

**FIRST REPORT, PATHOLOGY AND ANTIMICROBIAL
PROFILE OF AN INFECTION WITH *CITROBACTER*
YOUNGAE IN A SPECIES OF WILD-CAUGHT CARP
IN TÜRKIYE**

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Abstract

Citrobacter spp. are classified as Gram-negative, non-spore forming, rod-shaped, facultative anaerobic bacteria that have been identified in a variety of environmental and biological specimens. These include soil, sewage, mud, water, food, and the intestines of humans and animals. It has been determined that *Citrobacter* spp. accounts for 3–6% of all isolates from the Enterobacteriaceae family that have been implicated in nosocomial infections. The aquacultural industry produces fish as a highly nutritious foodstuff. This product is marketed and consumed worldwide in a variety of forms and culinary preparations, and constitutes a significant part of the human diet. Nevertheless, fish is also highly susceptible to deterioration and contamination by microorganisms at every stage of the food chain. The extant literature contains an insufficient amount of information regarding *Citrobacter youngae* and its implications for human and animal health. The present study detected *C. youngae* in four different carp species, which were obtained from natural sources by culture and MALDI-TOF method. Furthermore, the study determined the pathological findings and antibiotic sensitivity of the pathogen. A total of 48 fish samples were analyzed, and *C. youngae* was isolated from 26 samples, including liver, kidney, gill and muscle tissues. The fish samples exhibited symptoms such as abdominal bloating, exophthalmos, petechial haemorrhages on the

skin and haemorrhages at the bases of the fins, around the anus and mouth, especially in the upper and lower jaws. *C. youngae* demonstrated susceptibility to ampicillin, ampicillin-sulbactam, amoxicillin-clavulanate, piperacillin-tazobactam, cefepime, ceftriaxone, imipenem, ciprofloxacin, levofloxacin, moxifloxacin, gentamicin, amikacin, and tobramycin. However, resistance was observed for trimethoprim-sulfamethoxazole, cefotaxime, ceftazidime, ertapenem, and meropenem. Consequently, *C. youngae* was identified in wild-caught carp species, signifying the inaugural documentation of this bacterium with regard to both clinical observations and antibiotic profiles. Rigorous monitoring of this bacterium is imperative in the context of both aquaculture and public health.

Key words: antibiotic, carp, *Citrobacter youngae*, fish, MALDI-TOF

Introduction. The term “fish” is defined as any food that can be extracted from oceanic or terrestrial waters and utilized for the purpose of human or animal nutrition. Fish is regarded as a conventional and popular foodstuff in diverse regions of the world, with some countries utilizing it as the primary source of animal protein. Nevertheless, an increasing number of individuals are opting for fish as a health-promoting food option, not only due to its status as a source of protein with high biological value and digestibility, but also due to its content of polyunsaturated lipids, vitamins and minerals [1]. Microorganisms have the capacity to survive in a variety of natural environments and can be transferred to foodstuffs. Furthermore, the presence of hazards that compromise the health and perception of consumers has consequences for the acceptability of fish for consumption and the contribution of this food to human nutrition and health [2]. Significant economic losses are incurred as a consequence of bacterial diseases in the context of aquaculture. Seasonal fluctuations in water quality have been demonstrated to result in recurrent infections with opportunistic pathogens, which are precipitated by stress [3]. Enterobacteriaceae represents a significant group of infectious bacteria that have been identified as causative agents of disease in fish. Notable cases of disease have been associated with *Citrobacter* spp., which have been isolated from various sources including fish, animals, humans, soil, water, and food waste [4]. The genus *Citrobacter* comprises a total of 26 species [5]. Of these, *Citrobacter freundii* has been isolated from various fish species, including carp, Atlantic salmon, catfish, and rainbow trout [6]. As stated in the research by ALTUN et al. [7], *Citrobacter braakii* was isolated from the intestines of channel catfish and rainbow trout. *Citrobacter youngae* (*C. youngae*) was first described in 1993, and data and clinical findings on human diseases caused by this bacterium are still insufficient [8]. *Citrobacter* has been designated as an opportunistic hospital pathogen, frequently associated with urinary tract infections, bloodstream infections, intra-abdominal sepsis, brain abscesses, pneumonia and other neonatal infections, including meningitis, neonatal sepsis, joint infections and disseminated bacteremia [9]. As THAU et al. [10] note, most reports of *C. youngae* infections are of localized cases, with no evidence of bacteremia, particularly in immunocom-

promised patients. With regard to aquaculture, the literature contains a paucity of information regarding the clinical findings caused by the bacteria. The objective of this study is to furnish information on *C. youngae* from a comprehensive standpoint on animal health and the safety of fish and fish products. Diseases caused by *Citrobacter* sp. are incorporated in the control, prevention and hygiene legislation actions that are intended to promote animal health and the safety of aquatic foods, and to protect public health. In the present study, *C. youngae* was detected by culture and MALDI-TOF in four different carp species caught from nature, and clinical findings were determined, as well as the antibiotic susceptibility of the agent.

Material and method. In October 2024, a total of 48 carp samples were collected using nets from the Tigris River and subsequently identified to species. The species identified were *Carassius gibelio* Bloch, 1782, *Carassius auratus*, *Cyprinus carpio carpio* L., 1758, and *Cyprinus carpio*. The number of samples per species was 10, 9, 13, and 16, respectively. The average weight of the samples ranged from 100 to 300 grams. The necropsy method was executed in two distinct phases: an external examination and an internal examination. During the external examination, a comprehensive analysis was conducted of the skin, mouth, eyes, gills, anus, operculum, and fins, and the findings were meticulously recorded. The aforementioned data were recorded in accordance with the methodology described by SCHÄPERCLAUS et al. [11]. The internal (post-mortem) examination was then carried out in accordance with the procedures outlined by AUSTIN and AUSTIN [6]. Following the necropsy, a comprehensive examination of the internal organs was performed, including the liver, gallbladder, pancreas, kidney, spleen, gastrointestinal tract, swim bladder, gonads, and heart. The isolation of bacteriological samples involved the transportation of tissue samples to a microbiology laboratory, where they were cultivated in various agar media. Specifically, the samples were inoculated into blood agar (Merck, 110886, Darmstadt, Germany), MacConkey agar (Merck, 05465, Darmstadt, Germany), and eosin methylene blue agar (EMB agar) (Merck, 103858, Darmstadt, Germany). Following incubation under aerobic conditions at a temperature of 28 °C for a period of 18–24 hours [12], the samples were then subjected to serial passages on nutrient agar (Condalab, 1060, Madrid, Spain) and 5–7% sheep blood agar (Merck, 110886, Darmstadt, Germany). Purification was undertaken under identical conditions, and isolates were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry MALDI-TOF MS (VƏTEK MS, Biomerieux, France), (Fig. 1). The disk diffusion method was utilised for the purpose of antibiotic susceptibility testing, with ampicillin (10 µg), ampicillin-sulbactam (10–10 µg), amoxicillin-clavulanate (20–10 µg), piperacillin-tazobactam (30–6 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (10 µg), ceftriaxone (30 µg), ertapenem (10 µg), meropenem (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), moxifloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg) and Trimethoprim-

sulfamethoxazole (1.25–23.75 µg). The isolates were plated on 5% sheep blood agar and incubated for 24 h. Following this, bacterial suspensions with a turbidity of 0.5 McFarland were prepared from the colonies that grew purely, and antibiotic disks (Bioanalyse, Turkey) were placed after spreading on the surface of the medium. The zone diameter was measured after 16–20 hours of incubation (Fig. 1).

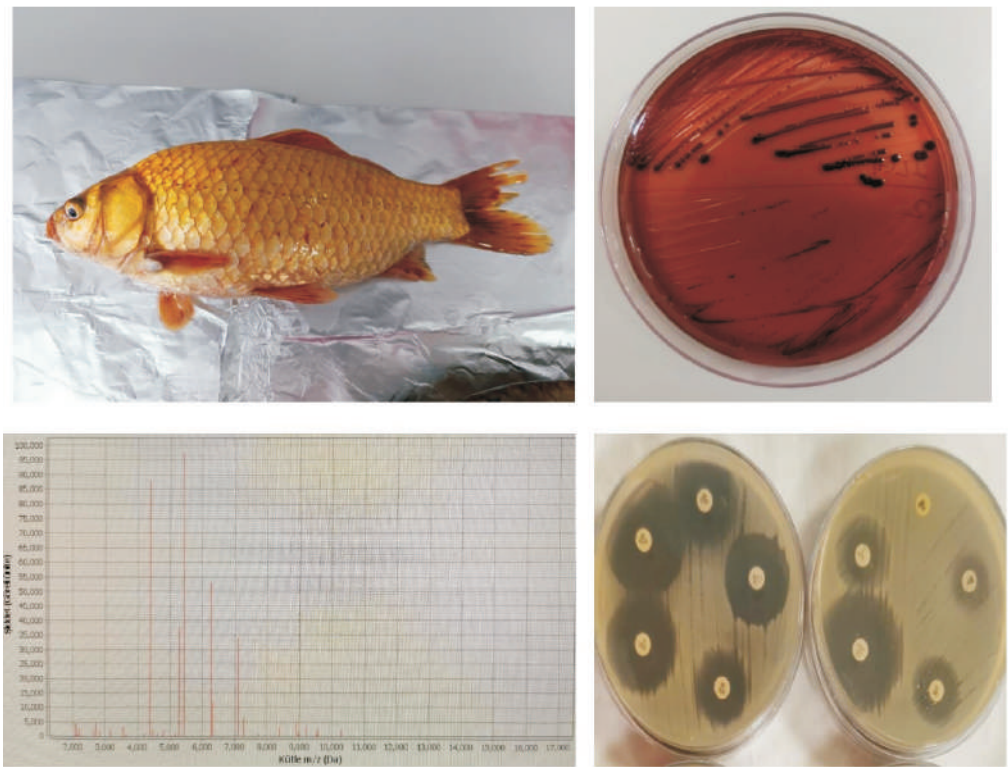


Fig. 1. Fish samples, colony morphology of *Citrobacter youngae*, MALDI-TOF image, and antibiotic zone diameters

Results. A comprehensive examination of the fish samples revealed several notable findings, including abdominal distension, exophthalmos, petechial haemorrhages in the skin, and haemorrhages at the bases of the fins, around the anus and mouth, particularly in the upper and lower jaws. Post-mortem examination revealed enteritis, a pale liver, and granulomas in the kidneys and spleens of infected individuals. The clinical findings observed for each species are presented in Fig. 2.

In this study, we looked at 48 fish samples and found *C. youngae* in the liver, kidneys, gills and tissues of 26 of them. We mostly found the pathogen in *Cyprinus carpio* and *Cyprinus carpio carpio* L., 1758, with some also being found in *Caras-*

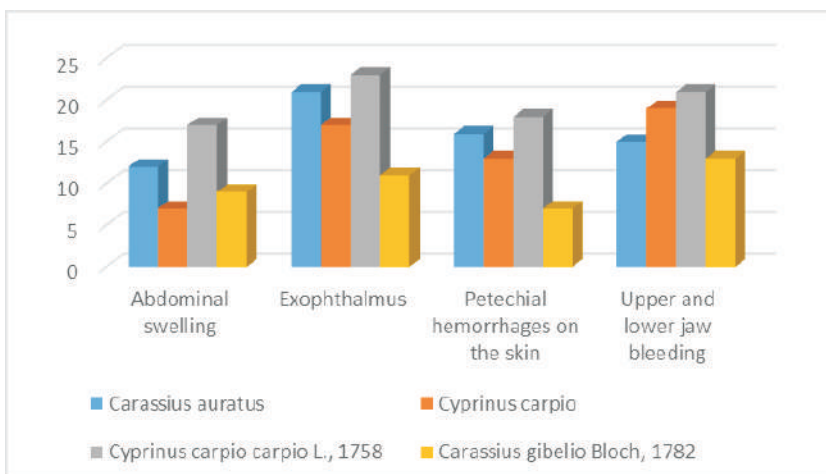


Fig. 2. The most common clinical findings for each species

sius auratus and *Carassius gibelio* Bloch, 1782. *C. youngae* was predominantly detected in liver, kidney, muscle and gill tissues, respectively. Antibiotic resistance patterns of *C. youngae* were evaluated by measuring the size of inhibition zones around each disc. The results demonstrated that *C. youngae* exhibited susceptibility to ampicillin, ampicillin-sulbactam, amoxicillin-clavulanate, piperacillin-tazobactam, cefepime, ceftriaxone, imipenem, ciprofloxacin, levofloxacin, moxifloxacin, gentamicin, amikacin, and tobramycin. However, resistance was observed to trimethoprim-sulfamethoxazole, cefotaxime, ceftazidime, ertapenem, and meropenem (Table 1).

Discussion. *Citrobacter* species are generally acknowledged within the field of human and veterinary medicine, including aquaculture, as opportunistic pathogens [13]. These pathogens are distinguished by their ability to produce immunosuppressive factors, such as stress and pollution, which contribute to the emergence of diseases. As a bacterial contaminant, *Citrobacter* spp. has been identified as a contributing factor to foodborne diseases and is frequently transmitted through food and water. In the human population, *Citrobacter* infection has been demonstrated to affect the central nervous system and is responsible for 75% of meningitis cases that result in brain abscesses. Its presence has also been identified in the gastrointestinal system, and due to its opportunistic character, it is known to cause urinary tract infections, septicemia and diarrhea [14]. The pathogen has been shown to be capable of causing hemorrhages and hemorrhagic enteritis in fish; the increase in the concentration of inorganic and organic substances in the water, environmental pollution and stress are reported as key parameters in the emergence of infection [15]. The study also yielded analogous findings in carp. Overall, petechial haemorrhages in the skin and haemorrhages at the bases of the fins, around the anus and mouth, especially in the upper and lower jaws, were detected

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Classification system (S for susceptible and R for resistant) has been employed to ascertain susceptibility to antimicrobial agents in *C. youngae*

Antimicrobial agent	Paper content (µg/piece)	Isolate 1	Isolate 2	Isolate 3
Ampicillin	10	S	S	S
Ampicillin-sulbactam	10	S	S	S
Amoxicillin-clavulanate	10–20	S	S	S
Piperacillin-tazobactam	6–30	S	S	S
Trimethoprim-sulfamethoxazole	1.25/23.75	R	R	R
Cefepime	30	S	S	S
Cefotaxime	30	R	R	R
Ceftazidime	10	R	R	R
Ceftriaxone	30	S	S	S
Ertapenem	10	R	R	R
Meropenem	10	R	R	R
Imipenem	10	S	S	S
Ciprofloxacin	5	S	S	S
Levofloxacin	5	S	S	S
Moxifloxacin	5	S	S	S
Gentamicin	10	S	S	S
Amikacin	30	S	S	S
Tobramycin	10	S	S	S

in the samples. A study conducted on Nile tilapia reported that the pathogen in question caused necrotising fasciitis and osteomyelitis. In this study, it was observed that the infection reached the muscle tissue. In general, alterations in abiotic environmental factors, such as diminished oxygen levels, frequent fluctuations in water temperature and pH, augmented ammonia and carbon dioxide concentrations, and elevated stocking density, have been demonstrated to engender animal stress and render animals susceptible to bacterial infections. These alterations can, in turn, promote the proliferation of pathogens and precipitate outbreaks. A body of research has identified sudden fluctuations in temperature, low dissolved oxygen levels, and high ammonia levels as common factors associated with cytotuberculosis [16]. *Citrobacter* species are naturally susceptible to a range of antimicrobial agents, including tetracycline, nitrofurantoin, chloramphenicol, sulfonamides, trimethoprim, nalidixic acid, polymyxins, fluoroquinolones and fosfomycin. However, other *Citrobacter* species have been observed to demonstrate resistance to these same agents [17] vancomycin, lincosamides, fusidic acid, erythromycin, and *C. braakii* is resistant to bacitracin, erythromycin, tetracycline, ampicillin, rifampin, and penicillin. The study revealed that *C. youngae* demonstrated sensitivity to ampicillin, ampicillin-sulbactam, amoxicillin-clavulanate, piperacillin-

tazobactam, cefepime, ceftriaxone, imipenem, ciprofloxacin, levofloxacin, moxifloxacin, gentamicin, amikacin, and tobramycin. However, resistance was observed for trimethoprim-sulfamethoxazole, cefotaxime, ceftazidime, ertapenem and meropenem. A high-allergy drug examination in various organisms is a feasible technique that can clearly decrease antibiotic dosage. However, there are some risks associated with the use of antibiotics. Firstly, the medicine can penetrate biological membranes and tissues, for example, it can pass through blood vessels in the brain. Secondly, prolonged use of antibiotics can lead to bacterial resistance, drug accumulation in fish tissues, and environmental problems [18].

Conversely, the extant literature review has not identified any instances of *Citrobacter youngae* infection in fish farms. The genus *Citrobacter* comprises 11 species: The *Citrobacter freundii*, *Citrobacter koseri*, *Citrobacter amalonaticus*, *Citrobacter braakii*, *Citrobacter farmeri*, *Citrobacter sedlakii*, *Citrobacter werkmanii*, *Citrobacter youngae*, *Citrobacter rodentium*, *Citrobacter gillenni*, and *Citrobacter murlinae* are the *Citrobacter* species under consideration. Of these, *Citrobacter freundii* has been reported to cause gastroenteritis and severe hemorrhagic septicemia in salmon, carp and rainbow trout farms. The recurring challenges posed by seasonal shifts in water quality and the intensive management of livestock result in a state of stress for the common carp (*Cyprinus carpio*) cultivated in this environment. This stress facilitates the reinfection of the fish with opportunistic pathogens [19].

In the human population, *Citrobacter* has been classified as an opportunistic hospital pathogen, frequently associated with urinary tract infections, bloodstream infections, intra-abdominal sepsis, brain abscesses, pneumonia, and other neonatal infections, including meningitis, neonatal sepsis, joint infections, and disseminated bacteremia. Consequently, regular monitoring of this bacterium is imperative.

Conclusion. In conclusion, *Citrobacter youngae* has been detected in carp species captured in the wild. There are clinical findings caused by the pathogen. The widespread and uncontrolled use of antimicrobial drugs can lead to the development of multiple resistance to antimicrobials. Therefore, antimicrobial susceptibility testing should be performed regularly in *Citrobacter* genus infections and other bacterial infections. Conversely, the pathogen should be monitored regularly for public health reasons.

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements.

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