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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].

## *Aeromona*s 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

111 immunocompromised patients [16,47]. While human infections are widely reported through
112 hundreds of published case reports [48], they are poorly documented compared to other
113 bacteria, with the notable exception of natural disaster situations, when thousands of wounded
114 individuals are exposed to contaminated waters with poor access to healthcare facilities [16].
115 No global study exists that compares prevalence among countries. The prevalence of
116 bloodstream infections seems higher in Asia compared with European countries [46,49], and
117 some species are more prevalent in tropical countries (e.g., *A. dhakensis*) [1]. One can expect
118 that use of gastrointestinal syndromic tests targeting aeromonads will increase documentation
119 and will provide a more exhaustive picture, as it was observed in this recent German study
120 with a prevalence of 2.9% of *Aeromonas* sp. from 5032 stool samples [50].
121 The particular case of healthcare-associated infections caused by ciprofloxacin-resistant
122 aeromonads following leech therapy is an example of the *One Health* challenges posed by
123 these bacteria, with resistant strains selected from the environment and spread to patients
124 (Figure 3). Medicinal leeches may be administered to patients after orthopedic surgery or
125 reconstructive surgery to treat venous congestion that threatens flap outcome through
126 necrosis. This salvage treatment actively removes blood and improves outcomes, at the
127 expense of a risk of serious wound infection leading to flap loss, amputation and sepsis.
128 Indeed, the leech regurgitates its symbiont during bloodlettings. While the most abundant
129 species among leech symbionts is by far *A. veronii*, *A. hydrophila* is the most frequently
130 reported species associated with infection following leech therapy (88%) [51]. This underlines
131 that the bacterial load during leech regurgitation is not the only factor to contribute to
132 infection and that some yet unknown virulence factors in *A. hydrophila* are likely involved
133 [52]. This risk of infection is controlled with ciprofloxacin-based prophylactic treatment
134 [51,53]. However, since the early 2010s, several cases of infections following leech therapy
135 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 3$^{rd}$ 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].

234 Finally, some questions also arise regarding the role of integron gene cassettes in *Aeromonas*.
235 While mostly associated with ARGs, they existed even prior to the antibiotic era, and they
236 operate as a general gene capture system. As such, they are likely to be an important factor in
237 the more general process of HGT during the evolution of bacterial genomes and in bacterial
238 adaptation [93], and they may contribute to shaping the plasticity, versatility and flexibility of
239 the *Aeromonas* genus [67].
240 Irrespective of the original functions in the genus and its evolutionary changes, *Aeromonas* to
241 date carries all the characteristics of a potential pathogen with resistance to multiple
242 antibiotics. Taking into account its weaponry and its lifestyles that encompass all the *One*
243 *Health* components, aeromonads have the potential to one day emerge as more prevalent
244 pathogens than they are currently, and to wreak havoc in the future. Therefore, it is essential
245 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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** 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.

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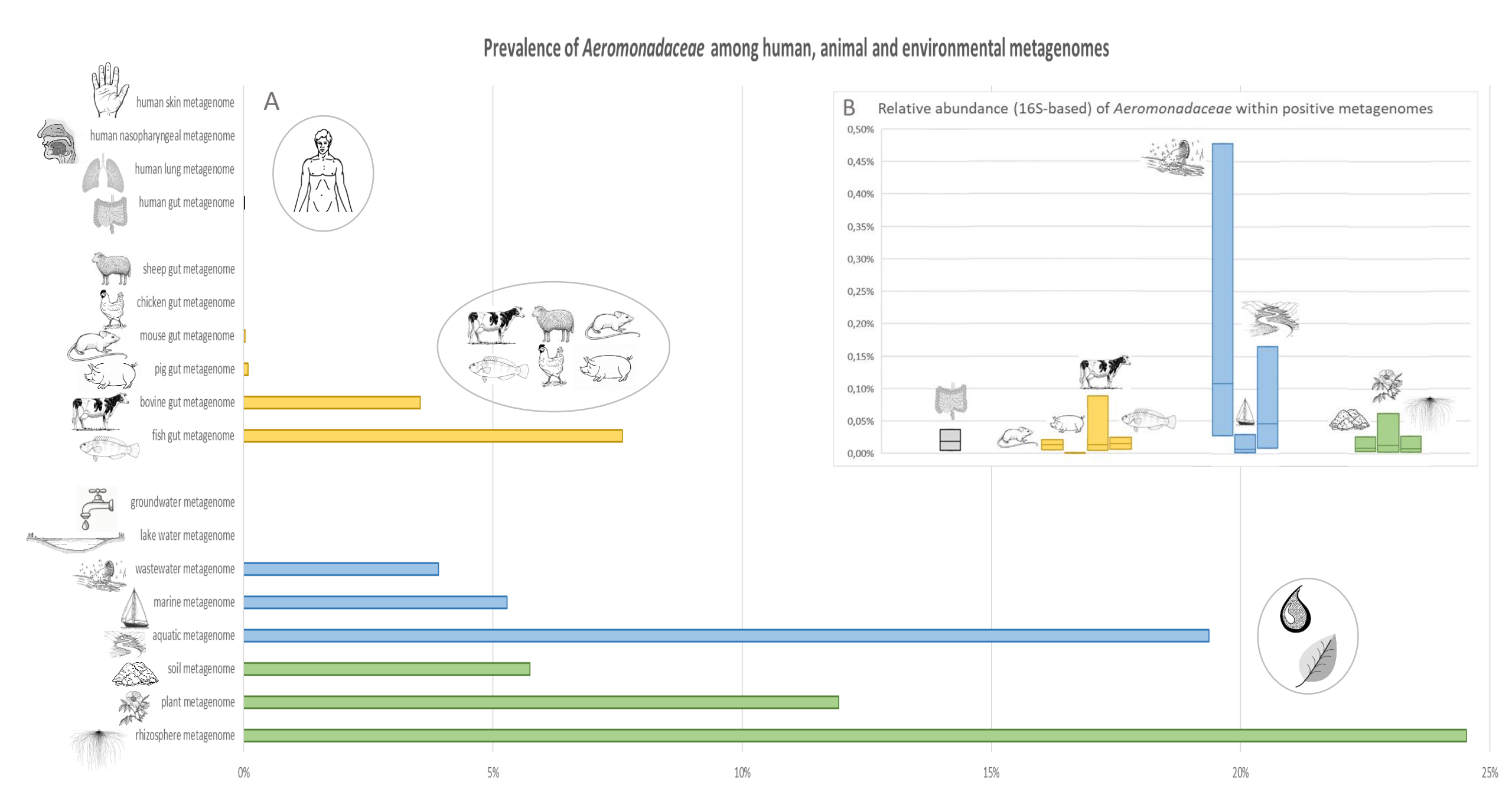

Prevalence of *Aeromonadaceae* among human, animal and environmental metagenomes
A
human skin metagenome
human nasopharyngeal metagenome
human lung metagenome
human gut metagenome
sheep gut metagenome
chicken gut metagenome
mouse gut metagenome
pig gut metagenome
bovine gut metagenome
fish gut metagenome
groundwater metagenome
lake water metagenome
wastewater metagenome
marine metagenome
aquatic metagenome
soil metagenome
plant metagenome
rhizosphere metagenome
0%
5%
10%
15%
20%
25%
B
Relative abundance (16S-based) of *Aeromonadaceae* within positive metagenomes
0,50%
0,45%
0,40%
0,35%
0,30%
0,25%
0,20%
0,15%
0,10%
0,05%
0,00%

**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) [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.

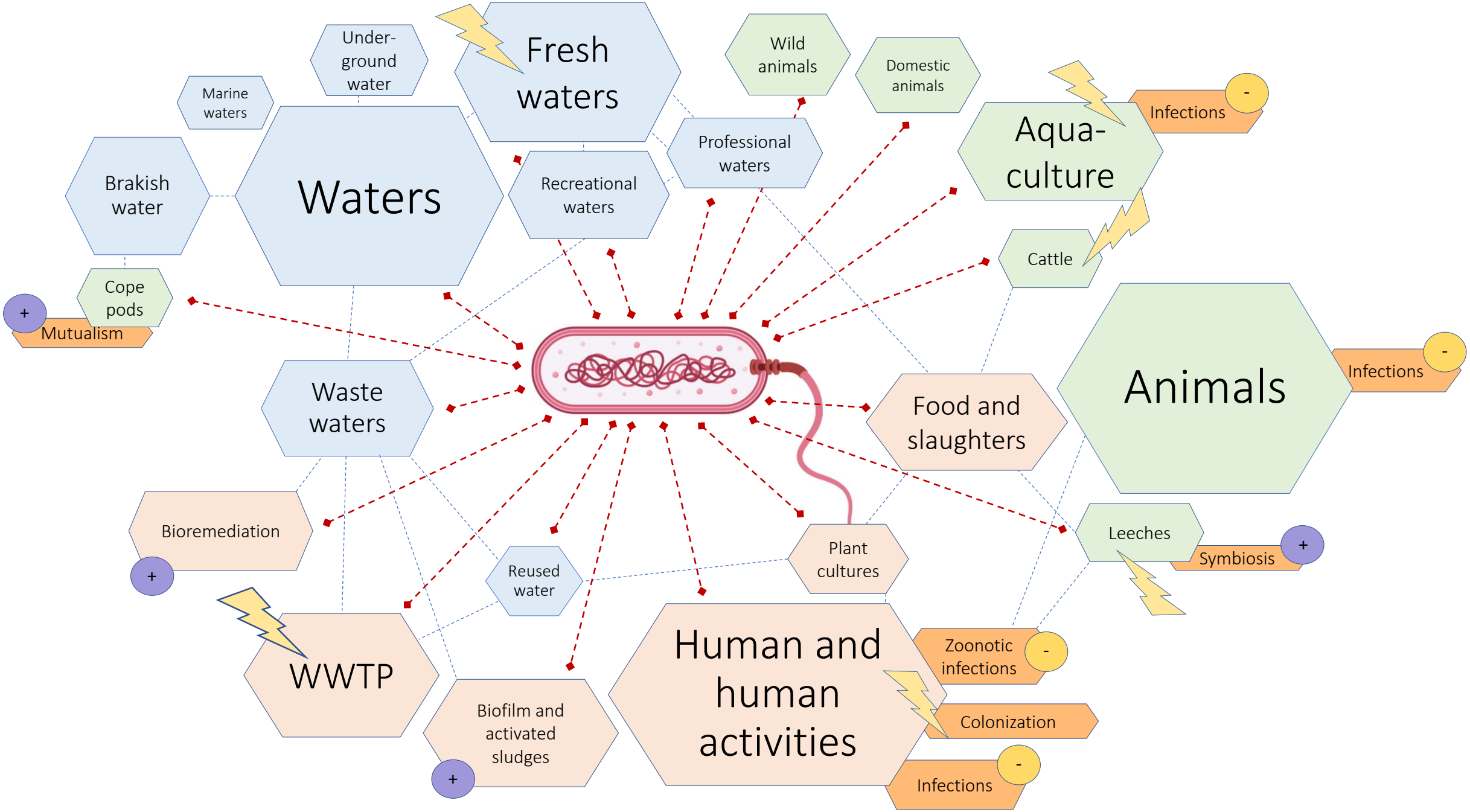
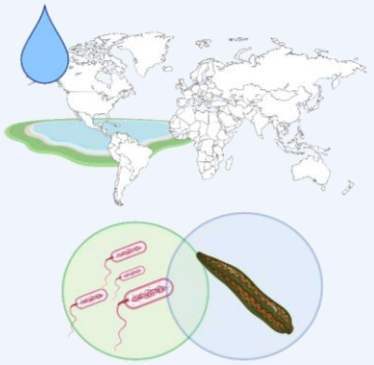
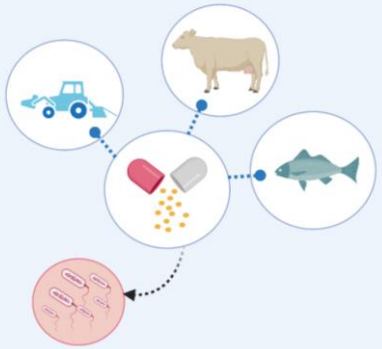

Under-ground water
Fresh waters
Wild animals
Domestic animals
Marine waters
Waters
Professional waters
Brakish water
Recreational waters
Aqua-culture
Infections
-
Cattle
Cope pods
+
Mutualism
Waste waters
Food and slaughters
Animals
Infections
-
Bioremediation
+
Leeches
Symbiosis
+
Plant cultures
Reused water
WWTP
Human and human activities
Zoonotic infections
-
Colonization
Biofilm and activated sludges
+
Infections
-

**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).

A



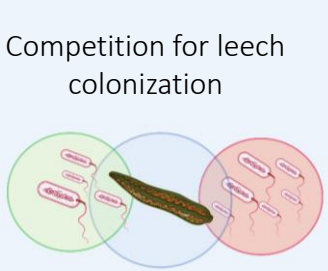
B

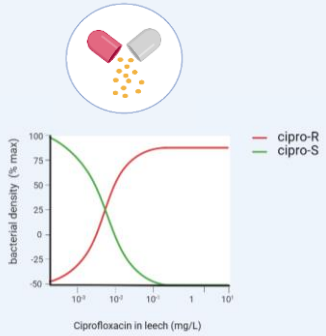


AMR selective pressure related to human activities

C

Competition for leech colonization





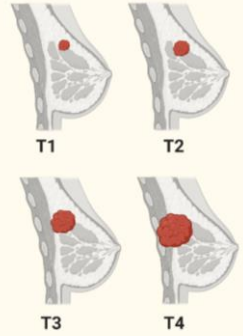

Advantage to cipro-R strains when traces of quinolone in leech environment

D

Leech global trade

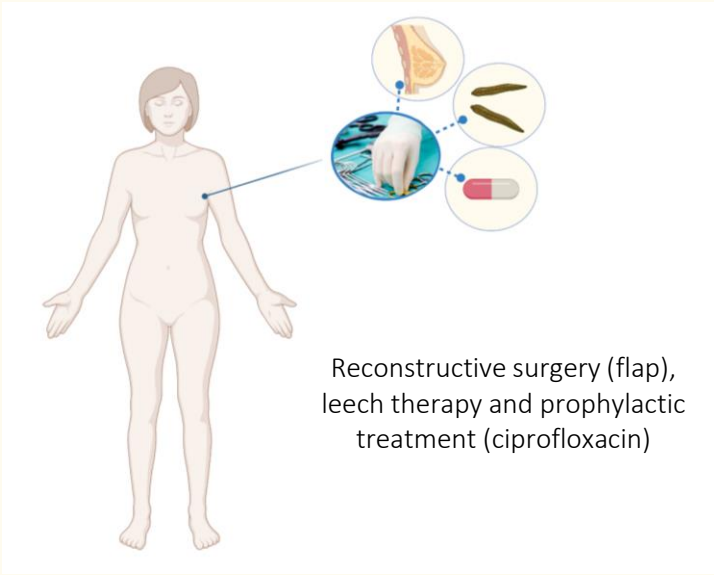
Leech colonized with cipro-R aeromonads

E




Patient with cancer admitted at hospital

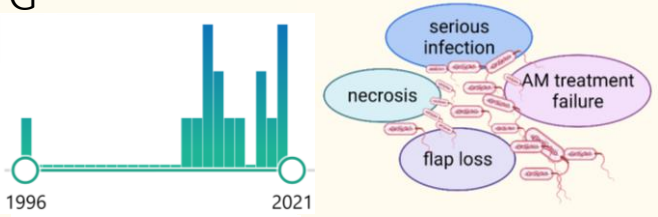
F



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

Leech regurgitates (multi-) drug resistant aeromonads

G




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

**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.