



Study of some Bacterial Pathogens that Infect Common Carp (*Cyprinus Carpio*) in the Diwaniya River and their Relationship to Water Characteristics

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Abstract | A total of 100 fishes was collected in the Diwaniya River during the period extending from December 2022 to November 2023, Fish were caught using three types of nets The seine net, gill nets and cast net. Seven diseases were bacterial It is diagnosed by clinical signs that appear from severe infections of external and internal organs (Bacterial gill disease, Tail and fin rot, Infection abdominal dropsy, Columnaris Disease, Enteric red mouth disease, Bacterial enteritis, Vibriosis). Bacteria were identified according to their shape, characterization and use of the Vitek II system, and they include: *A. hydrophila*, *A. veronii*, *P. putida*, *A. hydrophila*, *B. columnaris*, *F. columnaris*, *C. columnaris*, *F. columnare*, *E. cloacae*, *P. fluorescens Intestinalis*, *Streptococcus sp.* The temperature of the water varied between 11.2°C to 33.8°C, the salinity values varied from 0.50°C to 0.69°C, and the O₂ levels vary from 4.5 mg/L to 9.0 mg/L, pH levels, 8 at 6.1. The results of CCA showed the identification of temperature, ammonia, salinity, pH, nitrite, and BOD₅ as the most important water specifications due to a correlation in the spread of bacteria. The study findings underscore the to determine the effect of water quality on the spread of bacteria in common carp (*Cyprinus carpio*. L).

Keywords | Pathogenic bacteria, *C. carpio*, Water quality, Vital test

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INTRODUCTION

Bacteria are the main cause of diseases in farmed fish and cause significant economic losses. The actual role of bacteria in causing such diseases may vary or differ from primary to secondary causes, as some of these bacteria live in a putrefactive form, but when the fish are weakened, they infect them and cause a specific disease state (Ara-venaromán *et al.*, 2014). The incidence of disease is higher

in fish farms than in natural waters No matter how much humans try to bring the living conditions of fish closer to those in their usual environment, these conditions remain far from natural conditions, due to the overcrowding of fish resulting from intensive farming, and the competition of fish for food and environmental space All agricultural operations are accompanied by specific factors and changes in the physical, chemical and biological properties of the water, as well as the accumulation and decomposition of feed

or pollution resulting from external factors (Basha *et al.*, 2013). Inglis (2014) showed that bacterial diseases in fish farming depend on the presence of pathogenic fish and the quality of the water in general, although these factors may or may not affect the health of the fish. Water, especially water with a high organic content, is a good medium for the growth of many types of bacteria, and many researchers consider the bacteria naturally present on fish to be a reflection of the bacteria present in water in general. (Hassan *et al.*, 2017, Jonikaite *et al.*, 2018). Al-Shemmari (2017) noted that information on the effect of water quality on the presence of bacteria under actual field conditions is limited, and that most previous studies have only described bacteria isolated from either water, sediments, or various fish organs.

Pathogenic bacteria associated with fish can be classified into two groups, indigenous bacteria and non-indigenous bacteria (those that contaminate fish or otherwise pollute their environment). Examples of non-indigenous bacteria include *Clostridium botulinum*, *Listeria monocytogens*, *Shigella sp*, *Staphylococcus aureus*, and *Salmonella sp*, while indigenous bacteria are those that naturally inhabit the fish environment, such as *Vibrio spp* and *Aeromonas spp* (Eze *et al.*, 2011).

Study objective: The effect of water quality on the spread of bacteria that infect common carp (*Cyprinus carpio* L.) in the Diwaniya River.

MATERIALS AND METHODS

FISH SAMPLES COLLECTION

AL-Diwaniya River is an extension of the Al-Hilla River which is a branch of Euphrates River, in the middle of Iraq. It is 123 km long, 25-30m wide and 3-5m depth. The samples were collected from two sites in the river between Daghghara barrier through Sinniyah district to the AL-Diwaniya city (Figure 1).

The samples were collected monthly from during the period extending from December 2022 to November 2023, was carried out along the river between Daghghara barrier to the AL-Diwaniya city using seine net (3m long and 2.5m depth with a 20mm mesh size), gill nets (25m long with 20x20, 30x30 and 50x50mm mesh sizes), cast net (9m diameter with 15x15mm mesh size). The fish were transported alive to the Pathology Laboratory/College of Veterinary Medicine/Al-Qasim Green University within a few hours, and were killed. Fish damage the spinal cord. I took swabs from the skin, gills, intestines, and lesions found on the skin and gills of the fish for bacterial culture. The total length of each fish was measured to the nearest 0.1 cm, and the total weight of each fish was measured to the nearest gram using a one-pan scale.

WATER SAMPLES

Water samples were collected monthly from different locations in the river using sterile glass bottles of one liter capacity for testing some water specifications. Water samples were also collected in a 500 ml bottle and the water was filtered with 0.45-micron filter paper to measure chlorophyll and other water samples were collected in opaque bottles for testing (BOD5). Nitrate was measured according to the method described by Parson *et al.* (1984) in the laboratory which is based on reducing nitrate to nitrite by passing the sample through a cadmium column after activation where 50 ml of sample water is collected and the concentration is read using a spectrophotometer at a wavelength of 543 nm and the result is expressed in micrograms of nitrogen atoms/liter after taking the average of two readings for each sample. Reactive phosphate the method described by Parson *et al.* (1984) was followed using a spectrophotometer at a wavelength of 885 nm, and the result was expressed in micrograms of phosphorus atoms/liter after taking the average of the two readings for each sample.



Figure 1: Map of field sampling sites in River.

The method described in APHA (2005) was followed for the determination of ammonia, where a drop of 50% EDTA was added to 5 ml of water sample in a 25 ml standard beaker and mixed well, then 2 ml of Nessler reagent was added and completed to the mark with deionized water, the resulting yellow color was measured after 10 minutes by spectrophotometer at a wavelength of 420 nm.

MEASURING CHARACTERISTICS OF RIVER WATER

Six environmental characteristics of water were measured in the field using a multi-Meter device, including water temperature (C°), pH, and salinity concentration (mg/L). Dissolved oxygen (mg/L) was also measured using a DO-meter. Current velocity and Transparency (cm/s) were also measured.

ETHICAL CONSIDERATION

The current study has followed the accepted principles of ethical conduct by College of Veterinary Medicine / Al-Qasim Green University (No: 2023, 5/09/2023).

BACTERIAL ISOLATION AND IDENTIFICATION

The fish were collected, dissected, and aseptic samples were obtained from the skin, gills, fins, and intestine using sterile

loops once they arrived at the laboratory. MacConkey agar medium was used for the isolation of bacteria. To encourage bacterial growth, the infected plates were incubated for 24 hours at 37°C.

TOTAL COUNT OF BACTERIA

I prepared quartets of sterile test tubes and put nine ml of sterile distilled water in each one. Then I added one ml of river water to the first tube, and shook the tube well so that it became 1×10^{-1} . One milliliter was taken from the first test tube and added to the second test tube and collected. Well and it became 1×10^{-2} , so that it could be transferred to the eighth test tube and it became 1×10^{-8} . The solid nutrient agar medium was prepared and sterilized in the mu-ezzin. Once it cooled, it was poured into two Petri dishes containing 1 milliliter of 1×10^{-6} and 1×10^{-8} , and the water in the ears was connected to the nutrient medium. After it reached the nutrient medium, it was placed in a thermal incubator at a temperature of 37°C for 48 hours. The bacterial colonies were counted on a bacterial counting device, according to the total number of bacteria from the result is recording the number of colonies in the reciprocal of the unused one, and the result is the total number of bacteria in 1 ml of river (Andrews, 1992).

PREPARING THE CULTIVATION MEDIA

SOLID BLOOD MEDIUM: It is the most widely used medium and supports most common fast-growing organisms. This medium is prepared by dissolving 40 grams of powder in 1000 ml of distilled water, then placing it in the autoclave for sterilization at a temperature of 121 °C and a pressure of 15 lbs for 15 minutes. Then cool to 50°C, add 5% blood in a sterile environment, mix well, then pour into sterile dishes (Atals *et al.*, 1995).

McCONKEY SOLID MEDIUM: This culture medium is considered one of the most widely used selective and differential media for Gram-negative intestinal bacteria. The medium is prepared by dissolving 51.53 grams of the powder in 1000 ml of distilled water, then placed in the incubator for sterilization at a temperature of 121°C and a pressure of 15 pounds for 15 minutes, and it is cooled to 47 °C. 0 and mix well then pour into dishes

DIAGNOSIS BACTERIA USING VITEK 2: Gram-negative and Gram-positive bacteria were diagnosed using the VITEK2 in the Public Health Laboratory of the Babylon Health Department. Three cards were used in this study to diagnose various microorganisms:

- **GN:** Used to diagnose Gram-negative aerobic bacteria, fermenting and non- fermenting lactose.
- **GP:** It is used to diagnose spherical aerobic bacteria and non-spore-forming Gram-positive bacilli and bacilli.
- **AST-GN82:** It is used to test the sensitivity of Gram-negative bacteria to antibacterial agents.

STATISTICAL ANALYSIS

The ready-made statistical program for statistical analysis SPSS Statistics V. 19 was used to test the differences between the means for all tests, and standard correspondence analysis (CCA) was used to study the relationship between bacterial species and environmental factors at the study stations.

RESULTS AND DISCUSSION

During the current study, which extends from December 2022 to November 2023, 100 common carp fish were collected and examined from the Diwaniyah River. The highest average for total length was (46.31 cm) and the lowest average for total length was (22.83 cm). As for total weight, the highest average was (1100.5 g) and the lowest average was (345.63 g).

CHARACTERISTICS OF RIVER WATER

The temperature of the water varied significantly, from a minimum of 11.2°C in Feb to a maximum of 33.8°C in Aug. The highest water temperature was in the Aug and the lowest in the Feb. The temperature rises in the summer months and decreases in the winter. This is due to the nature of the climate of Iraq and is consistent with many studies, including (Al-Amari, 2011; Al-Khalidi, 2012; Khadiri, 2014; Hussein *et al.*, 2015; Yassin *et al.*, 2017; Al-Jabouri, 2019). The salinity values varied from 0.5o°C in Apr to 0.69°C in Oct, the high pH values in the winter months are attributed to the presence of aquatic plants and phytoplankton, which leads to an increase in the efficiency of photosynthesis and then leads to the consumption of carbon dioxide in the water, which raises the pH values towards the base (Al-Lami *et al.*, 2001). the O2 levels vary from 4.5 mg/L in Aug to 9.0 mg/L in Feb. The decrease in dissolved oxygen values in summer with the rise in temperature is due to the decrease in its solubility when temperatures rise, in addition to the effect of the river water by the organic wastes discharged into it, the analysis of which requires the consumption of dissolved oxygen (Al-Fahrawi, 2010). As for the increase in dissolved oxygen values in winter, which is due to the decrease in temperatures that lead to an increase in the solubility of oxygen (Dabrowski *et al.*, 2014), the results of the current study agreed with the studies of (Al-Amari, 2011; Khadiri, 2014; Al-Hali, 2019). Although pH levels showed relatively narrow fluctuations, with March recording the high average value of 8 and Aug the lowest at 6.1, these variations are typical in the river (Figure 2). The results of the statistical analysis showed no significant differences ($P > 0.05$) between the study sites. The study observed a rise in bacterial numbers in water corresponding to elevated water temperatures between 25°C and 32°C, increase organic the topic, salinity, and a pH range of 5 to 9, aligning (Chankaew *et al.*, 2023).

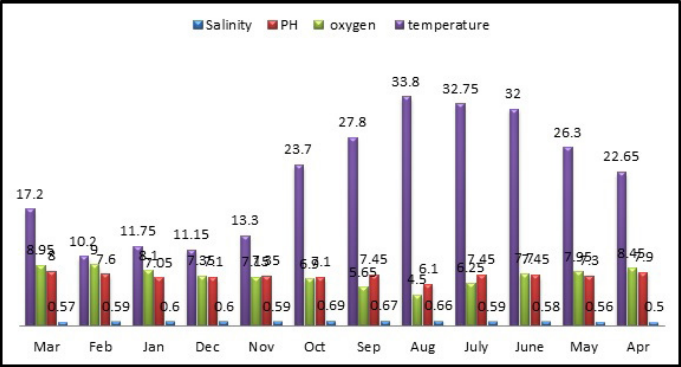


Figure 2: Monthly changes in water Tem, O2, salinity, and pH in River.

Table 1: Pathogenic bacteria and disease in C. carpio.

Pathogenic bacteria	Diseases	Fish spp
A. hydrophila, A. veronii	Bacterial gill disease	Cyprinus carpio
P. putida	Tail and fin rot	
A. hydrophila	dropsy	
Bacillus columnari	Columnaris Disease	
Flexibacter, columnaris		
Cytophaga columnaris,		
Flavobacterium columnare		
E. cloacae	Enteric red mouth disease	
Pseudomonas fluorescens. Intestinalis	Bacterial enteritis	
Streptococcus sp	Vibriosis	

Changes in the values of BOD₅ in the Diwaniya River The lowest values were recorded at 0.8 mg/L in February. While the highest values were recorded at 3.1 mg/L in September. Changes in nitrate values, ranging from 1.205-2.33 micrograms of nitrogen atom - nitrate / liter. The highest values were recorded as 0.23 micrograms of phosphorus-phosphate atoms/liter in the effective phosphate values, while the lowest values were recorded as 0.002 micrograms of phosphorus-phosphate atoms/liter in the month of May. While the monthly changes in ammonia values were, the highest value was 2.8 mg/L in August and the lowest value was 0.7 mg/L in December. Monthly changes in chlorophyll values were 4.2-6.7 µg/L. The results showed some environmental characteristics, some of which were significant and others were not significant. Suboptimal physical and chemical properties of water such as oxygen solubility, pH, salinity, ammonia, and temperature are an important aspect of an aquaculture system and can cause stress to farmed fish, reducing their resistance to diseases (Al-Jubouri, et al., 2023). fish exposed to various stressors become highly susceptible to infections (Hade et al., 2022). Zhang et al. (2016) showed that bacterial growth increases in water due to high levels of organic matter, high salinity, high water temperature from 25°C to 32°C, and pH between 5-9. The results of the present study are consistent with the

study of Ismail et al. (2016), as temperature and ammonia affected the diversity and presence of bacteria in hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) cultured in floating cages in Malaysia.



Picture 1: C. carpio with infection Bacterial gill disease.

BACTERIAL DIAGNOSIS

During the current study, which extends from December 2022 to November 2023, there were seven bacterial diseases of common carp fish at the study stations (Table 1) that were diagnosed. They include: Bacterial gill disease, the pathogen is *A. hydrophila* and *A. hydrophila. veronii* symptoms were swelling and congestion of the gills and distortion of the gill capillaries in the fish (Picture 1). Tail and fin rot disease, the pathogen is the *bacterium Pseudomonas putida*, and the symptoms are corrosion and wasting of the fins and skin (Picture 2). Infection abdominal dropsy the pathogen is *A. hydrophila* bacteria. Symptoms include bulging of the eyes, in addition to an enlargement of the abdomen (swelling) to collect pink fluids with foul odors, along with the presence of hemorrhagic lesions on the outer surface of the body and fins (Picture 3). Columnaris Disease The pathogen is *Bacillus columnaris*, *Flexibacter columnaris*, *Cytophaga columnaris*, and *Flavobacterium columnare*. Symptoms include brown to yellowish lesions on the gills, skin, and fins (Picture 4). Enteric red mouth disease, the pathogen is the bacterium *E. cloacae*. The symptoms were inflammation and redness in and around the mouth, in addition to the lining of the mouth (Picture 5). Bacterial enteritis is caused by the bacteria *P. fluorescens. Intestinalis* and the symptoms were redness and swelling in the exit area (Picture 6) Vibriosis The pathogen is *Streptococcus sp* bacteria and the symptoms were bleeding ulcers on the skin, redness of the skin, loss of scales, and fin rot (Picture 7).

In this study, the bacterial pathogens that cause *Cyprinus carpio* in carp recorded in the current study. were consistent with the study of Al-Haider et al. (2019) on carp in Hilla River, (Hadi et al., 2022; Al-Jabouri et al., 2023;

Alwan *et al.*, 2023; Al-Jabouri *et al.*, 2024) on carp in Di-waniyah River. It is consistent with what Marcel *et al.* (2013) found on tilapia fish cultured in floating cages in Malaysia. The researcher attributed this to the fact that water quality and animal and human activities play a role in the susceptibility of red tilapia fish to pathogenic bacteria. The current study agreed with (Tesfaye *et al.*, 2018) *Pseudomonas aeruginosa* was isolated from the skin and intestine, *Escherichia coli* from the skin, gills and intestine, and *Klebsiella* from the gills and liver of three fish species from Lake Haik, Ethiopia (common carp, African trout, and Nile tilapia).

aca, *C. freundii*, *E. coli*, *Klebsiella* sp., *Proteus mirabilis* and *P. vulgaris*. Austin (2007) explained that explained *Vibrio* Bacteria cause economic losses of fish farms of about 50%. Bacterial disease outbreaks are largely responsible for high mortality rates in wild and farmed fish and cause severe economic damage Fish farm losses *Aeromonas*, *Vibrio*, *Edwardsiella* and *Streptococcus* species are among the most common fish species, causing economically devastating losses in aquaculture (Al-Jubouri *et al.*, 2023). Mao *et al.* (2012) and Sebastião *et al.* (2015) pointed out that serious diseases threaten the farming of various fish, such as *Oncorhynchus clarkii* trout, *Oncorhynchus mykiss* rainbow trout, and the European eel *Ahlstromi Monognathus* due to the bacteria *Pseudomonas* spp. These bacteria, *Aeromonas* spp. As well as a number of other pathogenic bacteria, isolated from sick fish, become fatal if not treated early.



Picture 2: *C. carpio* with infection Tail and fin rot disease.



Picture 4: *C. carpio* with infection Columnaris disease.



Picture 5: *C. carpio* with infection Columnaris disease.



Picture 3: *C. carpio* with infection abdominal dropsy.



Picture 6: *C. carpio* with infection Bacterial enteritis.

Abdel Latif and Siddiq (2017) isolated several species of Enterobacteriaceae from Nile tilapia *Oreochromis niloticus* suffering from skin bleeding and ulcers with obvious focal bleeding and areas of liver necrosis and congestion in gills and spleen in Beheira Governorate in the Arab Republic of Egypt. These species are *K. aerogenes*, *E. clo-*

THE RELATIONSHIP BETWEEN WATER QUALITY AND BACTERIAL PROLIFERATION

Figure 3 shows the Canonical Correspondence Analysis (CCA) of the relationship between bacteria species and environmental factors during the current study period, which amounted to 12 months. The highest Eigenvalue for the

horizontal axis Axis 1 was 17.44% and it reached 15.24% for the vertical axis Axis 2. The bacterial groups were divided into two groups. The first includes *A. veronii* (AeVe), *Pseudomonas putida* (EnAe), *Aeromonas hydrophila* (EnCl), and *Pseudomonas putida* (CrDu), whose spread was positively affected by the following environmental factors: nitrate (NO₃) and biological oxygen requirement (BOD). Ammonia (NH₃), temperature (Temp.), salinity (Salinity), and phosphate (PO₄), while they were negatively affected by the following environmental factors: dissolved oxygen (DO), transparency (Trins), current velocity (Vel.), pH, and chlorophyll A (Chl). During the fall semester (September, October, and November) and July. The second group included the bacteria *Flexibacter columnaris* (StAu), *Bacillus columnaris* AlFa, *Cytophaga columnaris* (StPs), *Flavobacterium columnare* (StTh), *E. cloacae* (KySe), *Klebsiella oxytoca* (KIOx), and *A. sobria* (AeSo) and *Pseudomonas fluorescens*. *Intestinalis* (CiFr) and *Streptococcus* sp (PrHa), the spread of which was positively affected by the following environmental factors: dissolved oxygen, transparency, current speed, pH, and chlorophyll A, while it was negatively affected by the following environmental factors: nitrate (NO₃), biological oxygen demand (BOD), ammonia (NH₃), and temperature. Salinity and phosphate during the spring (March, April, and May), the winter (December, January, and February) and June. The results of the current study are consistent with the study of [Ismail et al. \(2016\)](#), as temperature and ammonia affected the diversity and presence of bacteria in hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) cultured in floating cages in Malaysia. In the current study stations, temperature, ammonia, nitrite, biological oxygen demand (BOD₅) and salinity were found to be the most environmental factors that positively affected the diversity and presence of bacteria. and study are consistent with the study of [Al-Haider \(2019\)](#), as temperature and ammonia, nitrite, biological oxygen requirements, and salinity are the most important specifications that were monitored, due to the strong associations between them and the presence of bacteria in common carp cultured in floating cages in Hilla River. This is because most of the fresh surface water, including the Diwaniyah River, is polluted with sewage water, which is a source of bacterial pollution, especially near residential areas, as a result of the discharge of sewage water without treatment. Therefore, fresh water is a carrier of most types of disease-causing bacteria ([Amal et al., 2015](#)).

[Marjabujat, 2013](#); [Ismail et al., 2016](#); [Oliveira et al., 2017](#); [Wanga et al., 2019](#)) that managing and monitoring water quality is important to ensure fish health, and the quality of aquaculture in general is affected by various chemical and bacteriological factors, because water quality directly affects the productivity and health of fish. Differences were found in the specifications of culture water at the study stations, which affected the presence and spread of bacteria. High

concentrations of non-ionized ammonia may increase susceptibility to bacterial infections, as well as other environmental factors involved, such as temperature changes, low dissolved oxygen, and harmful algal blooms, which likely contribute to increased susceptibility to disease. However, high ammonia concentrations may still be harmful. Ionization alone increases susceptibility to *S. agalactiae* ([Evans et al., 2006](#)). This was considered traditional Carp farms have a positive impact on aquatic ecosystems and aquatic environments negatively, as a result of the different types of farms that use the variable Degrees of water circulation and contaminated water with its waste ([Teodorowicz, 2013](#)). this may indicate the ways of transmission of bacteria from water to fish as a result of pollution or other factors such as stress. The aquatic environment is the final recipient of a greater number of pollutants as a result of the discharge of industrial, agricultural and urban wastes, the change in water quality results in sewerage water ([Al-Jubouri et al., 2024](#)).



Picture 7: *C. carpio* with infection Vibriosis disease.

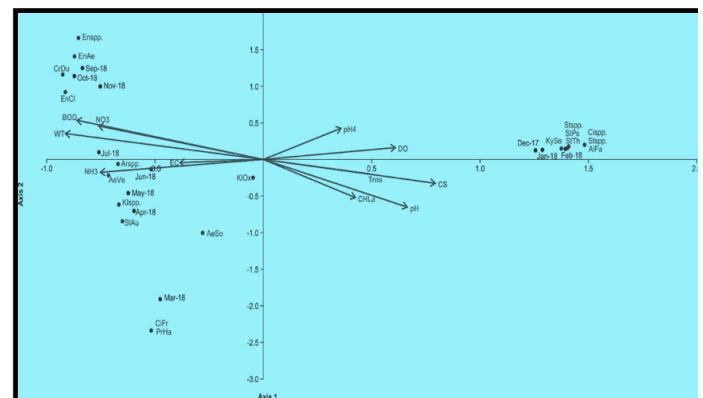


Figure 3: Canonical correspondence analysis (CCA) of the relationship between bacteria types and water characteristics.

CONCLUSIONS AND RECOMMENDATIONS

The presence of an effect of environmental factors on the spread of bacteria in fish, especially temperature, ammonia, nitrite, biological oxygen demand and salinity in the Diwaniya River. Seven bacterial diseases belonging to ten genera of Gram-negative and Gram-positive aerobic bac-

teria were diagnosed, indicating the extent of the spread of these bacteria in the aquatic environment in the study area. Spread environmental awareness among citizens to avoid throwing waste from residential activities directly into the river without treatment, and conduct studies to determine the level of water pollution due to its important and dangerous impact on public health.

ACKNOWLEDGEMENTS

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NOVELTY STATEMENT

The majority of this study findings underscore to determine the effect of water quality on the spread of bacteria in common carp (*Cyprinus carpio*). Ideas differ over the best objectives to employ, and the inadequacy of diagnostic tools make it challenging to compare labs.

AUTHOR'S CONTRIBUTIONS

Contributions from each author were equal.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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