



# Aeromonas: the multifaceted middleman in the One Health world

Brigitte Lamy, Sandrine Baron, Olivier Barraud

## ► To cite this version:

Brigitte Lamy, Sandrine Baron, Olivier Barraud. Aeromonas: the multifaceted middleman in the One Health world. *Current Opinion in Microbiology*, 2022, 65, pp.24-32. 10.1016/j.mib.2021.09.012 . anses-03798758

**HAL Id: anses-03798758**

**<https://anses.hal.science/anses-03798758>**

Submitted on 5 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

# ***Aeromonas*: the multifaceted middleman in the *One Health* world**

**Brigitte LAMY<sup>1,2,3</sup>, Sandrine BARON<sup>4</sup> and Olivier BARRAUD<sup>5,\*</sup>**

<sup>1</sup> Université de Nice Côte d'Azur, INSERM, C3M, Nice, France

<sup>2</sup> Laboratoire de Bactériologie, CHU de Nice, Nice, France

<sup>3</sup> Centre for molecular bacteriology and infection, Imperial College of London, London,  
United-Kingdom

<sup>4</sup> Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail,  
Laboratoire de Ploufragan-Plouzané-Niort, Unité Mycoplasmologie-Bactériologie et  
Antibiorésistance, Ploufragan, France

<sup>5</sup> Université de Limoges, INSERM, CHU Limoges, RESINFIT, U1092, Limoges, France

\*Corresponding author. Tel: +33-5-55-05-61-65; Fax: +33-5-55-05-67-22; E-mail:

[olivier.barraud@unilim.fr](mailto:olivier.barraud@unilim.fr)

## 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

## 26   **Abstract**

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

35

## ***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

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<sup>rd</sup> 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  $\beta$ -lactams, with extended-spectrum  $\beta$ -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].

### **Integrations 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 integrations 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 (integrations) could constitute a potential interesting tool. In fact, class 1 integrations can be easily detected [79] and are now considered as a good proxy for anthropic pollution [80,81]. Integrations 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 integrations 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.

## **Acknowledgments**

The authors are indebted to Akshay Sabnis who reviewed English language with great elegance. Authors would like also to thank Thomas Jové who provided with integron gene cassette arrays from *Aeromonas*.

## **Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## References

1. Fernandez-Bravo A, Figueras MJ: **An update on the genus *Aeromonas*: taxonomy, epidemiology, and pathogenicity.** *Microorganisms* 2020, **8**.
  2. Roger F, Marchandin H, Jumas-Bilak E, Kodjo A, col BVHsg, Lamy B: **Multilocus genetics to reconstruct aeromonad evolution.** *BMC Microbiol* 2012, **12**:62.
  3. Talagrand-Reboul E, Roger F, Kimper JL, Colston SM, Graf J, Latif-Eugenin F, Figueras MJ, Petit F, Marchandin H, Jumas-Bilak E, *et al.*: **Delineation of taxonomic species within complex of species: *Aeromonas media* and related species as a test case.** *Front Microbiol* 2017, **8**:621.
  4. Roger F, Lamy B, Jumas-Bilak E, Kodjo A, col BVHsg, Marchandin H: **Ribosomal multi-operon diversity: an original perspective on the genus *Aeromonas*.** *PLoS One* 2012, **7**:e46268.
  5. Reith ME, Singh RK, Curtis B, Boyd JM, Bouevitch A, Kimball J, Munholland J, Murphy C, Sarty D, Williams J, *et al.*: **The genome of *Aeromonas salmonicida* subsp. *salmonicida* A449: insights into the evolution of a fish pathogen.** *BMC Genomics* 2008, **9**:427.
  6. Gowda TK, Reddy VR, Devleeschauwer B, Zade NN, Chaudhari SP, Khan WA, Shinde SV, Patil AR: **Isolation and seroprevalence of *Aeromonas* spp. among common food animals slaughtered in Nagpur, Central India.** *Foodborne Pathog Dis* 2015, **12**:626-630.
  7. Igbinosa IH, Igbinosa EO, Okoh AI: **Antibiogram characterization and putative virulence genes in *Aeromonas* species isolated from pig fecal samples.** *Environ Sci Pollut Res Int* 2016, **23**:12199-12205.
  8. Lagkouvardos I, Joseph D, Kapfhammer M, Giritli S, Horn M, Haller D, Clavel T: **IMNGS: A comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies.** *Sci Rep* 2016, **6**:33721.
  9. Igbinosa IH, Igbinosa EO, Okoh AI: **Detection of antibiotic resistance, virulence gene determinants and biofilm formation in *Aeromonas* species isolated from cattle.** *Environ Sci Pollut Res Int* 2015, **22**:17596-17605.
  10. Ruzauskas M, Armalyte J, Lastauskiene E, Siugzdiniene R, Klimiene I, Mockeliunas R, Bartkiene E: **Microbial and antimicrobial resistance profiles of microbiota in common carps (*Cyprinus carpio*) from aquacultured and wild fish populations.** *Animals (Basel)* 2021, **11**.
  11. Maltz MA, Bomar L, Lapierre P, Morrison HG, McClure EA, Sogin ML, Graf J: **Metagenomic analysis of the medicinal leech gut microbiota.** *Front Microbiol* 2014, **5**:151.
  12. Seshadri R, Joseph SW, Chopra AK, Sha J, Shaw J, Graf J, Haft D, Wu M, Ren Q, Rosovitz MJ, *et al.*: **Genome sequence of *Aeromonas hydrophila* ATCC 7966T: jack of all trades.** *J Bacteriol* 2006, **188**:8272-8282.
  13. Zhong C, Han M, Yang P, Chen C, Yu H, Wang L, Ning K: **Comprehensive analysis reveals the evolution and pathogenicity of *Aeromonas*, viewed from both single isolated species and microbial communities.** *mSystems* 2019, **4**.
- \*\* 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.
14. Talagrand-Reboul E, Jumas-Bilak E, Lamy B: **The social life of *Aeromonas* through biofilm and quorum sensing systems.** *Front Microbiol* 2017, **8**:37.
  15. Talagrand-Reboul E, Colston SM, Graf J, Lamy B, Jumas-Bilak E: **Comparative and evolutionary genomics of isolates provide insight into the pathoadaptation of *Aeromonas*.** *Genome Biol Evol* 2020, **12**:535-552.

- \* 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.
16. Janda JM, Abbott SL: **The genus *Aeromonas*: taxonomy, pathogenicity, and infection.** *Clin Microbiol Rev* 2010, **23**:35-73.
  17. Awan F, Dong Y, Wang N, Liu J, Ma K, Liu Y: **The fight for invincibility: environmental stress response mechanisms and *Aeromonas hydrophila*.** *Microb Pathog* 2018, **116**:135-145.
- \* This paper presents an extensive review on the environmental stress response mechanisms of *A. hydrophila* and links it with its ability to survive and propagate under environmental various conditions
18. Nelson MC, Graf J: **Bacterial symbioses of the medicinal leech *Hirudo verbana*.** *Gut Microbes* 2012, **3**:322-331.
  19. Bandeira Junior G, Baldisserotto B: **Fish infections associated with the genus *Aeromonas*: a review of the effects on oxidative status.** *J Appl Microbiol* 2020.
  20. Menanteau-Ledouble S, Kumar G, Saleh M, El-Matbouli M: ***Aeromonas salmonicida*: updates on an old acquaintance.** *Dis Aquat Organ* 2016, **120**:49-68.
  21. Wallace IS, McKay P, Murray AG: **A historical review of the key bacterial and viral pathogens of Scottish wild fish.** *J Fish Dis* 2017, **40**:1741-1756.
  22. Zhang D, Xu DH, Shoemaker C, Hemstreet W: **Virulent *Aeromonas hydrophila* in channel catfish.** Edited by: Global Aquaculture Alliance; 2016.
  23. Charette SJ: **Microbe profile: *Aeromonas salmonicida*: an opportunistic pathogen with multiple personalities.** *Microbiology (Reading)* 2021, **167**.
- \* This article underlines the diversity of the species *A. salmonicida*, recognized as fish pathogen, described as psychrophilic bacteria. *A. salmonicida* able to growth at 37°C and to colonize/ infect mammals and birds is described here and linked to the genetic plasticity of the species.
24. Zhou H, Gai C, Ye G, An J, Liu K, Xu, Cao H: ***Aeromonas hydrophila*, an emerging causative agent of freshwater-farmed whiteleg shrimp *Litopenaeus vannamei*.** *Microorganisms* 2019, **7**.
  25. Abdelhamed H, Banes M, Karsi A, Lawrence ML: **Recombinant ATPase of virulent *Aeromonas hydrophila* protects channel catfish against motile *Aeromonas* septicemia.** *Front Immunol* 2019, **10**:1641.
  26. Abdelhamed H, Lawrence ML, Waldbieser G: **Complete genome sequence data of multidrug-resistant *Aeromonas veronii* strain MS-18-37.** *Data Brief* 2019, **23**:103689.
  27. Du X, Bayliss SC, Feil EJ, Liu Y, Wang C, Zhang G, Zhou D, Wei D, Tang N, Leclercq SO, et al.: **Real time monitoring of *Aeromonas salmonicida* evolution in response to successive antibiotic therapies in a commercial fish farm.** *Environ Microbiol* 2019, **21**:1113-1123.
  28. Sicuro B, Pastorino P, Barbero R, Barisone S, Dellerba D, Menconi V, Righetti M, De Vita V, Prearo M: **Prevalence and antibiotic sensitivity of bacteria isolated from imported ornamental fish in Italy: a translocation of resistant strains?** *Prev Vet Med* 2020, **175**:104880.
  29. Hossain S, Heo GJ: **Ornamental fish: a potential source of pathogenic and multidrug-resistant motile *Aeromonas* spp.** *Lett Appl Microbiol* 2021, **72**:2-12.
  30. Sreedharan K, Philip R, Singh IS: **Virulence potential and antibiotic susceptibility pattern of motile aeromonads associated with freshwater ornamental fish culture systems: a possible threat to public health.** *Braz J Microbiol* 2012, **43**:754-765.
  31. Koutsoumanis K, Lianou A, Sofos J: **Food Safety: Emerging Pathogens.** In *Encyclopedia of Agriculture and Food Systems*. Edited by Collection EPHE; 2014:250-272.
  32. Carlucci D, Nocella G, De Devitiis B, Viscecchia R, Bimbo F, Nardone G: **Consumer purchasing behaviour towards fish and seafood products. Patterns and insights from a sample of international studies.** *Appetite* 2015, **84**:212-227.



33. Lee HJ, Hoel S, Lunestad BT, Lerfall J, Jakobsen AN: ***Aeromonas* spp. isolated from ready-to-eat seafood on the Norwegian market: prevalence, putative virulence factors and antimicrobial resistance.** *J Appl Microbiol* 2021, **130**:1380-1393.
  34. Hoel S, Vadstein O, Jakobsen AN: **The significance of mesophilic *Aeromonas* spp. in minimally processed ready-to-eat seafood.** *Microorganisms* 2019, **7**.
  35. Umutoni N, Jakobsen AN, Mukhatov K, Thomassen GMB, Karlsen H, Mehli L: **Occurrence, diversity and temperature-dependent growth kinetics of *Aeromonas* spp. in lettuce.** *Int J Food Microbiol* 2020, **335**:108852.
  36. Fontes MC, Saavedra MJ, Monera A, Martins C, Martinez-Murcia A: **Phylogenetic identification of *Aeromonas simiae* from a pig, first isolate since species description.** *Vet Microbiol* 2010, **142**:313-316.
  37. Fontes MC, Saavedra MJ, Martins C, Martinez-Murcia AJ: **Phylogenetic identification of *Aeromonas* from pigs slaughtered for consumption in slaughterhouses at the North of Portugal.** *Int J Food Microbiol* 2011, **146**:118-122.
  38. Li L, Heidemann Olsen R, Ye L, Yan H, Nie Q, Meng H, Shi L: **Antimicrobial resistance and resistance genes in aerobic bacteria isolated from pork at slaughter.** *J Food Prot* 2016, **79**:589-597.
  39. Harnisz M, Korzeniewska E: **The prevalence of multidrug-resistant *Aeromonas* spp. in the municipal wastewater system and their dissemination in the environment.** *Sci Total Environ* 2018, **626**:377-383.
  40. Latif-Eugenin F, Beaz-Hidalgo R, Silvera-Simon C, Fernandez-Cassi X, Figueras MJ: **Chlorinated and ultraviolet radiation -treated reclaimed irrigation water is the source of *Aeromonas* found in vegetables used for human consumption.** *Environ Res* 2017, **154**:190-195.
  41. Solaiman S, Allard SM, Callahan MT, Jiang C, Handy E, East C, Haymaker J, Bui A, Craddock H, Murray R, et al.: **Longitudinal assessment of the dynamics of *Escherichia coli*, total coliforms, *Enterococcus* spp., and *Aeromonas* spp. in alternative irrigation water sources: a CONSERVE study.** *Appl Environ Microbiol* 2020, **86**.
- \* 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.
42. Kampfer P, Erhart R, Beimfohr C, Bohringer J, Wagner M, Amann R: **Characterization of bacterial communities from activated sludge: culture-dependent numerical identification versus in situ identification using group- and genus-specific rRNA-targeted oligonucleotide probes.** *Microb Ecol* 1996, **32**:101-121.
  43. Andersson S, Dalhammar G, Kuttuva Rajarao G: **Influence of microbial interactions and EPS/polysaccharide composition on nutrient removal activity in biofilms formed by strains found in wastewater treatment systems.** *Microbiol Res* 2011, **166**:449-457.
  44. Chong G, Kimyon O, Rice SA, Kjelleberg S, Manefield M: **The presence and role of bacterial quorum sensing in activated sludge.** *Microb Biotechnol* 2012, **5**:621-633.
  45. Chen M, Wang W, Feng Y, Zhu X, Zhou H, Tan Z, Li X: **Impact resistance of different factors on ammonia removal by heterotrophic nitrification-aerobic denitrification bacterium *Aeromonas* sp. HN-02.** *Bioresour Technol* 2014, **167**:456-461.
  46. Lamy B, Kodjo A, col BVHSG, Laurent F: **Prospective nationwide study of *Aeromonas* infections in France.** *J Clin Microbiol* 2009, **47**:1234-1237.
  47. Parker JL, Shaw JG: ***Aeromonas* spp. clinical microbiology and disease.** *J Infect* 2011, **62**:109-118.
  48. Bhowmick UD, Bhattacharjee S: **Bacteriological, clinical and virulence aspects of *Aeromonas*-associated diseases in humans.** *Pol J Microbiol* 2018, **67**:137-149.

49. Wu CJ, Chen PL, Tang HJ, Chen HM, Tseng FC, Shih HI, Hung YP, Chung CH, Ko WC: **Incidence of *Aeromonas* bacteremia in Southern Taiwan: *Vibrio* and *Salmonella* bacteremia as comparators.** *J Microbiol Immunol Infect* 2014, **47**:145-148.
  50. Zimmermann S, Horner S, Altwegg M, Dalpke AH: **Workflow optimization for syndromic diarrhea diagnosis using the molecular Seegene Allplex GI-Bacteria(I) assay.** *Eur J Clin Microbiol Infect Dis* 2020, **39**:1245-1250.
  51. Whitaker IS, Oboumarzouk O, Rozen WM, Naderi N, Balasubramanian SP, Azzopardi EA, Kon M: **The efficacy of medicinal leeches in plastic and reconstructive surgery: a systematic review of 277 reported clinical cases.** *Microsurgery* 2012, **32**:240-250.
  52. Beka L, Fullmer MS, Colston SM, Nelson MC, Talagrand-Reboul E, Walker P, Ford B, Whitaker IS, Lamy B, Gogarten JP, *et al.*: **Low-level antimicrobials in the medicinal leech select for resistant pathogens that spread to patients.** *MBio* 2018, **9**.
- \*\* 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.
53. Herlin C, Bertheuil N, Bekara F, Boissiere F, Sinna R, Chaput B: **Leech therapy in flap salvage: systematic review and practical recommendations.** *Ann Chir Plast Esthet* 2017, **62**:e1-e13.
  54. Patel KM, Svestka M, Sinkin J, Ruff Pt: **Ciprofloxacin-resistant *Aeromonas hydrophila* infection following leech therapy: a case report and review of the literature.** *J Plast Reconstr Aesthet Surg* 2013, **66**:e20-22.
  55. Barraud O, Robert A, Laval L, Ruimy R, Morquin D, Boyer L, Lamy B: **It takes two to tango: two *Aeromonas* isolates combine virulence and multidrug resistance in flap infection following leech therapy.** *Clin Microbiol Infect* 2020, **26**:793-794.
  56. Moraru R, Pourcher AM, Jadas-Hecart A, Kempf I, Ziebal C, Kervarrec M, Comunal PY, Mares M, Dabert P: **Changes in concentrations of fluoroquinolones and of ciprofloxacin-resistant Enterobacteriaceae in chicken feces and manure stored in a heap.** *J Environ Qual* 2012, **41**:754-763.
  57. Rutgersson C, Fick J, Marathe N, Kristiansson E, Janzon A, Angelin M, Johansson A, Shouche Y, Flach CF, Larsson DG: **Fluoroquinolones and *qnr* genes in sediment, water, soil, and human fecal flora in an environment polluted by manufacturing discharges.** *Environ Sci Technol* 2014, **48**:7825-7832.
  58. Floug Q, Sinna R, Fillatre A, Plesiat P, Hamdad F: **A case of leech-associated infection involving an extended-spectrum beta-lactamase-producing and extensively drug-resistant *Aeromonas hydrophila*.** *Clin Microbiol Infect* 2019, **25**:394-395.
  59. Kloub L, Gosselin S, Fullmer M, Graf J, Gogarten JP, Bansal MS: **Systematic detection of large-scale multigene horizontal transfer in prokaryotes.** *Mol Biol Evol* 2021, **38**:2639-2659.
- \* This study focus 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.
60. Piotrowska M, Popowska M: **Insight into the mobilome of *Aeromonas* strains.** *Front Microbiol* 2015, **6**:494.
  61. Rasmussen-Ivey CR, Figueras MJ, McGarey D, Liles MR: **Virulence factors of *Aeromonas hydrophila*: in the wake of reclassification.** *Front Microbiol* 2016, **7**:1337.
  62. Bello-Lopez JM, Cabrero-Martinez OA, Ibanez-Cervantes G, Hernandez-Cortez C, Pelcastre-Rodriguez LI, Gonzalez-Avila LU, Castro-Escarpulli G: **Horizontal gene transfer and its association with antibiotic resistance in the genus *Aeromonas* spp.** *Microorganisms* 2019, **7**.

\* This review focus 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.

63. Lorenz MG, Wackernagel W: **Bacterial gene transfer by natural genetic transformation in the environment.** *Microbiol Rev* 1994, **58**:563-602.

64. Huddleston JR, Brokaw JM, Zak JC, Jeter RM: **Natural transformation as a mechanism of horizontal gene transfer among environmental *Aeromonas* species.** *Syst Appl Microbiol* 2013, **36**:224-234.

65. Yuan S, Chen L, Liu Q, Zhou Y, Yang J, Deng D, Li H, Ma Y: **Characterization and genomic analyses of *Aeromonas hydrophila* phages AhSzq-1 and AhSzw-1, isolates representing new species within the T5virus genus.** *Arch Virol* 2018, **163**:1985-1988.

66. Park SY, Han JE, Kwon H, Park SC, Kim JH: **Recent insights into *Aeromonas salmonicida* and its bacteriophages in aquaculture: A comprehensive review.** *J Microbiol Biotechnol* 2020, **30**:1443-1457.

67. Vincent AT, Hosseini N, Charette SJ: **The *Aeromonas salmonicida* plasmidome: a model of modular evolution and genetic diversity.** *Ann N Y Acad Sci* 2021, **1488**:16-32.

\* This review focus on the latest knowledge in the field of plasmid studies and in particular on the knowledge of the *A. salmonicida* plasmidome. The key role of the plasmidome to better understand the biology, diversity, pathogenicity and the evolution of *A. salmonicida* is highlighted and discussed.

68. Veening JW, Blokesch M: **Interbacterial predation as a strategy for DNA acquisition in naturally competent bacteria.** *Nat Rev Microbiol* 2017, **15**:621-629.

69. Jaboulay C, Godeux AS, Doublet P, Vianney A: **Regulatory networks of the T4SS control: from host cell sensing to the biogenesis and the activity during the infection.** *J Mol Biol* 2021, **433**:166892.

70. Chourashi R, Das S, Dhar D, Okamoto K, Mukhopadhyay AK, Chatterjee NS: **Chitin-induced T6SS in *Vibrio cholerae* is dependent on ChiS activation.** *Microbiology (Reading)* 2018, **164**:751-763.

71. Aujoulat F, Roger F, Bourdier A, Lotthe A, Lamy B, Marchandin H, Jumas-Bilak E: **From environment to man: genome evolution and adaptation of human opportunistic bacterial pathogens.** *Genes (Basel)* 2012, **3**:191-232.

72. Nwaiwu O, Aduba CC: **An in silico analysis of acquired antimicrobial resistance genes in *Aeromonas* plasmids.** *AIMS Microbiol* 2020, **6**:75-91.

73. Ayobola ED, Oscar WO, Ejovwokoghene EF: **Occurrence of plasmid mediated fluoroquinolone resistance genes amongst enteric bacteria isolated from human and animal sources in Delta State, Nigeria.** *AIMS Microbiol* 2021, **7**:75-95.

74. Piotrowska M, Przygodzinska D, Matyjewicz K, Popowska M: **Occurrence and variety of beta-lactamase genes among *Aeromonas* spp. isolated from urban wastewater treatment plant.** *Front Microbiol* 2017, **8**:863.

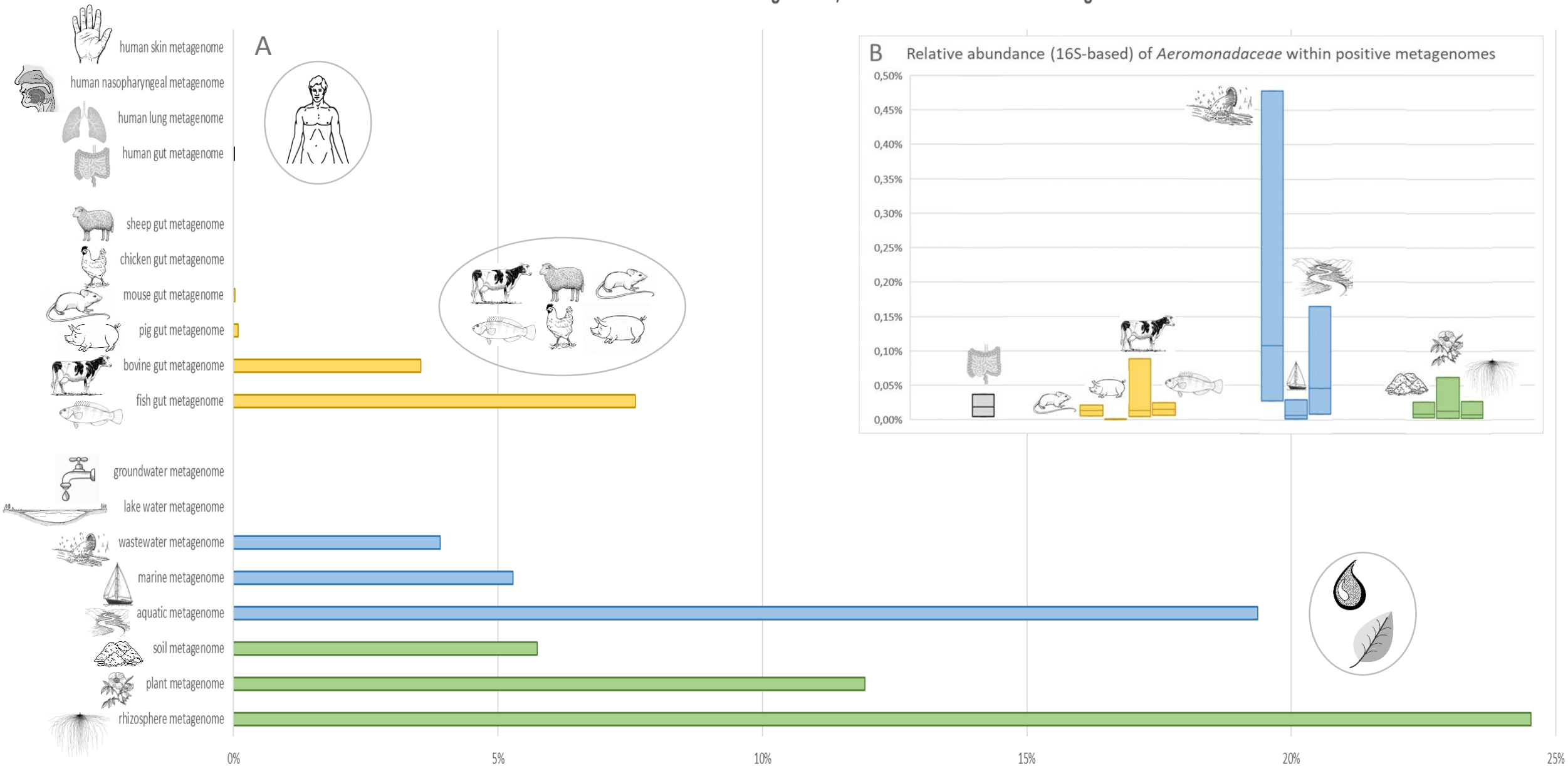
75. Bosch T, Schade R, Landman F, Schouls L, Dijk KV: **A blaVIM-1 positive *Aeromonas hydrophila* strain in a near-drowning patient: evidence for interspecies plasmid transfer within the patient.** *Future Microbiol* 2019, **14**:1191-1197.

76. Hu X, Yu X, Shang Y, Xu H, Guo L, Liang Y, Kang Y, Song L, Sun J, Yue F, et al.: **Emergence and characterization of a novel IncP-6 plasmid harboring blaKPC-2 and qnrS2 genes in *Aeromonas taiwanensis* isolates.** *Front Microbiol* 2019, **10**:2132.

77. Lin X, Lu J, Qian C, Lin H, Li Q, Zhang X, Liu H, Sun Z, Zhou D, Lu W, et al.: **Molecular and functional characterization of a novel plasmid-borne blaNDM-like gene, blaAFM-1, in a clinical strain of *Aeromonas hydrophila*.** *Infect Drug Resist* 2021, **14**:1613-1622.

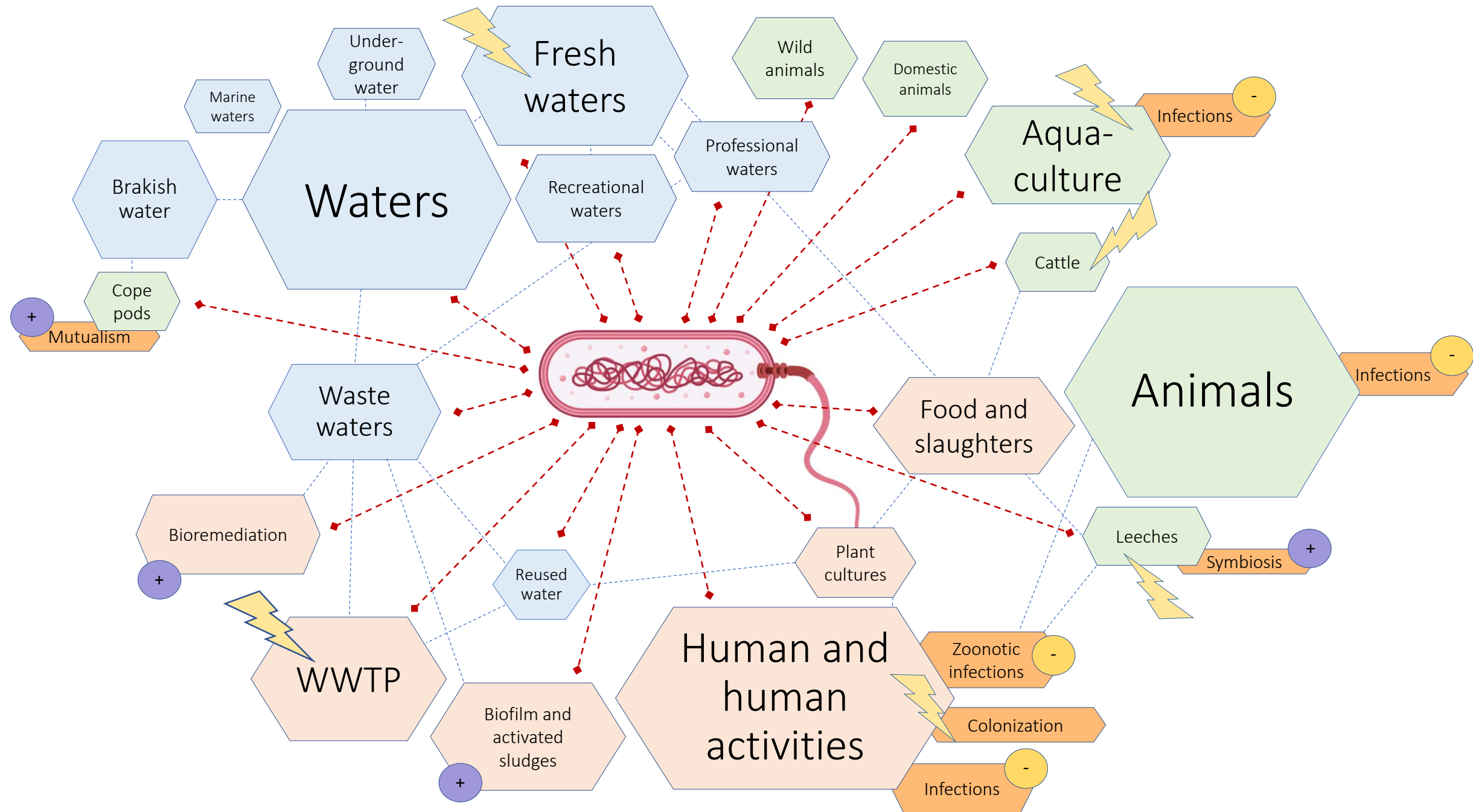
78. Tuo H, Yang Y, Tao X, Liu D, Li Y, Xie X, Li P, Gu J, Kong L, Xiang R, *et al.*: **The prevalence of colistin resistant strains and antibiotic resistance gene profiles in Funan River, China.** *Front Microbiol* 2018, **9**:3094.
79. Barraud O, Baclet MC, Denis F, Ploy MC: **Quantitative multiplex real-time PCR for detecting class 1, 2 and 3 integrons.** *J Antimicrob Chemother* 2010, **65**:1642-1645.
80. Gillings MR, Gaze WH, Pruden A, Smalla K, Tiedje JM, Zhu YG: **Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution.** *ISME J* 2015, **9**:1269-1279.
81. Stalder T, Barraud O, Jove T, Casellas M, Gaschet M, Dagot C, Ploy MC: **Quantitative and qualitative impact of hospital effluent on dissemination of the integron pool.** *ISME J* 2014, **8**:768-777.
82. Mazel D: **Integrons: agents of bacterial evolution.** *Nat Rev Microbiol* 2006, **4**:608-620.
83. Cambray G, Guerout AM, Mazel D: **Integrons.** *Annu Rev Genet* 2010, **44**:141-166.
84. Deng Y, Wu Y, Jiang L, Tan A, Zhang R, Luo L: **Multi-drug resistance mediated by class 1 integrons in *Aeromonas* isolated from farmed freshwater animals.** *Front Microbiol* 2016, **7**:935.
85. Lee MF, Peng CF, Lin YH, Lin SR, Chen YH: **Molecular diversity of class 1 integrons in human isolates of *Aeromonas* spp. from Southern Taiwan.** *Jpn J Infect Dis* 2008, **61**:343-349.
86. Carnelli A, Mauri F, Demarta A: **Characterization of genetic determinants involved in antibiotic resistance in *Aeromonas* spp. and fecal coliforms isolated from different aquatic environments.** *Res Microbiol* 2017, **168**:461-471.
87. Barraud O, Francois B, Chainier D, Vignaud J, Ploy MC: **Value of integron detection for predicting antibiotic resistance in patients with Gram-negative septicaemia.** *Int J Antimicrob Agents* 2014, **44**:351-353.
88. Mendes Moreira A, Couvé-Deacon E, Bousquet P, Chainier D, Jové T, Ploy MC, Barraud O: **Proteae: a reservoir of class 2 integrons?** *J Antimicrob Chemother* 2019, **74**:1560-1562.
89. Moura A, Soares M, Pereira C, Leitao N, Henriques I, Correia A: **INTEGRALL: a database and search engine for integrons, integrases and gene cassettes.** *Bioinformatics* 2009, **25**:1096-1098.
90. Pievani T, Serrelli E: **Exaptation in human evolution: how to test adaptive vs exaptive evolutionary hypotheses.** *J Anthropol Sci* 2011, **89**:9-23.
91. Cabello FC, Godfrey HP: **Aquaculture, exaptation, and the origin of *mcr*-positive colistin resistance.** *Antimicrob Agents Chemother* 2018, **62**.
92. Sanchez MB, Martinez JL: **Differential epigenetic compatibility of *qnr* antibiotic resistance determinants with the chromosome of *Escherichia coli*.** *PLoS One* 2012, **7**:e35149.
93. Rowe-Magnus DA, Mazel D: **Integrons: natural tools for bacterial genome evolution.** *Curr Opin Microbiol* 2001, **4**:565-569.

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



**Figure 1: Prevalence of *Aeromonadaceae* among human, animal and environmental metagenomes.**

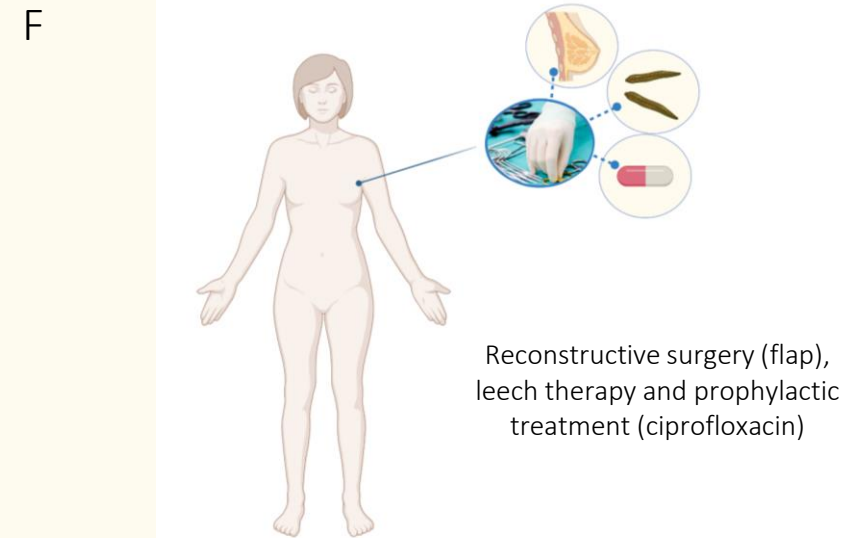
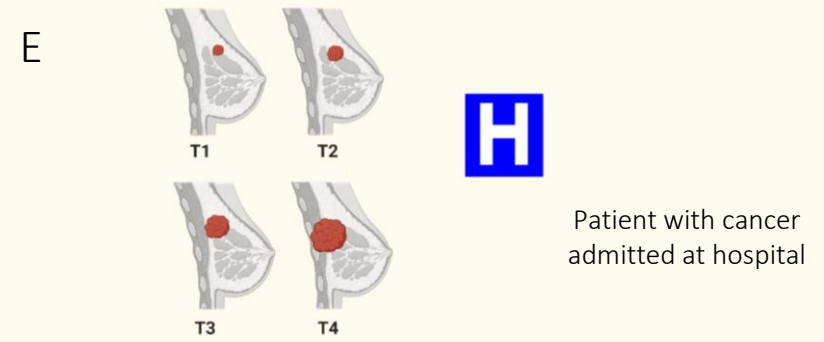
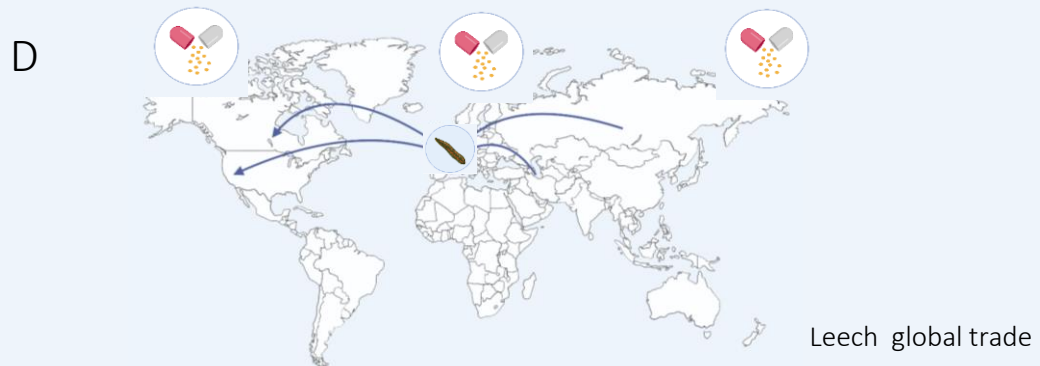
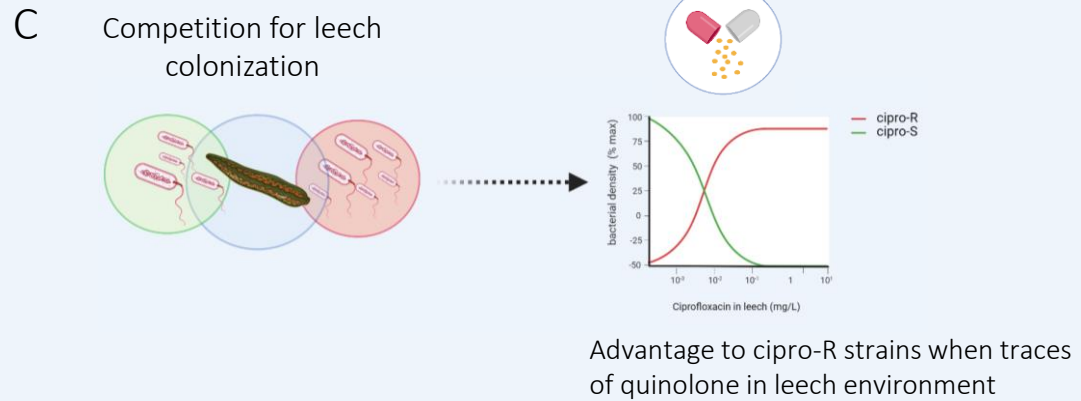
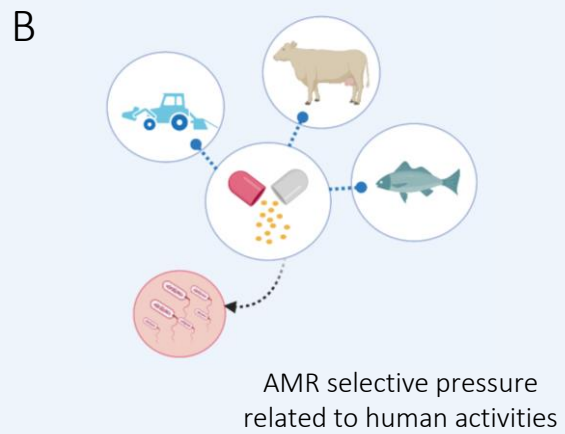
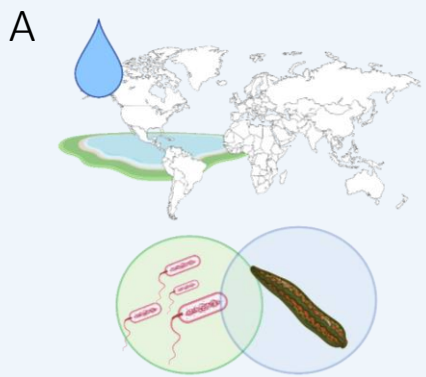
Figure 1A presents for the 3 compartments of the *One Health* world the percentage of metagenomes in which *Aeromonadaceae* are detected. This ecological distribution is based on 16S rRNA studies: grey, human metagenomes; yellow, fish and other animal metagenomes; blue, water metagenomes; green, soil 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.



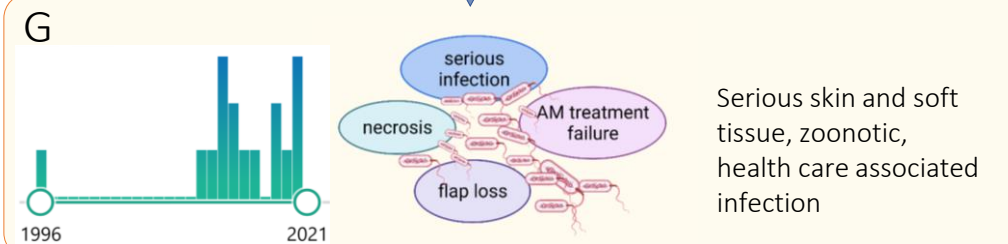
**Figure 2: *Aeromonas* in the *One Health* world, a complex network of relationships.**

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 regurgitates (multi-) drug resistant aeromonads



**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.