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# ISOLATION AND CHARACTERIZATION OF *CITROBACTER FREUNDII* FROM FRESH WATER COMMON CARP (*CYPRINUS CARPIO*) IN MOSUL CITY

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## ABSTRACT

*Citrobacter freundii* is an opportunistic pathogen in animals and humans. The current study aimed to isolate *C. freundii* from the intestines of common carp (Cyprinus carpio) and test their antibiotic susceptibility. For this purpose, 70 specimens of intestine were gathered from fish farms in Mosul city from October 2022 to February 2023. Initially, the samples were inoculated in Tryptone soya broth (TSB) and then cultured on both TSA and MacConkey agar. The suspected single colonies were streaked on the eosin methylene blue (EMB) and *Salmonella Shigella* agar. All suspected isolates were confirmed by manually prepared biochemical tests and the VITEK®2 compact, and then their sensitivity was determined against eight antibiotics. According to the cultural characteristics, biochemical tests, and VITEK®2 compact, 18 (12.6%) isolates out of 70 samples were *C. freundii***.** All isolates were highly susceptible to ciprofloxacin, amikacin, and gentamicin, moderately sensitive to tetracycline, ceftriaxone, highly resistant to amoxicillin and Cefixime, and low resistance to tobramycin. The presence of *C. freundii* in aquaculture represents many facets of challenges, with implications for public health, environmental stability, and the economic causes of fish farming.

***Keywords*****:** *Citrobacter freundii,* fish, VITEK$^2$**,** ciprofloxacin, amikacin

## INTRODUCTION

*Citrobacter freundii*, belongs to the family Enterobacteriaceae, is a Gram-negative rod that inhabits the digestive tract of fish (Liu *et al.*, 2020; Al-Asbahi, 2022). This bacterium acts as an opportunistic pathogen in animals and humans (Chen *et al*., 2018).

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Fish are considered an essential food for many world populations, because they contain a high percentage of animal protein (Wamala *et al.,* 2018). Many bacterial diseases can infect fish, leading to economic losses (Austin and Austin, 2007, Cortés-Sánchez *et al*., 2023).

Fish diseases are spreading due to the interaction between pathogens, hosts, and the aquatic environment. The prosperity of aquaculture relies on extensive research and active fish health management. (Türe and Kutlu, 2018; Jassim, 2024).

Aquatic products in many circumstances were correlated to epidemics of food borne illnesses everywhere in the world, wherever diverse causal agents, involving bacteria, have been ascertained **(**Al-Daraghi and Al-Behadili**,** 2021; Friesema *et al.,* 2022*;* Mahmmoud and Al-Dabbagh, 2022*)*. Deeming the animal healthiness theme, bacteria like *C. freundii* are common in freshwater, which can infect fish, also instigating diseases in other living organisms, mainly digestive diseases (Wang et al., 2018; Cortés-Sánchez *et al*., 2023). *C. freundii* is chiefly isolated in mingled infections and, occasionally, it is referred to as a subordinate and opportunistic invasive instigating injury and extreme mortality (Eissa, 2016). *Citrobacter braakii* and *C. freundii* are linked to illnesses in fish. The latter is linked to severe renal disease, severe enteritis, hemorrhagic septicemia, and catfish gill lesions. (Jia *et al*., 2020).

Antibiotics are currently utilized in fish farms to stop bacterial infections. However, researchers are quite concerned about the growth of antibiotic resistance, since it transfers bacterial drug resistance to consumers. (Ture and Alp, 2016; AL-dabbagh, *et al*., 2015).

*Citrobacter* spp. were allied to agents of a biological basis harmful to human and animal health (Aminharati *et al*., 2019). Therefore, this research aimed to isolate and identify C. *freundii* from common carp (*C. carpio)* intestines and test their antibiotic susceptibility.

## MATERIAL AND METHODS

### 1. Ethical approval:

All samples were taken in accordance with permission granted by Institutional Animal Care, University of Mosul, College of Veterinary Medicine, under authority number UM.VET.2021. 061.

### 2. Specimens Collection:

From October 2022 to February 2023, seventy fish samples were gathered from Mosul City's fish farms. Every sample was put in a sterile plastic bag and kept cold while being brought to the laboratory in the Microbiology Department, College of Veterinary Medicine.

### 3. Specimens Cultivation:

Intestine specimens were cultivated in Tryptone soya broth (TSB) with incubation at 25°C for 24 hr, and then the broth was cultivated on both TSA and MacConkey agar under the same previous conditions. A single colony was then cultured on the eosin methylene blue (EMB) and *Salmonella Shigella* agar. All plates were incubated at 28°C for 24-48 hr. (Chen *et al.,* 2018; Al-Haider *et al.,* 2019).

### 4. Phenotypic examination and biochemical reactions:

The putative isolates were examined microscopically to determine their morphology, motility, and Gram stain reactivity. The bacterial isolates underwent biochemical testing following (Al-Asbahi ,2022)

### 5. Identification of bacteria by Vitek $^{2}$ compact system.

All suspected isolates were examined by VITEK®2 compact, and the results were recorded and analyzed (Hashim and AlKhafaji, 2018; Ossman *et al*., 2024).

### 6. Antibiotic susceptibility test:

The test was done using the disc diffusion method on Mueller Hinton agar (40). Eight antibiotics supplied by (Bioanalyse) company were used, including ciprofloxacin (CIP 5µg), amikacin (Ak 30µg), amoxicillin (AX 10µg), ceftriaxone (CE 30µg), Cefixime (CFM 5), tobramycin (TOB 10 µg), tetracycline (TE 10µg), and gentamycin (CN 10µg). The results are classified following (CLSI, 2020).

## RESULTS

**1. Phenotypic characters and biochemical reaction results:**

Microscopically and phenotypically, features revealed Gram-negative bacilli. *C. freundii* colonies appeared large and irregular. On MacConkey agar, the bacteria grew and showed lactose-fermented colonies. On EMB agar, they showed brown colonies; on Salmonella Shigella agar, the colonies were black and smooth (Figure 1). According to the cultural characteristics of the studied isolates from *C. carpio* intestines, 18 (12.6%) isolates out of 70 samples were *C. freundii***.**

**2. Identification of bacteria by Vitek² compact system**:

The results of the Viteck® 2 compact system were similar to those of the bacterial culture and phenotypic characteristics, as the eighteen isolates were identified as *C. freundii* (Figure 2).

**3. Antibiotic susceptibility test:**

According to the resistance profile to antibiotics, the outcomes of this study revealed that all isolated bacteria were relatively highly sensitive to ciprofloxacin (100%), amikacin (88.8%), and gentamicin (83.3%), and moderately sensitive to tetracycline (77.7%) and ceftriaxone (66.6%). All of them were resistant to amoxicillin (100%) with high resistance to Cefixime (88.8%) and low resistance to tobramycin (11.1%) (Table 1).

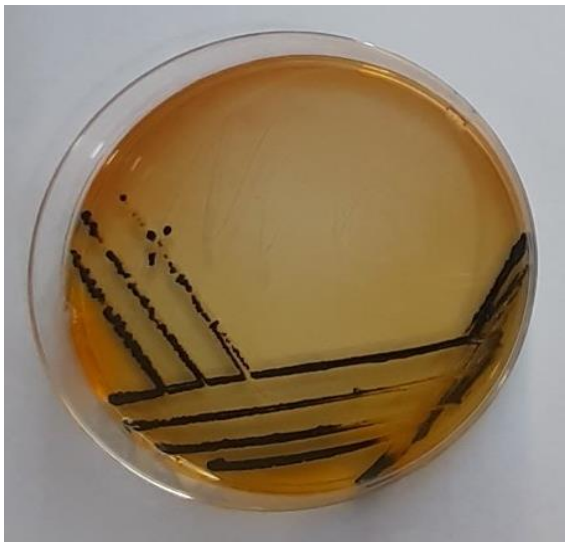

**Fig. 1:** Black colonies of *Citrobacter freundii* on Salmonella Shigella agar

bioMérieux Customer: Microbiology Chart Report

Patient Name: 5. 5 Patient ID: 747
Location: Physician:
Lab ID: 747 Isolate Number: 1

Organism Quantity:
**Selected Organism : Citrobacter freundii**

**Source:** **Collected:**

| **Comments:** | |
|---|---|

| **Identification Information** | **Analysis Time:** | 7.00 hours | **Status:** | Final |
|---|---|---|---|---|
| **Selected Organism** | 86% Probability | **Citrobacter freundii** | | |
| | **Bionumber:** | 4417611551526210 | | |
| **ID Analysis Messages** | | | | |

**Biochemical Details**

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | + | 5 | lARL | - | 7 | dCEL | - | 9 | BGAL | + |
| 10 | H2S | + | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | + | 14 | GGT | + | 15 | OFF | + |
| 17 | BGLU | - | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | - | 22 | BAlap | - |
| 23 | ProA | + | 26 | LIP | - | 27 | PLE | - | 29 | TyrA | + | 31 | URE | - | 32 | dSOR | + |
| 33 | SAC | + | 34 | dTAG | - | 35 | dTRE | + | 36 | CIT | + | 37 | MNT | - | 39 | 5KG | - |
| 40 | ILATk | + | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | - | 44 | AGAL | + | 45 | PHOS | - |
| 46 | GlyA | - | 47 | ODC | + | 48 | LDC | + | 53 | IHISa | - | 56 | CMT | + | 57 | BGUR | - |
| 58 | O129R | + | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

**Fig. 2:** Identification of *Citrobacter freundii* by Vitek 2 compact system

**Table 1:** Antibiotic sensitivity test of *Citrobacter freundii* isolated from the intestines of common carp (*Cyprinus carpio*) collected from fish farms

| Antibiotics (µg) | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | NO | % | NO | % | NO | % |
| Ciprofloxacin (CIP10) | 18 | 100 | 0 | 0 | 0 | 0 |
| Amikacin (AK 30) | 16 | 88.8 | 2 | 11.1 | 0 | 0 |
| Gentamicin (CN 10) | 15 | 83.3 | 3 | 16.6 | 0 | 0 |
| Tetracycline (TE 30) | 14 | 77.7 | 4 | 22.2 | 0 | 0 |
| Ceftriaxone (CE 30) | 12 | 66.6 | 6 | 33.3 | 0 | 0 |
| Tobramycin (TOB10) | 6 | 33.3 | 10 | 55.5 | 2 | 11.1 |
| Cefixime (CFM 5) | 0 | 0 | 2 | 11.1 | 16 | 88.8 |
| Amoxicillin (AX10) | 0 | 0 | 0 | 0 | 18 | 100 |

## DISCUSSION

Fish and fish products encourage the spread of pathogens, including *C. freundii*. Rigorous stock and heavy feeding systems impart a praising environment for the bacteria to multiply (Jia *et al.*, 2020). Controlling such conditions by enhancing biosecurity, improving water quality, and reducing stocking densities can help reduce the spread of pathogens (Mohammed *et al*., 2023). Studies have shown that fish in less stressful environments suffer fewer bacterial infections, making these practices essential for sustainable fish farming (Kousara *et al*., 2020). Therefore, fish-origin foodstuffs have been interconnected to eruptions of diseases worldwide because of many causes, involving bacteria (Imran *et al*., 2020).

In this study, the use of advanced identification techniques, like the Vitek$^2$ compact system, was crucial for rapid and accurate pathogen identification (Al-Daraghi and Al-Behadili, 2021; Ossman *et al*., 2024). The accessibility and cost of these methods can be easy and inexpensive, therefore recommended especially in resource-limited regions. The evolution and execution of fee-reasonable diagnostic tools could

significantly benefit fish farmers and magnify local biosecurity efforts (Jassim *et al.*, 2019; Mala and Abdullah, 2022).

The outcome of the current study revealed the fundamental appearance of *C. freundii* in fish samples with a reasonable ratio (12.6%). Much research has recorded the isolation of these bacteria as one of the pathogens of fish in different percentages (de Pádua *et al*., 2014; Abid and Al-Hamdani, 2016; Garabawi *et al.*, 2022). The seasonal variation in water characteristics and dense stock status results in stress that permits repeated infection with opportunistic pathogens in fish cultivated **(**Al-Haider *et al*., 2019**).** Outbreaks of diseases caused by pathogens like *C. freundii* can lead to considerable economic losses for fish farmers and disrupt food supply chains, impacting food security. Therefore, it requires extra attention to effective management to control pathogens in fish farms that protect local wealth and ensure a stable food supply, contributing firmly to public health and economic benefits (Aminharati *et al*., 2019; Jassim, 2024).

Although *C. freundii* isolates had previously been identified as multidrug–resistant (MDR) strains (de Pádua *et al*., 2014), the current isolates exhibited sensitivity to several antibiotics, including ciprofloxacin, amikacin, gentamycin, tetracycline, and ceftriaxone, with no signs of resistance. These findings align with previous studies by (Mohammed *et al*., 2023) and (El-Barbary and Hal, 2017). Conversely, the resistance was obvious with amoxicillin, cefixime, and a moderate rate resistance for tobramycin. This susceptibility to multiple antibiotics seen in our study may be attributed to the fish sources examined, potentially originating from farms that do not heavily rely on extensive antibiotics for treatment.

Recording this wide range of sensitivity to antibiotics paves the way for using these antibiotics in treating diseases that may be caused by these bacteria in fish farms (Smith, 2019).

# CONCLUSION

The presence of *Citrobacter freundii* in aquaculture poses numerous challenges, including implications for public health, environmental stability, and the economic viability of fish farming. Effectively controlling this pathogen necessitates a combination of scientific approaches and practical procedures tailored to specific aquaculture settings.

## CONFLICT OF INTEREST

According to the authors. There are no conflicts of interest.

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# عزل وتوصيف الستروباكتر فروينديي من اسماك المبروك العادي في مدينة الموصل

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تمثل السيتروباكتر فروينديي مُمْرِضات انتهازية في الحيوانات والبشر. هدفت هذه الدراسة إلى عزل بكتيريا سيتروباكتر فروينديي من أمعاء سمك المبروك العادي واختبار حساسيتها للمضادات الحيوية. لهذا الغرض، جُمعت سبعون عينة من امعاء سمك الكارب من مزارع الأسماك في مدينة الموصل بين تشرين الأول ٢٠٢٢ وشباط ٢٠٢٣. حُقنت العينات أولًا في بيئة التربتون صويا، ثم زُرعت في كلٍّ من وسط التربتون صويا أجار و الماكونكي اجار .المستعمرات المنفردة المشتبه بها خططت على كل من وسط اجار ازرق المثيلين والايوسين وأجار السالمونيلا شايغيلا. تم تأكيد جميع العزلات المشتبه بها من خلال الاختبارات البيوكيميائية المُحضرة يدويًا وباستخدام جهاز الفايتك ٢، وتم تحديد حساسيتها لثمانية مضادات حيوية. بناءً على الخصائص الزرعية والاختبارات البايوكيميائية واختبار الفايتك٢ ، كانت ١٨ (١٢,٦٪) عزلة من أصل ٧٠ عينة تعود لجراثيم سيتروباكتر فريونديي. أظهرت جميع العزلات حساسية عالية نسبيًا للسيبروفلوكساسين والأميكاسين والجنتاميسين، ومتوسطة الحساسية للتتراسيكلين والسيفترياكسون. في المقابل، أظهرت جميعها مقاومة عالية للأموكسيسيلين والسيفيكسيم ومقاومة منخفضة للتوبراميسين. يُشكل وجود جراثيم سيتروباكتر فريوندي في تربية الأحياء المائية تحديات متعددة الجوانب، تُشكل تهديدًا خطيرًا للصحة العامة، والاستقرار البيئي، والتأثيرالاقتصادي على تربية الأسماك.