



## Case Report

# *Klebsiella pneumoniae* as a Main Cause of Infection in Nishikigoi *Cyprinus carpio* (Carp) by Inadequate Handling

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## Abstract

*Klebsiella* spp. is a genus of the gram-negative Enterobacteriaceae family. They are found in many environments and are part of the natural microflora of soils, effluents and surface water and in some plants. *Klebsiella* spp. is an opportunistic agent and mainly attacks subjects hospitalized with severe diseases as well as many fragile animals. This paper reports the occurrence of *Klebsiella pneumoniae* infection in a nishikigoi carp, *Cyprinus carpio*, widely used as an ornamental fish in aquaria, for food, and for production of pituitary extracts for induction of reproduction. In a closed system used for the domestic cultivation of ornamental fish, a carp was found with an injury indicative of bacterial infection. A tissue sample from a lesion was triturated, plated on Blood Agar, and incubated at 37° C for 24 hours to confirm and isolate the possible pathogen. Biochemical tests were conducted to identify the strain based on manual methods described in the literature. In accordance with the phenotypic and biochemical tests conducted, *Klebsiella pneumoniae* was the organism present in the carp lesion.

**Key words:** aquaculture, ornamental fish, public health.

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## Introduction

The genus *Klebsiella* spp. (Enterobacteriaceae) was named in honor of Edwin Klebs in 1885 by Trevisan. *Klebsiella* spp. are gram-negative microorganisms found in different environments and are part of the natural microflora of soils, effluents, and natural water environments and in some plants. Among the species found in this genus, *Klebsiella oxytoca* and *Klebsiella pneumoniae* are known human pathogens and are responsible for several cases of severe infections and deaths in hospital settings (5).

*Klebsiella* spp. are opportunistic agents and mainly attack individuals hospitalized with severe diseases (e.g., *Diabetes mellitus* and AIDS) and other pathologies, such as urinary tract infections, bacteremia, chronic atrophic rhinitis, arthritis, enteritis, sepsis, and meningitis in children (3). *K. pneumoniae* is commonly known as the cause of pneumonia, and colonization in humans occurs from various sources, usually environmental, that have suffered some type of anthropic change (5). *K. pneumoniae* is a microorganism reported in hospital settings, but there are no reports in the literature showing the occurrence of these bacteria in infectious processes in fish.

This paper reports the occurrence of infection by *K. pneumoniae* in a nishikigoi carp, *Cyprinus carpio* Linnaeus, 1758, widely used as ornamental fish in aquaria and eventually as a food source.

### Case Report

In a closed system for the domestic cultivation of ornamental fish (an ornamental lake), a nishikigoi carp was found with an injury indicative of bacterial infection. For the confirmation and isolation of the possible pathogen, a tissue sample from the lesion on the animal (left side, near the caudal peduncle) was triturated, plated on Blood Agar and incubated at 37°C for 24 hours. The presence of a single colony type was observed after growth, rose colored, brilliant, with an elevated aspect and mucoid consistency.

Staining was performed by the Gram method and indicated that the organism was gram negative. Identification was facilitated by seeding additional samplings in MacConkey Agar to obtain a pure cell to carry out the manual biochemical tests (Urease, Citrate, Lysine, Gas and Lactose) based on the methodology described by (7).

The biochemical tests identified *K. pneumoniae* as the microorganism in the injured tissue. As opposed to most infections that occur by *Aeromonas* spp., we could observe the difference between *K. pneumoniae* and *Aeromonas* spp. through the biochemical tests. In our tests, *K. pneumoniae* showed positive results for urease, citrate, lysine, gas, and lactose, proving that the infection was caused by this microorganism. However, the hypothesis of an infection by *Aeromonas* spp. was rejected because *Aeromonas* spp. only presented a positive result for lysine, using the previously described tests. The hypothesis suggested that the colonization of *K. pneumoniae* might have initially occurred because of an infection contracted through improper handling, leading to the transmission of this bacterium to the fish. (Figure 1).



**Figure 1.** Infectious process caused by *Klebsiella pneumoniae*

Most of the infections caused by *Klebsiella* spp. occur in hospital settings. The number of infections by these microorganisms increases with the changes in antimicrobial susceptibility. The transmission of this organism can occur through direct contact or any other contact that will disperse the bacteria in the hospital environment and affect hospitalized patients (1, 4).

For this study, it can be inferred that the transmission path of the microorganism can be attributed to the improper handling of the fish, the improper maintenance of the culture system, or even the contamination of the water source of the supplying lake; nonetheless, a microorganism that is not part of the natural microflora environment was introduced, thus causing a serious infectious processes within the animal (6).

Researchers (8, 9) conducted a study on food handlers to search for possible causes of potential microbial contamination, where *K. pneumoniae* and *Enterobacter aerogenes* were found to be microorganisms that may be potential contaminants in the hands of food handlers.

Comparing these two studies, both studies show the danger of an inadequate asepsis in the transmission of pathogens in food and other products or organisms that may come into contact with humans.

Brown and Seidler, 1973 (2) reported the isolation of *K. pneumoniae* in the Oregon River near a pulp mill that casts its effluents into the river. Therefore, it can be stated that this bacteria comes from anthropogenic sources and not from natural sources.

### Conclusion

*K. pneumoniae* was present in the lesion on the carp. The infectious process caused by this microorganism occurred due to inadequate manipulation by food handlers. It can be concluded that an inadequate asepsis can cause many infections leading to problems in fish health.

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### References

1. SEIDLER RJ., TALBOT HW JR., MORROW JE. Isolation of Klebsiellae from within living wood. **Appl. Environ. Microbiol.**, 1978, 36, 178-85.
2. BROWN C., SEIDLER RJ. Potential pathogens in the environment: *Klebsiella pneumoniae*, a taxonomic and ecological enigma. **Appl. Microbiol.**, 1973, 25, 900-4.

3. CARPENTER JL. *Klebsiella* pulmonary infections: occurrence at one medical center and review. **Rev. Infect. Dis.**, 1990, 12, 672-82.
4. DIAS RS., SANTOS DN., FERNANDES TMG., FERREIRA JGG. Infecção hospitalar-IH-causas múltiplas e fatores de risco associados a microrganismos de veiculação hídrica. **Rev. Tecer.**, 2012, 1, 54-60.
5. FIERER J. Biofilm formation and *Klebsiella pneumoniae* liver abscess: True, true and unrelated? **Virulence**, 2012, 3, 241-2.
6. MERCANTE CTJ., MARTINS YK., CARMO CF., OSTI JS., PINTO CSRM., TUCCI A. Qualidade da água em viveiro de Tilápia do Nilo (*Oreochromis niloticus*): caracterização diurna de variáveis físicas, químicas e biológicas, São Paulo, Brasil. **Bioikos**, 2012, 21, 79-88.
7. MURRAY PR., BARON EJ. **Manual of clinical microbiology**. Washington, DC: ASM Press, 2007.
8. SANTOS BHC., SOUZA EL., SOUSA CP., SERRÃO LHC., AMARAL WC. Manipuladores como causas potenciais de contaminação microbiana de alimento enteral. **Infarma**, 2004, 15, 11-2.
9. ZEGARRA JJQ., BOTTEON RCCM., OLIVEIRA BCRS., BOTTEON PTL., SOUZA MM. Pesquisa de microrganismos em utensílios, leite e queijos de produção artesanal em unidades de produção familiar no município de seropédica, rio de janeiro. **Ciênc. Anim. Bras.**, 2009, 10, 312-21.