

DETERMINATION OF THE ANTIBIOTIC SUSCEPTIBILITY
PROFILE OF *AEROMONAS VERONII*, WHICH CAUSES
SEPTICEMIA IN WILD-CAUGHT CARP SPECIES, USING
MALDI-TOF AND CULTURE METHODS

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Abstract

The genus *Aeromonas* is recognised as containing numerous pathogenic species, with the bacterium *Aeromonas veronii*, an example of this genus, having been documented as the causative agent of disease in both humans and animals, either as a primary infection or as a concurrent infection with other pathogens. In this study, *A. veronii* was detected by culture and MALDI-TOF in four different species of carp caught in the wild. In addition, pathological and antibiotic susceptibility of the pathogen were determined. Eighty fish samples were analysed and *A. veronii* was isolated from 52 samples including liver, kidneys and muscle tissues. It was observed that the gills, kidneys and livers of the fish examined were pale in colour. In addition to erythema, haemorrhages and superficial ulcerative lesions on the skin of the fish, petechial haemorrhages were observed on the tongue, upper jaw and operculum. The results demonstrated that *A. veronii* exhibited susceptibility to ciprofloxacin, levofloxacin, ceftazidime, cefepime, trimethoprim-sulfamethoxazole, enrofloxacin, florfenicol, gentamicin, streptomycin, and neomycin. However, *A. veronii* demonstrated resistance to amikacin, oxytetracycline, and erythromycin. It is imperative to acknowledge the potential risk of contamination of fruits and vegetables irrigated with irrigation water contaminated with *Aeromonas*, which may result

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in a human infection. Consequently, the implementation of regular monitoring programmes is essential to ensure produce safety.

Key words: *Aeromonas veronii*, antibiotic profile, carp, MALDI-TOF

Introduction. Aquaculture is of significance as a primary source that can be relied upon to ensure food security for countries. This is especially the case in terms of providing animal protein with high nutritional value, preserving and strengthening natural resource stocks and protecting the environment [1]. The interplay between the environment, host and pathogen is a critical factor in the emergence and progression of diseases. Stressful conditions, including population density, changes in water temperature and oxygen levels, have been demonstrated to accelerate the spread of pathogenic bacteria and precipitate large-scale disease outbreaks [2]. Infectious diseases pose a significant threat to the sustainability of aquaculture industries, giving rise to both direct financial costs arising from fish losses and indirect costs associated with disease management practices and the depreciation of product value. It is an established fact that bacterial pathogens are the most frequently encountered potential disease agents [3]. The number of new pathogens identified in the Mediterranean basin has increased rapidly over time. *Aeromonas* species are of particular note as they are among the most widespread infectious diseases in farmed and wild marine fish [4]. *Aeromonas* species are one of the pathogens that affect many fish species living in freshwater and tropical environments [5]. This organism is transmitted to humans through various dietary and water sources, as well as recreational activities in water. The genus *Aeromonas* has been identified as a high-risk pathogen carrier [6]. It is also naturally found as a flora bacterium of many animals. As a secondary biological agent, this opportunistic bacterium contributes to the initiation and progression of fish diseases. The disease caused by *Aeromonas* bacteria in fish is known as Motile *Aeromonas* Septicemia (MAS). These pathogens have also been identified as a significant cause of disease in amphibians, reptiles, birds, and mammals, including humans. It has been demonstrated that *Aeromonas veronii* exhibits the widest host range in terms of virulence among *Aeromonas* species [7]. *Aeromonas veronii* is classified as a Gram-negative, rod-shaped, mesophilic, motile bacterium [8]. In the present study, *A. veronii* was detected in four different carp species caught from nature by culture and MALDI-TOF. Furthermore, pathological and the antibiotic susceptibility of the agent was also ascertained.

Material and methods. In the course of the study, 80 carp samples were obtained through the use of nets from the Tigris River in October 2024. Subsequent to this, the average weights of the samples were recorded using a digital scale with 0.1 g sensitivity (Mettler Toledo ML6001T, Switzerland). The species identification of the samples was then conducted. The species identified included *Carassius auratus*, *Cyprinus carpio*, *Cyprinus carpio carpio* L., 1758, and *Carassius gibelio* Bloch, 1782. The number of samples according to species are 22, 19,

24, and 15, respectively. The necropsy method was conducted in two distinct phases: an external examination and an internal examination. In the external examination, a comprehensive analysis was conducted on the skin, mouth, eyes, gills, anus, operculum and fins, with the findings meticulously recorded. The aforementioned data were recorded in accordance with the methodology delineated by SCHÄPERCLAUS [9]. The internal (post-mortem) examination was conducted in adherence to the procedures outlined by AUSTIN and AUSTIN [10]. Following necropsy, a comprehensive examination of the internal organs was conducted, encompassing the liver, gallbladder, pancreas, kidney, spleen, gastrointestinal tract, swim bladder, gonads, and heart.

Bacteriological isolation. Tissue samples were brought to the microbiology laboratory and cultured on blood agar (Merck, 110886, Darmstadt, Germany) containing 5–7% sheep blood, MacConkey agar (Merck, 05465, Darmstadt, Germany) and eosin methylene blue agar (EMB agar) (Merck, 103858, Darmstadt, Germany). The samples were then subjected to incubation under aerobic conditions at a temperature of 28 °C for a period of 18–24 h [11]. Following incubation, mixed cultures were 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. Isolates were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry [MALDI-TOF MS (VITEK MS, Biomerieux, France)]. The disc diffusion method was utilised for the purpose of antibiotic susceptibility testing, with cefepime (30 µg), ceftazidime (10 µg), levofloxacin (5 µg), ciprofloxacin (5 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg), enrofloxacin (10 µg), florfenicol (30 µg), neomycin (10 µg), amikacin (10 µg), oxytetracycline (30 µg), erythromycin (15 µg), gentamycin (10 µg), streptomycin (10 µg) being used to assess the antibiotic susceptibility of *Aeromonas veronii* isolates. 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 discs (Bioanalyse, Turkey) were placed after spreading on the surface of the medium. The zone diameter was measured after 16–20 h of incubation.

Results. Signs of fish with disease. The gills, kidneys and livers of the examined fish were observed to be pale in colour. The fish exhibited erythema, haemorrhage and superficial ulcerative lesions on the skin, as well as petechial haemorrhage on the tongue, upper jaw, and operculum. The spleen was enlarged and exhibited multiple abscesses. Some fish displayed evidence of ecchymosis and fatty degeneration in the liver. Additionally, the abdominal cavity contained bloody exudates, and the intestinal wall was observed to be transparent. Other notable findings included the presence of yellowish liquid within the intestinal tract, as well as multiple granulomas in both the spleen and kidney. A perusal of the clinical findings revealed that the most prevalent manifestations were ery-

thema, haemorrhage, ulcerative lesions on the skin, and petechial haemorrhage on the tongue. The incidence of these clinical findings according to fish type is illustrated in Fig. 1.

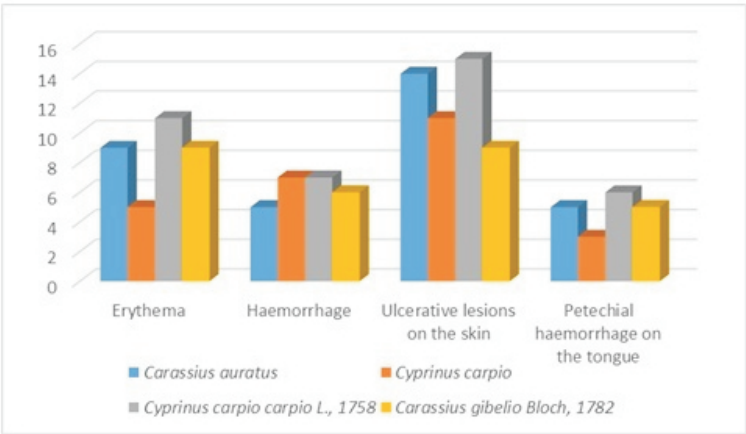


Fig. 1. The most common clinical findings for different types of fish

In the present study, a total of 80 fish samples were examined, and *A. veronii* was detected in the liver, kidneys, and tissues of 52 of these samples. The pathogen was predominantly detected in the *Carassius auratus* species, followed by *Cyprinus carpio*, *Cyprinus carpio carpio* L., 1758 and *Carassius gibelio* Bloch, 1782, respectively. Within these species, *A. veronii* was predominantly detected in the liver, kidneys, and muscle tissue, respectively (Fig. 2).

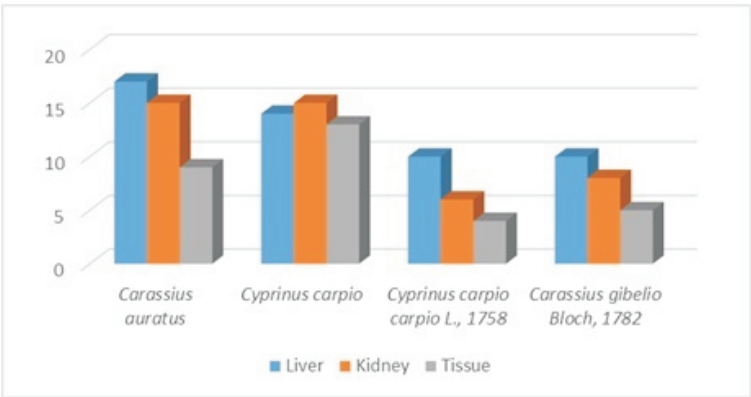


Fig. 2. Detection rate of *A. veronii* in organs according to species

The antibiotic resistance patterns of *A. veronii* were evaluated by measuring the size of the inhibition zones around each disc. The results indicated that *A. veronii* exhibited sensitivity to ciprofloxacin, levofloxacin, ceftazidime, cefepime, trimethoprim-sulfamethoxazole, enrofloxacin, florfenicol, gentamicin, streptomycin,

and neomycin. However, *A. veronii* demonstrated resistance to amikacin, oxytetracycline, and erythromycin (Table 1).

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The aforementioned classification system (S for susceptible and R for resistant) has been employed to ascertain susceptibility to antimicrobial agents in *A. veronii*

Antimicrobial agent	Paper content (µg/piece)	Isolate 1	Isolate 2	Isolate 3
Ciprofloxacin	5	S	S	S
Levofloxacin	5	S	S	S
Ceftazidime	10	S	S	S
Cefepime	30	S	S	S
Trimethoprim-sulfamethoxazole	1.25/23.75	S	S	S
Enrofloxacin	10	S	S	S
Florfenicol	30	S	S	S
Neomycin	10	R	R	R
Amikacin	10	R	R	R
Oxytetracycline	30	R	R	R
Erythromycin	15	R	R	R
Gentamycin	10	S	S	S
Streptomycin	10	S	S	S

Discussion. It is an irrefutable fact that the *Aeromonas* genus comprises a number of opportunistic pathogens. These pathogens are responsible for the causation of diseases in both aquatic and terrestrial animals, and, it must be added, in humans [12]. Of these, *A. veronii* has been identified as an important pathogen for humans and aquatic animals [13]. As stated by GASHGARI and SELIM [14] among the *Aeromonas* species, *A. veronii* appears to demonstrate the most extensive host range in terms of virulence. *A. veronii* has been identified as a highly opportunistic pathogen that is distributed widely in water environments and among fish populations. It has been demonstrated to cause diseases in fish under conditions of stress, such as overcrowding, low oxygen levels, and high water temperatures [15]. According to the clinical findings obtained from studies on *A. veronii* in sea bass, the main organs affected by the disease were found to be the muscle, liver, and gill [16]. The most affected organs were found to be similar in the study. The disease, caused by bacteria of the *Aeromonas* genus in fish, is known as Motile *Aeromonas* Septicemia (MAS). The majority of MAS outbreaks have been linked to an environmental condition that compromises the fish’s capacity to combat infections. Given that these bacteria inhabit various ecological niches, including free-floating states in aquatic environments, biofilms, and frequently within the gastrointestinal tract of fish, they are well-positioned to exploit hosts with compromised immune systems. Risk factors for *Aeromonas* infections include heat stress, poor water quality, inadequate nutrition, injury and infection

with other bacteria, viruses, parasites or fungi. Therefore, combining associated conditions, *Aeromonas* are among the most common pathogens contributing to disease outbreaks in fish farming. In the majority of cases, primary infections are initiated when bacteria penetrate the site of injury or breach the mucus barrier that envelops the fish. These bacteria then establish a localised infection, characterised by the breakdown of skin, muscle, and connective tissue due to the release of proteolytic enzymes. These manifestations often take the form of deep ulcers, scale loss, fin erosion, or abscesses. It is important to note that bacteria can spread from these localized infections to cause systemic infections (septicemia or bacteremia). *Aeromonas* has also been observed to invade fish through the gut or gills, resulting in systemic infection. Depending on the predisposing factor, rapidly increasing mortality may occur; however, losses usually decrease relatively quickly if the predisposing factor can be corrected [8]. Abdominal swelling and skin ulcers were detected in carp infected with *A. veronii*. Necropsies revealed enteritis, mild congestion in the liver, and enlarged spleen and kidneys. The clinical and histopathological findings detected in the present study are consistent with those reported in other studies [17]. The clinical observations recorded included anorexia, reduced swimming speed on the water surface, darker skin colouration, and exophthalmos. In the case of the fish under consideration, the gills are pale in colouration, and the majority of fish exhibit an icteric appearance, with yellowish skin, fins and blood serum. Epidermal lesions are rarely superficial, with ulcers extending to the underlying muscles being the most common. Further clinical signs observed in affected fish include haemorrhaging in the head, mouth and jaw, as well as erythema in the fins and operculum. Post-mortem examination revealed that the principal organs affected by the disease were the spleen, kidney and liver. Necrosis and diffuse hemorrhages in the liver have been reported. The histological analysis of infected carp in the present study revealed significant changes in the gill, kidney and liver. These changes are consistent with those reported in previous studies [18]. For instance, the changes observed, including blood congestion and epithelial hyperplasia in the gill, were analogous to those reported in carp infected with *A. veronii*. The observed necrosis and haemorrhage may be associated with bacterial toxin production. In earlier literature on the subject of disease reports, the susceptibility of *A. veronii* isolates to antibiotics has been shown to vary according to both the fish host and the environmental conditions present. The results indicated that *A. veronii* exhibited sensitivity to ciprofloxacin, levofloxacin, ceftazidime, cefepime, trimethoprim-sulfamethoxazole, enrofloxacin, florfenicol, gentamicin, streptomycin, and neomycin. However, *A. veronii* demonstrated resistance to amikacin, oxytetracycline, and erythromycin. *Aeromonas* has been identified in various types of water bodies, including surface water, groundwater, potable water, bottled water, seawater, and irrigation water [19]. The prevalence of *Aeromonas* in wastewater has been documented, and it has been observed that the bacterial concentration in water may be associated with

terrestrial wastewater. Conventional treatment methods employed in wastewater treatment plants for the purpose of water recycling have been observed to be ineffective in reducing the concentration of *Aeromonas*. It has been documented that the combination of conventional treatments with additional treatments such as chemical (ozone, chlorination) and physical (ultraviolet radiation) treatments results in the complete elimination of *Aeromonas* [19]. Another natural treatment method is lagooning. This treatment process involves the temporary storage of wastewater in shallow pools or lagoons, thereby reducing *Aeromonas* levels. In their 2022 study, GUERRA et al. [20] examined *Aeromonas* strains isolated from river water, soil, and plant materials; virulence genes and antimicrobial resistance genes were also detected in some strains. This type of environmental study aims to demonstrate the potential for human or animal pathogenicity of *Aeromonas* strains found in environmental sources (water, soil, plants). While it does not provide direct evidence of human pathogenicity, it supports the concept of "potential pathogenicity." Therefore, this study demonstrates that the claim in our article, "waterborne contamination risk of human infection" is based on similar considerations in the literature. This information has been added to the study. The utilisation of recycled water as a source of irrigation is contingent upon its microbiological quality. However, there is a risk of contamination of fruits and vegetables irrigated with irrigation water containing *Aeromonas*, which can cause infection in humans.

Conclusions. *A. veronii* has been detected in various species of carp captured in the wild. The clinical findings are consistent with those reported in previous studies. The most affected organs by the pathogen were determined to be the liver, gills and tissue. The susceptibility of *A. veronii* to various antimicrobial agents was also investigated, with the results indicating sensitivity to ciprofloxacin, levofloxacin, ceftazidime, cefepime, trimethoprim-sulfamethoxazole, enrofloxacin, florfenicol, gentamicin, streptomycin, and neomycin. However, *A. veronii* demonstrated resistance to amikacin, oxytetracycline and erythromycin. It is imperative to acknowledge the potential risk of contamination of fruits and vegetables irrigated with irrigation water containing *Aeromonas*, which may result in human infection. Consequently, it is imperative to implement regular monitoring programmes to ensure the safety of produce.

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 they followed all pertinent ethical and legal procedures and requirements.

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