sources such as Wastewater, waste, or the sack of feed provided to fish contaminated with bacteria [17].

When *E.coli* re cultured on CHROMagar™ ESBL, ESBL *E.coli* diagnosed in 39.5% (19 out of 48) as in figure (3)

In the study of Mahmmoud and Al-Dabbagh [18] shows that 34% of E. coli isolated from fish in Mosul are resistant to cefotaxime, while in the study of Kumar *et al*. [19] showed that 38% of fish were contaminated with ESBLs E. coli. While in the study of Tyasningsih *et al*. [20] showed that ESBLs *E. coli* formed 1.7% of all *E. coli* isolated from animal products in Indonesia.

According to the PCR test results , Stx1 gene detection on 31 isolates out of 48 isolates at the rate of 64.5% (Fig. 4) while Stx2 gene detection on 39 isolates at rate of 81.2% (Fig. 5).

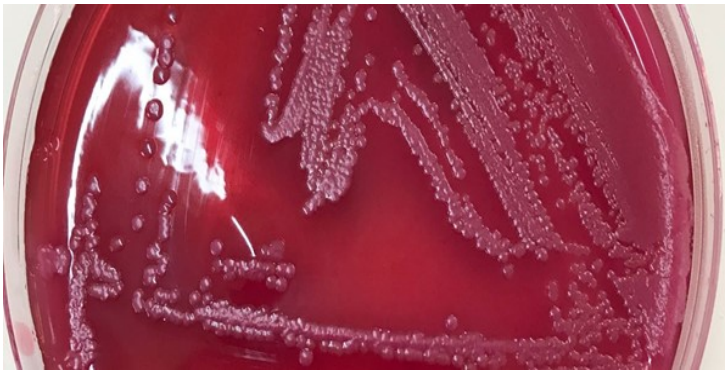

Fig. 1. MacConkey agar , shows pink colony of *E.coli*

Fig. 2. EMB agar , shows green metallic sheen colony of *E.coli*

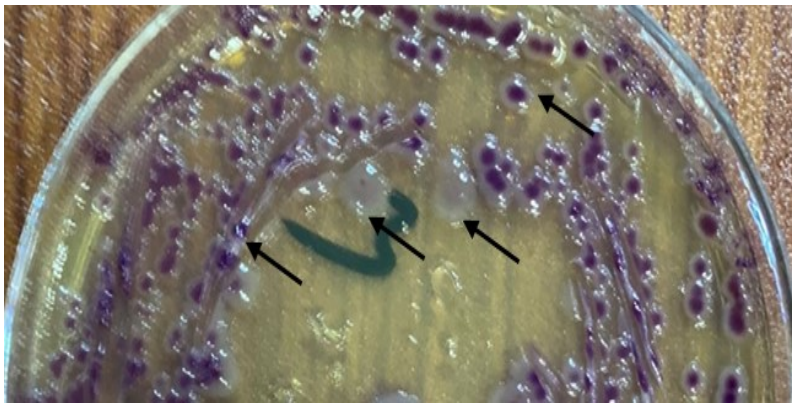

Fig. 3. CHROMagar™ ESBL agar, shows purple colony of ESBL *E.coli*

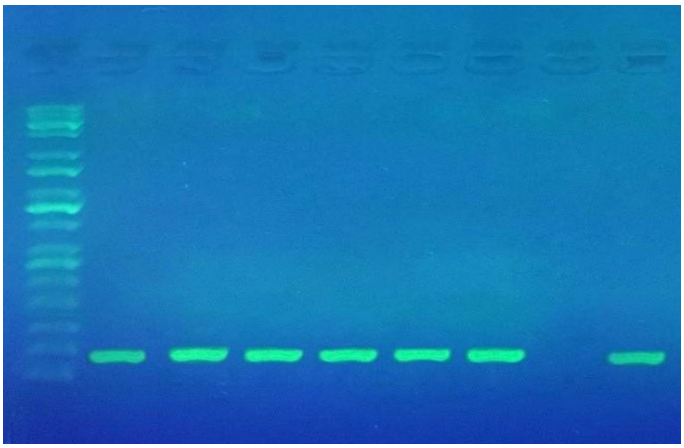

Fig. 4. Results of *E.coli* producing stx1 gene, Lens 1,2,3,4,5,6, and 8 positive results with band in size of 180bp . M:100pb DNA marker.

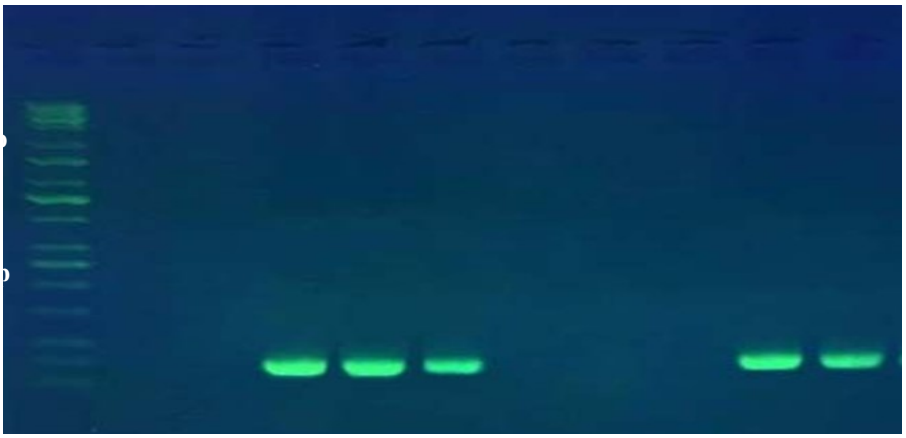

Fig. 5. Results of *E.coli* producing stx2 gene, lens 3,4,5, 9 and 10 positive results with band in size of 255bp, M:100pb DNA marker.

In study of MS *et al*. [21] showed that *E.coli* produced stx1 gene and stx2 gene in rates of 60% and 70% when isolated from Fish Markets in Egypt, while in study of Pradhan *et al*. [22] showed that the presence of *E.coli* isolates producing stx1 gene and stx2 gene in rate of 44% and 77%, these difference may be due geographic differences of study location. stx-1 and stx-2 seem to be important in human infections. E. coli O157:H7 is the principal serotype of this group Enteroinvasive E. coli (EIEC) causes a diarrheal illness similar to shigellosis [23,24]. This result may be attributed to the possible attachment and penetration of E. coli O157 from 209 mucus into the skin. Although the mucopolysaccharide structure of the mucus layer in the fish 210 skin is reported to protect fish against infections [25] indicated that E. coli O157: H7 cells were able to penetrate through the mucus layer of 212 fish and become a source of contamination during processing. [26] Howbeit, injury of the fish 213 body during stressful harvesting and direct contact with the contaminated ice during storage 214 could lead to fish muscle contamination.

Conclusion

We can conclude the high contamination rate of fish and its farms with *E.coli* in Salhaldeen province. most isolates are ESBL, Stx2 gene are more frequent than Stx1 gene.

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Conflict of interest

There are no conflicts of interest to be declared.

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Author contributions

Conceptualization, study design, sample collection, data analyses, Manuscript drafting, and manuscript finalization: Qusai Saleh Jumma

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الكشف عن بكتيريا الاشريشيا القولونية المقاومة لطيف واسع من المضادات الحياتية والحاملة للجنين STX1 و STX2 من سمك الكارب الشائع (*Cyprinus carpio*) في محافظة صلاح الدين

قصي صالح جمعه

فرع الأمراض والدواجن - كلية الطب البيطري - جامعة تكريت - العراق.

تعتبر الإشريكية القولونية من أهم البكتيريا الملوثة للمزارع السمكية وتؤدي إلى تلوث وفساد الأسماك مما يشكل خطرا على الصحة العامة، هدفت الدراسة الحالية إلى إيجاد انتشار بكتيريا الاشريشيا القولونية المقاومة لطيف واسع من المضادات الحياتية التي تحمل STX1وSTX2من أسماك الكارب الشائع (Cyprinus carpio) .في محافظة صلاح الدين، ولهذا الغرض تم جمع 100 عينة من الأسماك، وتم استخدام الطرق التقليدية والوراثية. أظهرت نتائج الدراسة الحالية أنه من أصل 100 عينة من الأسماك تم عزل 41 عزلة من الإشريكية القولونية بنسبة 41% و 19 عزلة من أصل 48 عزلة تم تشخيصها على أنها *E.coli* ESBL بنسبة 39.5% حسب اختبار PCR لجين .Stx1 تم الكشف عن 31 عزلة من أصل 48 عزلة بنسبة 64.5% بينما تم الكشف عن جين. Stx2 على 39 عزلة بنسبة 81.2%.

الاستنتاجات: وجود نسبة تلوث عالية ببكتريا الاشريشيا القولونية في الاسماك واحواضها في محافظة صلاح الدين، اغلب العزلات كانت مقاومة لطيف واسع من المضادات الحياتية. الجين STX2 اكثر ظهورا من الجين STX1

الكلمات الدالة: الإشريكية القولونية ، الشبوط الشائع، سموم شيكا. المقاومة لطيف واسع من المضادات الحياتية