Isolation of *Citrobacter* species from common carp, *Cyprinus carpio* cultivated in floating cages at Al-Hilla river, Babylon province

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Abstract. About 144 moribund of the common carp were examined were brought to the laboratory for examination after an epizootic of citrobacteriosis on cultivated in floating cages at Al-Hilla river, during the period December 2017- November 2018. The bacterium was identified by Vitek II system. The fish displayed dark color and hyperemia of the mouth. The possible etiologic agent was identified by Vitek II system as *Citrobacter* spp. *Citrobacter* species were identified with a different percentage in common carp, which are *C. freundii*, *C. amalonaticus* and *C. braakii*. Antibiotic susceptibility test of 12 antibiotics, which are piperacillin/ tazobactan, amikacin, cefazolin, gentamicin, cefoxitin, ciprofloxacin, ceftazidime, levofloxacin, cefepime, tigecycline, imipenem and trimethoprim/ sulfamethoxazole by showed variable resistances for *Aeromonas* species. *Aeromonas* species were identified and examined for antibiotic susceptibility using the Vitek II system. The results indicate that the bacteria sensitive to Minimum Inhibitory Concentration (MIC) of levofloxacin (<= 0.12 μg/ml), imipenem and ciprofloxacin (<= 0.25 μg/ml), but resistant to cefazolin (>= 64 μg/ml) and trimethoprim/ sulfamethoxazole (40 μg/ml).

1. Introduction
Economic casualties consequent to bacterial diseases are considerable in aquaculture. The seasonal changes in water quality and intensive stock conditions result in stress which allows repeated infection at opportunist pathogens in common carp cultivated [1; 2; 3].

Enterobactericeae is an infectious bacteria and important group of infectious bacteria, able of causing disease in fish industry [4]. Notable cases of disease was concerning to *Citrobacter* spp., which was isolated form fish, animal, human, soil, water and food waste [5]. Bacteria from genus *Citrobacter* was examined for studies of resistance mechanisms of antibacterial in catfish [6] and pathogenesis in rainbow trout caused by *C. freundii* [7]. *Citrobacter* spp. can cause infection in under stressful conditions, animals and humans [8; 9].

Outbreaks of the infectious disease are usually caused changing in environmental state and stress. Sudden fluctuation of temperature, crowding, low dissolved oxygen level, high ammonia level is the common factors related with citrobacteriosis [10; 11]. Due to the little information pertains to *Citrobacter* infection in the fish farms particularly in Iraq, the aim of the present study is to identify the prevalence and antibiotic resistance in common carp, *C. carpio* cultivated in floating cages in Al-Hilla river and the susceptibility pattern of bacteria to 12 antimicrobial drugs.
2. Materials and Methods
A total of 144 fish of common carp from four farms of floating cages at Al-Hilla, the first and second farms, before the city center and the third and fourth farms, after the city center. Were sampled during the period December 2017- November 2018. The fish length ranging between 25- 40 cm and the weight was 303-1006 g. The live fish were transported to oxygenated pond water. Before transferred to the laboratory in College of Veterinary Medicine, University of Al-Qasim Green.

The collected fish were dissected and bacterial swabs were taken aseptically using a sterile loop from skin, gill and intestine. For isolation of bacteria, MacConkey Agar medium was used. The inoculated plate was incubated at 37 °C for 24 h. Bacteria were identified and antibiotic susceptibility (piperacillin/ tazobactan, amikacin, cefazolin, gentamicin, cefoxitin, ciprofloxacin, cefazidime, levofoxacin, cefepime, tigecycline, imipenem and trimethoprim/ sulfamethoxazole by using the Vitek II system and biochemical tests (glucose fermentation, oxidase, lactose fermenting, methyl red and citrate).

3. Results and Discussion
The results of present study showed that C. freundi, C. amalonaticus, C. braakii and Citrobacter sp. isolated and identified from the four floating cages farms in Al-Hilla river are a dangerous and may be unhealthy for public health when consumed. Citrobacter species (Table 1) were identified with different percentages from common carp (Table 2). The Citrobacter species causes main disease problems in the carp farms [12].

The study was conducted from December 2017 till the end of November 2018 in which there are fluctuations in water quality parameters in the studied farms (Table 3). The mean of temperature fluctuated from 10.7 °C to 32.9°C. The mean of salinity was recorded ranged from 460 to 580 ppt, ammonia ranged from 1.3 to 2.8 mg.l⁻¹. The pH value was from 6.4 to 8.1. In culture system of fish that always exposed an assortment of stresses become over sensitive to disease infection [13; 14]. The numbers of bacteria in water increased by rising levels of water temperature from 25 °C to 32°C, organic matters, salinity and pH 5-9 [15]. These levels of growing bacteria were observed in the current study.

Table 1. Citrobacter spp. isolated from the skin, gills and intestines of common carp fish

| Bacteria            | skin | Gills | Intestines |
|---------------------|------|-------|------------|
| Citrobacter freundii| +    | +     | +          |
| C. amalonaticus      | +    | +     | -          |
| C. braakii           | -    | -     | +          |
| Citrobacter spp.     | +    | -     | +          |

(+) found, (-) not found

Table 2. Percentage (%) of isolated Citrobacter spp. isolated from the skin, gills and intestines of common carp

| Station | C. freundii | C. amalonaticus | C. braakii | Citrobacter sp. |
|---------|-------------|-----------------|------------|-----------------|
| 1       | 1.96        | 1               | 0          | 2.94            |
| 2       | 1.83        | 0               | 0.96       | 1.83            |
| 3       | 0.96        | 0.96            | 0.96       | 0.96            |
| 4       | 2.85        | 0.95            | 0          | 2.85            |
| Total   | 8.58        | 1.92            | 2.91       | 7.6             |
Table 3. The water parameters of studied stations.

| Station | Temp (°C) | Salinity (ppt) | Ammonia (mg.l⁻¹) | pH |
|---------|-----------|----------------|------------------|----|
|         | Range (mean) |                |                  |    |
| 1       | 11.9 - 32.7 (22.66) | 480 - 560 (517.5) | 1.3-1.8 (1.62) | 6.8 - 8.1 (7.36) |
| 2       | 10.7 - 32.9 (22.9) | 460 - 570 (516.66) | 1.4-1.9 (1.69) | 6.5 - 8.1 (7.33) |
| 3       | 13 - 32.6 (23.8) | 480 - 580 (530.8) | 2.0-2.8 (2.42) | 6.4 - 7.6 (7.09) |
| 4       | 12.5 - 31.2 (22.86) | 460 - 560 (513.33) | 0.7-1.4 (1.21) | 7.1 - 7.9 (7.35) |

The best antibiotics susceptibility were Levofloxacin (<= 0.12 μg/ml), Imipenem and Ciprofloxacin (<= 0.25 μg/ml), when tested in vitro on *C. freundii* and *C. braakii* while, it was resistant to Cefazolin (>= 64 μg/ml) on *C. freundii* (Table 4 and 5). In vitro, the more antibiotic susceptibility to *C. braakii* was Levofloxacin (<= 0.12 μg/ml), resistant to Cefazolin and Cefoxitin (<= 4 μg/ml) on *C. braakii* (Table 4 and 5). Of them is antimicrobial drugs, it is the common antibiotic for controlling the aquatic bacteria, so antimicrobial sensitivity experiments are crucial for an efficient therapy [9; 16].

Table 4. Antibiotic susceptibility of *Citrobacter freundii*.

| Antimicrobial | MIC* | Interpretation | Antimicrobial | MIC | Interpretation |
|---------------|------|---------------|---------------|-----|---------------|
| Ampicillin    |      |               | Amikacin      | <= 2 | S             |
| Piperacillin/ Tazobactan | <= 4 | S             |               |     |               |
| Cefazolin     | >=64 | R             | Gentamicin    | <= 1 | S             |
| Cefoxitin     | <=64 | R             | Nitrofurantion| <= 16 | S           |
| Ceftazidine   | <=1  | S             | Ciprofloxacin | <= 0.25 | S        |
| Ceftiaxone    | <=1  | S             | Levofloxacin  | <= 0.12 | S        |
| Cefepime      | <=1  | S             | Tigecycline   | <= 0.5  | S         |
| Ertapenem     | <=0.5| S             | Trimethoprim/Sulfa methoxazole | <= 20 | R  |
| Imipenem      | <= 0.25 | S         |               |     |               |

*MIC: Minimum Inhibitory Concentration (μg/ml), S: Sensitive, R: Resistant.

Table 5. Antibiotic susceptibility of *Citrobacter braakii*.

| Antimicrobial | MIC | Interpretation | Antimicrobial | MIC | Interpretation |
|---------------|-----|---------------|---------------|-----|---------------|
| Ampicillin    |     |               | Amikacin      | <= 2 | S             |
| Piperacillin/ Tazobactan | <= 4 | S             |               |     |               |
| Cefazolin     | <=4  | R             | Gentamicin    | <= 1 | S             |
| Cefoxitin     | <=4  | R             | Nitrofurantion| <= 16 | S           |
| Ceftazidine   | <=1  | S             | Ciprofloxacin | <= 0.25 | S        |
| Ceftiaxone    | <=1  | S             | Levofloxacin  | <= 0.12 | S        |
| Cefepime      | <=1  | S             | Tigecycline   | <= 0.5  | S         |
| Ertapenem     | <=0.5| S             | Trimethoprim/Sulfa methoxazole | <= 20 | R  |
| Imipenem      | <= 0.25 | S         |               |     |               |

*MIC: Minimum Inhibitory Concentration (μg/ml), S: Sensitive, R: Resistant.

*Citrobacter* species are naturally susceptible to tetracycline, nitrofurantoin, chloramphenicol, sulfonamides, trimethoprim, nalidixic acid, polymyxins, fluoroquinolones and fosfomycin, other *Citrobacter* species, they are resistant to vancomycin, lincosamides, fusidic acid, erythromycin and *C. braakii* is resistant to bacitracin, erythromycin, tetracycline, ampicillin, rifampin and penicillin [6].
High allergy of drug examination in various organisms is feasible technique that can clearly decrease antibiotic dosage, on the other hand, there are some risks for using antibiotics, firstly, the medicine can penetrate the biological membrane and tissues, for example, it can pass through blood vessels in the brain, secondly, using antibiotics for a long time causes the resistance of bacteria, drug accumulation in fish tissues and environmental problems [7; 18; 19; 20; 21; 22].

4. Conclusion
Extensive and uncontrolled use of antimicrobial drugs may generate a multiple resistance for antimicrobial. So, the test of antimicrobials susceptibility in genus Citrobacter infection and other bacterial infections must be under intensive in cultivated fish. The water should be orderly changed and stress and overcrowding in the fish population should be avoided to prevent the infection.

5. Acknowledgements
Sincere thanks to Assis. prof. Dr. Abdul Kareem Salman Al-Yassari from the College of Veterinary Medicine, University of Al-Qasim Green, Iraq, for the help with some bacterial analyses.

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