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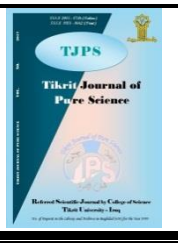

Bacterial Content in Gut For Different Species of Fish Collecting From Tigris River in Baghdad City, Iraq

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Abstract

A bacteriological study was conducted on fish from May to October 2015. The total of 50 fishes were collected from Tigris River passing through Kurayat, Shawakah and Utaifiah at Baghdad City. There are seven species of fish recorded were: *Acanthobrama marmid*, *Alburnus mossulensis*, *Carasobarbus luteus*, *Cyprinus carpio*, *Garra elegans*, *Garra rufa* and *Planiliza abu*. Selective media were used to identify the bacteria such as: CHROMagar™ Orientation (for Enterobacteriaceae and non- Enterobacteriaceae group), CHROMagar™ Vibrio and TCBS media (for *Vibrio* group), CHROMagar™ ESBL medium (forβ- lactamase production), and Xylose lysine deoxycholate agar (XLD) (for *Salmonella* spp. In current study, the bacterial total count in the gut of examined fishes ranging from 31 to 456 $\times 10^3$ cell/ml. The isolated bacteria belonged to Enterobacteriaceae are *Citrobacter amalonobicas*, *C. diversus*, *C. frundii*, *Edwardsiella tarda*, *Enterobacter agglomerans*, *E.cloacae*, *E.gergoviae*, *E.sakazaki*, *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumonia*e, *Proteus mirabilis*, *P. vulgaris*, *Providencia vettigen*, *Serratia marcesens* and *S. rubidaea*, while for Non- Enterobacteriaceae are *Acinetobacter* spp., *Pseudomonas* spp., *Vibrio alginolyticus*, *V. cholera*, *V. parahaemolyticus* and *V. vulinficus*. This study provides an overview of the bacterial gut contents of economic and non-economic fish.

Introduction

Rivers are the most important freshwater resource for human, plant and animals, Water is contaminated by industrial waste, sewerage, and plethora of human activities and all of which effect on the physical and chemical properties of water [1]. The presences of bacteria in the aquatic environment which can be used to evaluate its sanitary and bacteriological state also reflect the state of the fish living in it [2]. Pathogenic bacteria associated with fish include *mycobacteria*, *Escherichia coli*, *Enterobacter aeromonads*, *Salmonella spp*., *Pseudomonas*, *Vibrio spp*., *Streptococcus spp.* [3]. Enterobacteraceae in fish are considered as an indicator to sewage pollution and has been reported as opportunistic pathogen in fish [4]. *Escherichia coli* is the most common contaminant and is often encountered in high numbers in intestine of fish [5]. *Vibrio* species are also known to cause disease in human especially *V. cholerae* species, most often following the consumption of contaminated fish [6]. Several of these bacteria are opportunistic pathogens and may cause diseases when the fish are under unfavorable condition, the genera *Pseudomonas* and *Vibrio* are commonly isolated from normal healthy fish, but only certain strains of these bacteria possess the virulence factor necessary to induce disease [7]. Previous local report showed that water at Shawakah was contaminated by industrial waste water and municipal wastewater more than in Kurayat and Utaifiah area[8].

Due to the lack of a local guide identifies bacterial species found in local fish, and multiple sources of pollution of the river, the study was designed to determine the bacterial species present in the local gut of fish as this has an impact on consumer health .

Materials and methods

Sample collection area

The Samples were collected from Tigris river passing through Shawakah, Kurayat & Utafiah in Baghdad city as shown in figure (1).

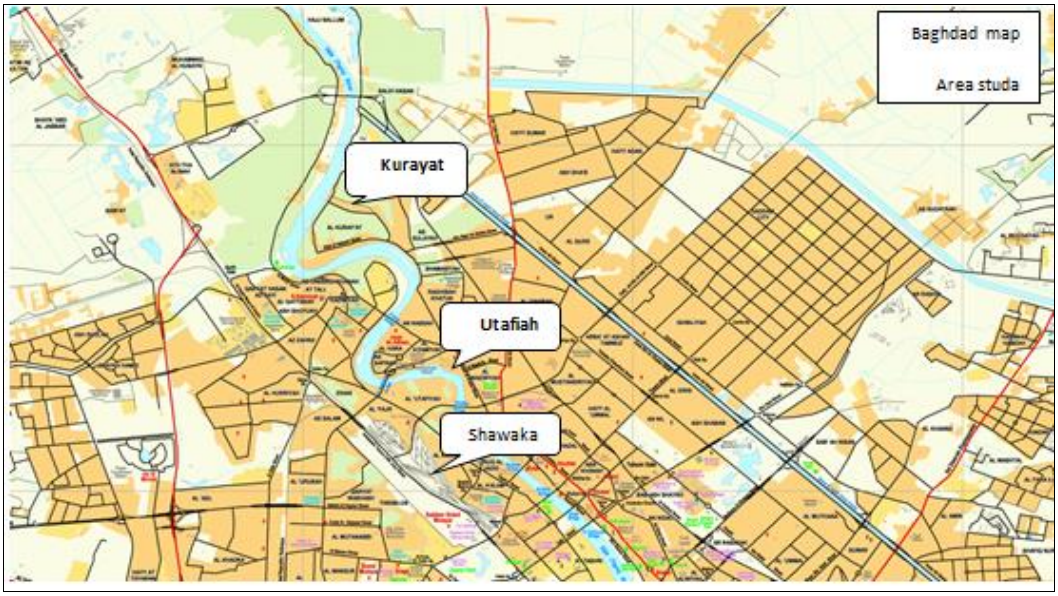

Figure1: A map showing the sampling area.

Fish sampling

Fish Samples were collected during the period from May to October 2015. The fish caugh by fish net and transported to the laboratory to conduct the required tests for the detection of bacterial infection.

Total bacteria count

The gut samples(10 gm of gut) removed from all examined fishes and putted in sterile containers (containing sterile normal saline). Five serial dilutions was conducted in normal saline (0.85% NaCl) then cultured on Nutrient agar media [9].

Bacterial Identification

Different differential and selective media have been used for isolation Enterobacteriaceae and non-Enterobacteriaceae group, there are the following:

a- Lactose and non- lactose ferment medium: CHROMagar™ Orientation used for identification Enterobacteriaceae and non- Enterobacteriaceae isolates, prepared according to the instruction for use. Identification the isolates according to the color change.

b- *Vibrio spp*. identification: CHROMagar™ Vibrio used for isolation and detection of *V. cholera*, *V. parahaemolyticus*, *V. vulnificus* Prepared and identification according to the instruction for use, and the color change. Alkaline peptone (pH 8.6), TCBS also used for identification *Vibrio spp.*

c- *Salmonella spp.* and *Shigella spp.* : Tetrathionate broth and XLD media used for isolate *Salmonella* spp. and *Shigella spp.*

All media were incubated at 37 ℃ for 24 hours.

Gram stain and Biochemical tests

Gram stain and various biochemical tests were used to identify the bacterial isolates. Biochemical tests include: oxidase test, IMVIC tests (indol, methyl red, Voges- Proskauer, citrate utilization), Kligler Iron agar test and Urease test [10], Red ring tests used for identification *V. cholera*e [8].

β- Lactamase production

CHROMagar™ ESBL medium (Extended Spectrum β-Lactamases) was used, and prepared according to the instruction for use.

Result and Discussion

This study included isolating bacteria from seven types of fish and two species *G. elegans* and *A. marmid* were more frequent than other types careless, as shown in Table (1).

Table (1): Type and number of isolated fish

Type of fish	Total number	Region
Acanthobra mamarmid	13	Shawakah, Kurayat, Utafiah
Alburnus mossuleusis	6	Shawakah, Utafiah
Carasobarbus luteus	3	Shawakah
Cyprinus carpio	3	Shawakah, Kurayat
Garra elegans	19	Shawakah, Kurayat, Utafiah
Garra rufa	1	Kurayat
Planiliza abu	5	Shawakah, Kurayat

This study identified 16 species of Enterobacteriaceae and 6 species of non- Enterobacteriaceae included four *Vibrio spp*. (Table 2, 3). Figures explains that *E. coli* presence is where are recorded in 96 % of the examined fish as well as *E. agglomerans* but at rates lower. *Vibrio* species most recording were *V. parahaemolyticus*, *V. alginolyticus* and *V. vulinficus*

either type *V. cholera* not recorded only twice in *A. marmid* and *G. elegans*.

Table (2): Type of Enterobacteriaceae isolated from fish sample

Type of fish	Type of Enterobacteriaceae	Total number
Acanthobrama marmid	*Citrobacter diversus*	2
	Enterobacter agglomerans	1
	Enterobacter gergoviae	1
	E.coli	10
	Proteus vulgaris	2
	Serratia rubidaea	1
Alburnus mossulensis	*Citrobacter frundii*	2
	Edwardsiella tard	1
	E.agglomerans	1
	E.coli	4
	Proteus vulgaris	1
	Providencia vettigen	1
Carasobarbus luteus	*E.coli*	2
	E. cloacae	1
Cyprinus carpio	*Citrobacter frundii*	1
	Enterobacter	1
	E.coli	3
	Klebsiella pneumonia	1
	Serratia marcesense	1
Garra elegans	*Citrobacter amalonobicas*	1
	Citrobacter diversus	1
	Citrobacter frundii	1
	Enterobacter agglomerans	2
	Enterobacter sakazaki	1
	E. coli	15
	Klebsiella oxytoca	2
	Proteus mirabilis	2
	Proteus vulgaris	2
	Serrati arubidea	2
	Serratia marcesens	1
Garra rufa	*E.coli*	1
Planiliza abu	*Citrobacter amalonobicas*	1
	Citrobacter diversus	1
	E.agglomerans	1
	E.coli	5
	Proteus vulgaris	1

Table (3): Type of *vibrio* spp. isolated from fish samples.

Type of fish	Type of *vibrio* spp.	Total number
Acanthobrama marmid	*Vibrio alginolyticus*	8
	Vibrio cholera	1
	Vibrio parahaemolyticus	5
	Vibrio vulinificus	7
Alburnus mossulensis	*Vibrio alginolyticus*	1
	Vibrio parahaemolyticus	1
	Vibrio vulinificus	2
Carasobarbus luteus	*Vibrio alginolyticus*	2
	Vibrio parahaemolyticus	2
	Vibrio vulinificus	2
Cyprinus carpio	*Vibrio alginolyticus*	1
	Vibrio parahaemolyticus	2
	Vibrio vulinificus	2
Garra elegans	*Pseudomonas* spp.	2
	Acinetobacter spp.	6
	Vibrio alginolyticus	10
	Vibrio cholera	1
	Vibrio parahaemolyticus	5
	Vibrio vulinificus	9
Garra rufa	*Vibrio alginolyticus*	1
	Vibrio vulinificus	1
Planiliza abu	*Vibrio alginolyticus*	1
	Vibrio parahaemolyticus	2
	Vibrio vulinificus	1

The isolated bacteria comprised spoilage bacteria such as:
P. mirabilis, *C. freundii* and *Acinetobacter spp*. and other opportunistic pathogens such as: *E. coli* and *E. cloacae*, and *K. oxytoca*, spoilage agent sometimes exist as an opportunistic pathogen under suitable condition [11]. The *E. coli* isolates were the common among the Enterobacteriaceae isolated from all type of fish samples [12]. The total bacteria count load in the gut of *G. elegans* fish was higher than in the gut of *A. marmid* and other types of fish as shown in Table (4).

Table (4): The upper and lower values of total bacteria count.

Type of fish	Highest count	Lowest count
Acanthobrama marmid	414×10^3	53×10^3
Alburnus mossulensis	220×10^3	50×10^3
Carasobarbus luteus	432×10^3	75×10^3
Cyprinus carpio	360×10^3	50×10^3
Garra elegans	456×10^3	103×10^3
Garra rufa	250×10^3	31×10^3
Planiliza abu	377×10^3	60×10^3

[13] explained that the total bacteria count in fish that exceeds ^{8}10 making them unfit for consumption therefore, the results of the present study can not be considered as a risk to the consumer. The contamination observed may result from rupturing fish gut during poor processing or inadequate washing agent for food spoilage [14]. Many previous study reported that presence of isolated bacteria, mainly *E. coli*, *Citrobacter*, *Enterobacter*, *Proteus*, *Klebsiella* and some species of *Vibrio* in fish present a health hazard to human [15].
The present results showed that 90% of isolated bacteria produce β- Lactamase which includes four groups (Carbapenems, Cephalosporins, Monobactams and Penicillins), prepared according to the instruction for use and Identification the isolates according to the color change as shown in table (5).

Table (5): Type of Isolated bacteria on CHROMagar™ ESBL medium.

Type of bacteria	Color of colony
Acinetobacter	Cream
Citrobacter	Metallic blue
Enterobacter	Metallic blue
Escherichia	Dark pink to reddish
Klebsiella	Metallic blue
Proteus	Brown halo
Pseudomonas	Translucent

Many *Pseudomonas spp*. and *Acienetobacter spp*. widely-known to be frequently multi drug resistant bacteria [16]. The resistance of bacteria to antibiotics in fishes was recorded in several publications throughout the world [17]. Disease outbreaks in marine organisms appear to be escalating worldwide [18]. Widespread antibiotic usage for treatment of human infection has resulted in an extensive spread of multidrug bacteria in various environment including water[19]. The consumption of fishes may be risk to the human health If the fish habitats are contaminated by pathogenic bacteria [20].
This study clearly showed variation in bacteria load in the gut of different specie fish sample therefore, the causes of bacterial diversity in the gut of fish can be due to the fact that the bacteria may be an essential part of the fish food as well as their presence as a normal flora in the fish gut [21]. bacterial infections play an important role as secondary infection and lead as a major risk that can result in very large losses of fisheries [22]. precaution should be taken to prevent water contamination during harvesting as well as post-harvest handling of fish, As well as the bacterial ecology of fish be linked to several environmental factors such as fish feed quality, handling, water pollution, transport and storage condition [23].

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المحتوى البكتيري للقناة الهضمية لأنواع مختلفة من الاسماك، جمعت من نهر دجلة في مدينة بغداد

ندى وليد حمود[1] ، اسراء عبد الجبار ابراهيم[2]

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الملخص

تم اجراء الدراسة البكتريولوجية على الأسماك للفترة من ايار الى تشرين الاول 2015, حيث جمعت 50 عينة من الأسماك من نهر دجلة المار في منطقة الكريعات, الشواكة والعطيفية في مدينة بغداد. وتم تسجيل سبعة انواع من الأسماك وهي: اسماك الحمري *Carasobarbus luteus*, اسماك السمنان الطويل *Alburnus mossulensis*, اسماك العرّيضة *Acanthobrama marmid*, اسماك الكارب الاعتيادي *Cyprinus carpio*, اسماك الكركور الاحمر *Garra rufa* واسماك كركور بلاد ما بين النهرين *Garra elegans* واسماك الخشني *Planiliza abu*. استعملت اوساط زرعية انتقائية لتشخيص البكتريا مثل وسط CHROMagarTM Orientation (لتشخيص مجموعة العائلة المعوية وغير المعوية), CHROMagarTMVibrio TCBS media (لتشخيص مجموعة الضمات), CHROMagarTM ESBL (لإنتاج انزيم البيتالاكتم), ووسط XLD (لتشخيص انواع السالمونيلا). اظهرت نتائج التحليل البكتيري ان التعداد الكلي للبكتريا في امعاء الاسماك تراوح بين 31 الى 456× 10^3 خلية/مل. وكانت البكتريا المعزولة تنتمي الى العائلة المعوية وهي:

Cirobacter amalonobicas ,*C. diversus* ,*C. frundii* ,*Edwardsiella tarda* ,*Enterobacter agglomerans*, *E. cloacae* , *E. gergoviae* ,*E. sakazaki* ,*Escherichia coli* ,*Klebsiella oxytoca*, *K. pneumonia* ,*Proteus mirabilis* ,*P. vulgaris* , *Providencia vettigen*, *Serratia marcesens* و *S. rubidaea* وشملت وغير المعوية *Acinetobacter* spp. ,*Pseudomonas* spp. ,*Vibrio alginolyticus* ,*V. cholera* ,*V. parahaemolyticus* و *V. vulinficus* .

هذه الدراسة زودتنا بنظرة عامة عن المحتوى البكتيري للقناة الهضمية للأسماك الاقتصادية وغير الاقتصادية.

الكلمات المفتاحية: الأسماك, الأمعاء, بكتريا الأسماك, نهر دجلة.