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Aeromonas: the multifaceted middleman in the One Health world

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Highlights

- *Aeromonas* is an ubiquitous hydrotelluric bacterium
- *Aeromonas* is at the interface between humans, animals and the environment
- *Aeromonas* can be responsible for human infections following leech therapy
- *Aeromonas* has a high level of genome plasticity and capability of acquiring genes
- *Aeromonas* contributes to antimicrobial resistance gene dissemination
Abstract

*Aeromonas* is at the interface of all the *One Health* components and represents an amazingly sound test case in the *One Health* approach, from economic loss in aquaculture to challenges related to antibiotic-resistant bacteria selected from the environment. In human health, infections following leech therapy is an outstanding example of such *One Health* challenges. Aeromonads are not only ubiquitous environmental bacteria, able to rapidly colonize and cause opportunistic infections in humans and animals, they are also capable of promoting interactions and gene exchanges between the *One Health* components. This makes this genus a key amplifier of genetic transfer, especially of antibiotic resistance genes.
Aeromonas in the One Health world: a ubiquitous “Jack of all trades”

The genus Aeromonas belongs to the Aeromonadaceae family, which itself is part of the Aeromonadales order and Gammaproteobacteria class [1]. This genus, which currently comprises 36 species (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi), is organized in a complex of species, i.e., heterogeneous groups of closely-related species with unclear boundaries [2], as observed for other Gram-negative bacteria like Vibrio, Acinetobacter, Pseudomonas, Burkholderia, and several Enterobacterales [3]. From this complex of species, some more specialized clones may emerge [4]. Of these, the complex A. salmonicida, which is responsible for fish diseases, is to date the only species that has successfully become specialized to cause a specific disease [5]. While the primary reservoir of Aeromonas is aquatic environments and soils where it is almost always present, it is also capable of rapidly colonizing a wide range of niches and hosts [1]. Aeromonas has a notable ability to colonize the animal gut, including the guts of leeches, fish, cows and humans [6,7] as well as many other host organisms.

Data from Pubmed, using close to 10,000 descriptions since 1944, (https://pubmed.ncbi.nlm.nih.gov/?term=aeromonas&sort=date&size=20) provides a biased view of this epidemiology, with >50% of Aeromonas publications linked to humans or animals including livestock, companions and wildlife. The IMNGS 16S rRNA-based metagenomic website provides a more accurate and realistic picture of Aeromonas epidemiology [8]. Aeromonas is mostly detected from water and soil samples, and is present in 20% of rhizosphere and aquatic metagenomes (Figure 1). Detection from animal samples is infrequent, except from fish gut metagenomes and, to a lesser extent, from bovine gut metagenomes [9,10]. In humans, detection remains exceptionally rare. Relative abundance data from positive metagenomes also highlights the importance of Aeromonas in hydrotelluric environments with anthropic pollution. The highest relative abundance is found in wastewater
metagenomes (Figure 1), as a partial consequence of gut-related waste. Relative abundance of
Aeromonadaceae in animals is very low, below 0.1% (Figure 1). One exception is leech gut
where relative abundance of Aeromonas seems very important, estimated at 36% [11].
The environmental reservoir of Aeromonas allows it to be at the interface of all the One
Health components (Figure 2). With its ability to produce biofilm and its large metabolic
capacity that facilitates its rapid adaptation to environmental changes [12,13], Aeromonas is
viable in a large range of conditions. These extended capabilities that allow Aeromonas to
persist and rapidly propagate in continuously changing environments [12-17] has earned this
genus the nickname of a “Jack-of-all-trades” [12].

Aeromonas in close contact with human activities
Aeromonas is primarily able to develop long-term symbiotic relationships (e.g. with leeches),
or to transiently colonize other animals and humans, where it can trigger cutaneous or
digestive infections [16,18]. Aeromonas spp. are responsible for economic burdens of dozens
of millions of dollars in aquaculture due to mortality, lost feeding days and costs associated
with chemical and antibiotic therapy [19-22]. Among fish pathogens, A. salmonicida subsp.
salmonicida (referred to as “typical”) is responsible for furunculosis disease in salmonids
[23], while some hypervirulent clones have emerged (e.g., A. hydrophila ST251) in the catfish
farming and shrimp industry [24,25].
Another Aeromonas-impacted area concerns ornamental fish that are traded worldwide (2
billion/year). These exchanges occur mainly between Asian countries (the primary producer)
and USA/Europe. In this industry, as observed for several years in fish farms [26,27], the
misuse or overuse of antibiotics to fight bacterial diseases has selected for drug-resistant
pathogens [28-30]. Recent studies underline the potential role of ornamental fish in the
dissemination of antibiotic-resistant bacteria, with possible consequences for private
aquariums and human public health in importing countries [28].

*Aeromonas* spp. have also been isolated from a wide range of foods of both plant and animal
origin [31], including minimally processed and ready-to-eat seafood products that are gaining
popularity [32]. The prevalence of *Aeromonas* in ready-to-eat products has been estimated to
be between 8 and 52%, with resistance to clinically-relevant antibiotics also emerging [33-35]. This raises concerns of the potential for food spoilage and foodborne infections.

*Aeromonas* is associated with human activities such as animal husbandry, animal slaughter
[36-38], wastewater treatment [39], reclaiming irrigation water [40,41], recreational or
occupational activities that are related to water exposure and those that exploit
microbiological metabolic capabilities in biotechnological applications (Figure 2). These
include the treatment of sewage and industrial wastewater with activated sludge that contains
aeromonads at up to 2% of the total biomass [14,42]. Here, aeromonads are organized in
aggregates and can resist the extreme conditions of pH, salinity, heavy metal toxicity, and
temperature [14]. These aggregates are inserted in mixed species biofilms that are able to
remove organic pollutants and improve both denitrification and phosphorus removal [43-45].

**Human infections: by accident or by Trojan horse**

In humans, *Aeromonas* infections are primarily associated with contact with soil and/or water
e.g., during recreational or occupational activities, or following road accidents [16,46].

Cutaneous, eye or digestive infections are the most frequent, although almost any type of
infection is possible. Aeromonads are weak opportunistic pathogens in humans, and most
infections are associated with a barrier disruption. Aeromonads can also spread from a
 colonized niche, typically the gut, and cause biliary or bloodstream infections notably in
immunocompromised patients [16,47]. While human infections are widely reported through hundreds of published case reports [48], they are poorly documented compared to other bacteria, with the notable exception of natural disaster situations, when thousands of wounded individuals are exposed to contaminated waters with poor access to healthcare facilities [16]. No global study exists that compares prevalence among countries. The prevalence of bloodstream infections seems higher in Asia compared with European countries [46,49], and some species are more prevalent in tropical countries (e.g., *A. dhakensis*) [1]. One can expect that use of gastrointestinal syndromic tests targeting aeromonads will increase documentation and will provide a more exhaustive picture, as it was observed in this recent German study with a prevalence of 2.9% of *Aeromonas* sp. from 5032 stool samples [50].

The particular case of healthcare-associated infections caused by ciprofloxacin-resistant aeromonads following leech therapy is an example of the *One Health* challenges posed by these bacteria, with resistant strains selected from the environment and spread to patients (Figure 3). Medicinal leeches may be administered to patients after orthopedic surgery or reconstructive surgery to treat venous congestion that threatens flap outcome through necrosis. This salvage treatment actively removes blood and improves outcomes, at the expense of a risk of serious wound infection leading to flap loss, amputation and sepsis. Indeed, the leech regurgitates its symbiont during bloodlettings. While the most abundant species among leech symbionts is by far *A. veronii*, *A. hydrophila* is the most frequently reported species associated with infection following leech therapy (88%) [51]. This underlines that the bacterial load during leech regurgitation is not the only factor to contribute to infection and that some yet unknown virulence factors in *A. hydrophila* are likely involved [52]. This risk of infection is controlled with ciprofloxacin-based prophylactic treatment [51,53]. However, since the early 2010s, several cases of infections following leech therapy were suddenly reported worldwide by aeromonads resistant to ciprofloxacin [54,55]. As
detailed in Figure 3, investigations showed that these events resulted from complex relationships between the antibiotic that disseminates in the environment, the leech microbiota where there is competition between fluoroquinolone (FQ)-resistant and FQ-susceptible bacteria, a leech trade based on a limited number of suppliers that disseminate leech with closely related FQ-resistant strains, and immunocompromised patients [52].

The use of antimicrobials in a natural setting facilitates the spread of resistance markers in *Aeromonas*, even when used at very low-levels [56,57], and this spread may occur between distant locations through a vector and Trojan horse such as the leech. This example highlights the consequences of the selective pressure in the leech environment with subsidiary consequences to human health. Such resistance spread is not limited to FQ, with some reports recently identifying *Aeromonas* that harboured resistance to 3rd generation cephalosporins or cotrimoxazole, which exemplifies the capability of aeromonads to acquire diverse antibiotic resistance genes (ARGs) [55,58].

How to uptake DNA: the Swiss Army knife

Bacteria of the *Aeromonas* genus have a 4-5 Mbp genome, which is smaller than other Gram-negative bacteria like Enterobacterales or *Pseudomonas*. *Aeromonas* compensates for this relatively small genome size by its tremendous capacity to acquire several mobile genetic elements (MGE), including plasmids, transposons, genomic islands or integron gene cassettes. The core genome of these bacteria is represented by only 16% of the genes in the whole genome, with the remaining (84%) consisting of variably represented genes. This highlights the exceptional level of genome plasticity in *Aeromonas* due to horizontal gene transfers (HGT) both from close relatives in the genus and from several other families [13,59]. An exciting yet unanswered question is to know whether the environment shapes aeromonad
plasticity and whether their accessory genome correlates with their origin. This mobilome [60] allows for the acquisition of hundreds of antibiotic or heavy metal resistance genes, genes involved in molecular function or biological process, as well as genes encoding metabolic enzymes, transporters and bacterial defense mechanisms [59,61]. These genes enable the survival of Aeromonas in hostile environments. All known mechanisms of HGT have been described within the Aeromonas genus [62]: transformation (as most Aeromonas species are naturally competent [63,64]), transduction and conjugation. A wide variety of lysogenic phages has been described especially amongst the species A. salmonicida and A. hydrophila [65,66]. Conjugation remains the most efficient mechanism of HGT, with hundreds of conjugative plasmids identified in aeromonads. This plasmidome remains poorly characterised, with the exception of that of A. salmonicida [67].

Aeromonads are diversely equipped with the type II, III, IV and VI secretion systems (TSS) [13]. Whilst the study of these nanomachines in Aeromonas has mostly focused on their virulence properties, the T4SS and T6SS are also likely to be implicated in gene transfer, as they are typically involved in Gram-negative bacterial conjugation and transformation respectively [68,69]. For instance, competence-induced T6SS-mediated bacterial killing has been demonstrated in V. cholerae, and occurs through a complex system that involves chitin-composed exoskeleton-associated-biofilms (e.g. in copepods), quorum sensing and the metabolic activity of chitinase [68,70]. Because Aeromonas shares aforementioned features with the closely-related Vibrio spp., it is conceivable that the T6SS plays a role, albeit still unravelled, in Aeromonas DNA uptake. Whatever the mechanisms involved, the lifestyle of aeromonads is associated with biofilm in sheltering organisms (e.g., copepods) that are hotspots for genetic exchanges [71].

Given its importance in human and animal health, the acquisition of ARGs is the most described event of this genetic exchange capability of Aeromonas. The genus can be
considered as a “sponge” because it can easily absorb and redistribute genes [67]. A recent in silico analysis on plasmids retrieved from Aeromonas species revealed that more than one third of plasmids carry ARGs [72]. These ARGs can affect all antibiotic families, including β-lactams, with extended-spectrum β-lactamases (CTX-M, GES, VEB) and carbapenemases (VIM, KPC, NDM-like), but also aminoglycosides, cyclins, trimethoprim, sulfonamides, FQ (with notably the qnr and qepA genes [73]) and even colistin, with the recent description of the mcr genes carried on Aeromonas plasmids [55,58,74-78].

**Integrons in Aeromonas: witnesses of horizontal gene transfers?**

Due to the environmental location of Aeromonas and its capability to acquire multiple MGE under selective pressure, it can be expected that detection of virulence or ARGs in aeromonads could constitute potential biomarkers of anthropic pollution. Unfortunately, these genes are too diverse for this approach to be feasible. One alternative attractive possibility in the search for a unique biomarker of anthropic pollution would be to detect integrons from Aeromonas isolates. Combining a relevant microorganism capable of acquiring hundreds of genes under selective pressure (Aeromonas) and a marker of antimicrobial resistance frequently hosted by Aeromonas isolates and easy to detect (integrons) could constitute a potential interesting tool. In fact, class 1 integrons can be easily detected [79] and are now considered as a good proxy for anthropic pollution [80,81]. Integrons are non-mobile genetic elements carried by plasmids or transposons [82,83] that can carry hundreds of mobile gene cassettes, most of them involved in antimicrobial resistance. The prevalence of class 1 integrons within the Aeromonas genus is difficult to determine and can greatly vary (from 7 to 72%) depending on the origins of the samples [84-86]. Irrespective, their description among the Aeromonas species is frequent, as is their association with multidrug resistance, as
previously demonstrated for Enterobacterales [87,88]. Whilst gene cassettes are relatively
diverse in *Aeromonas* [89] (Table S1), the presence of these integrons demonstrates that
aeromonads readily acquire MGEs following selective pressure.

**Conclusions**

Although aeromonads are often described as possessing a significant pathogenic potential,
these opportunistic pathogens seldom cause infections, and when they do, it is usually in the
context of a disrupted barrier or a compromised immune system.

One intriguing question is why a microorganism that bears many so-called virulence factors
rarely causes infection. Understanding pathogenicity in *Aeromonas* remains elusive [15], with
virtually all studies failing to successfully predict pathogenicity in aeromonads from the
expression of so-called virulence factors. One assumption to reconcile this paradox is that the
genes that we, from a very anthropocentric analysis, consider to encode virulence factors may
indeed have other primary functions. Several arguments suggest that exaptation, the process
by which features acquire functions for which they were not originally adapted or selected
[90], may have occurred in *Aeromonas*. For example, aeromonad hydrolytic enzymes are now
thought to be primarily involved in essential functions of general cell metabolism [15].

Exaptation may have then changed the function of these genes during the course of
evolutionary succession. Other examples of possible exaptation are *mcr-3* or *qnr* genes, which
evolved long before the synthesis and clinical use of colistin and quinolones, respectively
[91,92]. A complementary hypothesis is that the multiple varied environments and organisms
that aeromonads colonize act as a nursery to pre-adapt to pathogenicity, because such
relationships need to overcome the innate defense of their hosts [71].
Finally, some questions also arise regarding the role of integron gene cassettes in *Aeromonas*. While mostly associated with ARGs, they existed even prior to the antibiotic era, and they operate as a general gene capture system. As such, they are likely to be an important factor in the more general process of HGT during the evolution of bacterial genomes and in bacterial adaptation [93], and they may contribute to shaping the plasticity, versatility and flexibility of the *Aeromonas* genus [67].

Irrespective of the original functions in the genus and its evolutionary changes, *Aeromonas* to date carries all the characteristics of a potential pathogen with resistance to multiple antibiotics. Taking into account its weaponry and its lifestyles that encompass all the One Health components, aeromonads have the potential to one day emerge as more prevalent pathogens than they are currently, and to wreak havoc in the future. Therefore, it is essential that we maintain a close watch on these fascinating bacteria.
Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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** This study based on Whole Genome Sequencing of 29 *Aeromonas* isolates from different species revealed a limited core genome and a high level of genome plasticity. More than four fifth of the pan-genome was variable with hundreds of gene expansions and contractions, horizontally transferred genes, and mobile genetic elements, which emphasized the heterogeneity of the genus. This is one of the first study that deeply compared genome contents; this bioinformatics analysis highlighted differences between acquired genes and abundance of some genes that may reflect environmental selection.


The authors analysed 64 genome from strains representative of the genus *Aeromonas* to investigate the correlation between genome-based phylogeny and virulence associated factors to provide insight into pathoadaptation through the evolutionary process of virulence associated genes.


* This 26-month longitudinal study assessed the prevalence of bacteria indicating water quality, fecal contamination, and crop contamination risk. It included *Escherichia coli*, total coliforms, *Enterococcus*, and *Aeromonas*. This study provides comprehensive microbial data on alternative irrigation water and serves as an important resource for food safety planning and policy setting.


** Investigating the causes of ciprofloxacin-resistant *Aeromonas* infection following leech therapy, this is the first study to bring some understanding on the recent epidemiological change. This work highlights the role of low-level ciprofloxacin in the leech, and as corollary in the leech environment, and consequences on the leech microbiota.


* This study focuses on horizontal multigene transfers. It evaluates the characteristics and frequencies of HGT and provides a more complete understanding of HGT and microbial evolution using the genus *Aeromonas* as a test case and 103 genomes as a dataset. Interesting relationships between gene function, phylogenetic distance, and frequency of multigene transfer were identified. This work introduce the first computational framework to further study the scale of HGT events.


This review focuses on the mechanisms allowing acquisition of antimicrobial resistance genes. This article extensively reviewed transformation, transduction, and conjugation mechanisms within the genus *Aeromonas* with several examples from different species and several antibiotic resistance genes.


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Prevalence of *Aeromonadaceae* among human, animal and environmental metagenomes

![Graph showing the relative abundance of *Aeromonadaceae* in different metagenomes: human skin, human nasopharyngeal, human lung, human gut, sheep gut, chicken gut, mouse gut, pig gut, bovine gut, fish gut, groundwater, lake water, wastewater, marine, aquatic, soil, plant, rhizosphere. The graph indicates that the highest abundance is in the aquatic metagenome, followed by the plant, rhizosphere, and human gut metagenomes.](image)
Figure 1: Prevalence of *Aeromonadaceae* among human, animal and environmental metagenomes.

Figure 1A presents for the 3 compartments of the *One Health* world the percentage of metagenomes in which *Aeromonadaceae* are detected. This ecological distribution is based on 16S rRNA studies: grey, human metagenomes; yellow, fish and other animal metagenomes; blue, water metagenomes; green, soil and plant metagenomes. These data were obtained using the IMNGS database ([http://imngs.org](http://imngs.org)) [8] queried in May 2021 using reads matching the *Aeromonadaceae* taxonomy. Figure 1B indicates the relative abundance of *Aeromonadaceae* retrieved for each type of positive metagenome, presented with box plots.
Figure 2: Aeromonas in the One Health world, a complex network of relationships.
The figure presents the 3 parts of the One Health world where aeromonads are present: environment, mainly waters (blue), animals (green), human beings and related activities (light orange) and the compartments to which they are connected are shown with red dotted connectors. The intricate and complex network in which aeromonads interface the different compartments, mostly through waters, and in which they play a role of courier is shown with blue dotted lines. Flashes highlight the compartments where aeromonads contribute to disseminate antimicrobial resistance, dark orange specific host-bacteria interactions; beneficial relationships with host (e.g., human being) are highlighted in purple while detrimental relationships are yellow highlighted. WWTP, Wastewater treatment plant. Some parts of the figure were made with biorender (https://biorender.com).
Leech colonized with cipro-R aeromonads

AMR selective pressure related to human activities

Competition for leech colonization

Advantage to cipro-R strains when traces of quinolone in leech environment

Leech regurgitates (multi-) drug resistant aeromonads

Reconstructive surgery (flap), leech therapy and prophylactic treatment (ciprofloxacin)

Serious skin and soft tissue, zoonotic, health care associated infection

Patient with cancer admitted at hospital
Figure 3: the One Health perspective of aeromonad infection following leech therapy, an emblematic health care associated zoonotic infection caused by multi-drug resistant environmental opportunistic bacteria.

A, aeromonads are ubiquitous bacteria present primarily in waters. They usually exhibit very high rate of susceptibility to ciprofloxacin (green circles) [46]. B, Human activities with either misuse or overuse of antimicrobials, including quinolones, lead to antimicrobial selective pressure on aeromonads that can become resistant to ciprofloxacin (red circles). C, during leech colonization by Aeromonas, its main symbiont, ciprofloxacin resistant strains outcompete the susceptible strains when some trace of quinolones are present in the leech environment [52]. D, the leech trade is a global trade with diverse distant supplying sources, which allows the dissemination of bacteria from distanced parts of the world. E, patients with cancer are managed in hospital. F, during care pathway, cancer patients may benefit from reconstructive surgery, which may include flap surgery and may be associated with leech therapy to circumvent flap venous congestion. There is a risk of aeromonosis when leech regurgitates bacteria into the flap during bloodletting. Risk of infection is controlled with an antimicrobial prophylactic treatment, mainly ciprofloxacin [53]. G, because leeches are now frequently colonized with ciprofloxacin-resistant aeromonads, infection occurs despite prophylactic treatment and results in serious skin and soft tissue infection with flap loss and sepsis. Such event are posterior to 2010 and are regularly reported [55]. Some parts of the figure were created in biorender.com. Bibliometry histogram in G was obtained from Pubmed with keywords Aeromonas, leech therapy, infection and antimicrobial resistance. R: resistant. AMR: antimicrobial resistant. AM: antimicrobial.