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K1

LESONS LEARNED AFTER WORKING ON THE TAXONOMY AND EPIDEMIOLOGY OF *Aeromonas* FOR 25 YEARS.

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The celebration of our ISAP 2023 coincides with the 80th anniversary of the description of the genus *Aeromonas* and its type species *Aeromonas hydrophila* in 1943 by Stainer. The latter is one of the more abundant species recovered from almost all the environments. However, the prominent role of this species that eclipsed all other species for many years is a result of misidentifications using API, Vitek, MicroScan, BBLCrystal. The MALDI-TOF method is a fast and highly precise identification method at the genus level but still relatively imprecise at the species level because the database include a limited number of strains and species and old names that have long ago been synonymised. However, these limitations have been ignored by the producers. In 1992 the phylogeny of the genus using the sequences of the 16S rRNA gene was published indicating that some had an almost identical sequence. Later on, it was discovered that the different copies of this gene presented microheterogeneities, an additional complication that generated misidentifications. The introduction of housekeeping genes (*gyr*B, *rpo*D etc.) with a high-resolution and the use of a multilocus phylogenetic analysis (MLPA) derived from the concatenated sequences of multiple genes lead to the discovery of many new species, four are still in the process of description. The availability of many *Aeromonas* genomes and tools like the *in silico* DNA-DNA hybridization (isDDH) and the Average Nucleotide Identity (ANI) for their comparison revealed to us the existence of many mislabelled genomes deposited at NCBI database. Guidelines for their validation were proposed. Molecular identification revealed that 95.4% of the clinical strains belong to *A. caviae*, *A. dhakensis*, *A. veronii* and *A. hydrophila*, but the prevalence depends upon the geographical region. Willing to explain why some of the latter are the most prevalent species it was discovered that strains of these species possess a T3SS and some produce a strong immune response in human monocytic cells (THP-1) with a higher cell damage. Recently, we have described the virulence potential of clinical isolates of mesophilic *Aeromonas salmonicida* and of *Aeromonas trota,* the only susceptible species to ampicillin. The latter two form part of the 11 additional species recovered from clinical cases. Gastroenteritis, bacteremia and wound infections remain the dominating presentations. The role of *Aeromonas* as etiological agent of diarrhea has been questioned. However, using data from outbreaks, challenging studies and dose response models of microbial infection it was demonstrated that the enteropathogenicity of *Aeromonas* was similar to the one of *Campylobacter* or *Salmonella*. Infections affect also the respiratory tract, the bone and/or joints, the urinary tract, in addition to necrotizing fasciitis (NF) mainly in elderly patients with cirrhosis or hepatobiliary disease. Infections worsen with comorbidities and/or the involvement of antibiotic resistant strains. An NF in a young girl that required limp amputations was caused by two strains of *A. hydrophila* bearing T6SS and/or exotoxin A (ExoA) genes. Construction of T6SS and ExoA mutants from these strains showed that both virulence factors play a role after monomicrobial and polymicrobial infection in mouse peritonitis and necrotizing fasciitis models. Further research to determine the specific role of *Aeromonas* in monomicrobial and polymicrobial gastrointestinal infections is also needed. Metagenomic analysis revealed that *Aeromonas* are able to multiply in wastewater. The latter can act as reservoir of potential pathogenic species. The use of reclaimed water for irrigation of ready-to-eat vegetables may represent a potential threat for consumers considering that the same genotype of a *A. caviae* has been isolated from the irrigated tomatoes and the water. This can be relevant considering the impact of climate change in water scarcity.

K2

PLESIOMONAS SHIGELLOIDES A UNIQUE BACTERIUM?

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Plesiomonas shigelloides are rod-shaped, Gram-negative, facultative anaerobic, oxidase- and catalase-positive, flagellated bacteria emerging as important effectors in foodborne diseases. *P. shigelloides* was considered part of the *Vibrionaceae* family for many years, due mainly to its resemblance to aeromonads –the Greek prefix *plesio*- means “near”–. Nowadays, plesiomonads are classified as part of the *Enterobacteriaceae* family, given their genetic similarity to *Proteae*. Interestingly, as their species names suggest, plesiomonads present *Shigella*-like antigens and some *P. shigelloides* serotypes cross-react with *Shigella* spp. antisera. *P. shigelloides* is the only oxidase-positive member of its family. Furthermore, phylogenetic studies routinely place *P. shigelloides* in a peripheral branch or directly outside the enterobacterial cluster.

Plesiomonas infections can be gastrointestinal and extraintestinal. While gastrointestinal complications are the most common, limited information about extraintestinal *P. shigelloides* diseases is available. The exact mechanism of *P. shigelloides* infection is currently unknown, in part due to the lack of effective infection models. However, several virulence factors have been associated with infections, including the lipopolysaccharide (LPS) complex, flagella motility, toxins, and iron acquisition systems. We are going to focus in the study of the two first factors.

Our research team study the *P. shigelloides* flagella genomics. *P. shigelloides* have three or four polar flagella in liquid media (lophotrichus) but on solid media show flagellation consisting of a completely different peritrichous (or lateral) flagellum which may be present in large numbers. *P. shigelloides* is the only *Enterobacteriaceae* with a functional lateral flagella system. We also show that both flagella in *P.shigelloides* are glycosylated by a derivative of legionaminic acid. It has been shown that being the first enteric flagella described glycosylated, and the first flagella glycosylated with legionaminic acid. The role in pathogenesis of flagella is determinant, specially through motility used by both flagella types.

We report the identification of the genes required for the biosynthesis of the core lipopolysaccharides (LPS) of two *P. shigelloides* strains (*waa* clusters), also with the genomics of O1-antigen LPS (*wb* cluster). *P. shigelloides* and *Klebsiella pneumoniae, Serratia marcescens, Proteus sp.* share a core LPS carbohydrate backbone extending up at least to the second outer-core residue, which agree with their taxonomic position.

Our research team demonstrate that the presence of the O1-antigen LPS is crucial for to survive in serum mainly to become resistant to complement. Also, it is an important factor in the bacterial adhesion and invasion to some eukaryotic cells, and in the ability to form biofilms. *P. shigelloides* demonstrated an important variety of core LPS structures in the outer-core LPS with *waa* clusters with small modifications, despite being a single species of the genus, as well as high homologous recombination in housekeeping genes.

K3

REVIEW OF *AEROMONAS DHAKENSIS*: CLINICAL INFECTIONS AND PATHOGENESIS

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Aeromonas hydrophila subspecies dhakensis (*A. dhakensis*) is a member of the *A. hydrophila* complex. It is commonly found in aquatic environments and clinical samples worldwide, particularly in tropical regions. Despite often being misidentified as *A. hydrophila* using traditional phenotypic identification methods, accurate detection of *A. dhakensis* can be achieved through matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) and molecular typing. This pathogenic species is known to cause serious clinical infections, including gastroenteritis, septicemia, and severe soft tissue infections. Extensive studies using cell lines and animal models have demonstrated the virulence potential of *A. dhakensis*. Notably, patients with *A. dhakensis* bacteremia have shown higher mortality rates related to sepsis compared to those infected with other species. However, the underlying mechanism by which A. dhakensis induces tissue damage remains poorly understood.

A. dhakensis harbors various genes encoding virulence factors such as hemolysins, secretion systems, proteases, and collagenases. In our recent investigation, we discovered that the hemolysin Ahh1, a prominent virulence factor of A. dhakensis, triggers cellular damage by activating the NLR family pyrin domain-containing 3 (NLRP3) inflammasome signaling pathway. Targeting the NLRP3 inflammasome, in combination with antibiotics, holds promise as a potential therapy for severe soft tissue infections caused by toxin-producing bacteria. It is worth noting that *A. dhakensis* displays reduced susceptibility to third-generation cephalosporins and carbapenems due to the presence of AmpC β-lactamase and metalloprotease, respectively. Given the prevalence of genes encoding AmpC β-lactamase and carbapenemase, the preferred treatment options for severe *A. dhakensis* infections with a high bacterial burden include fourth-generation cephalosporins, fluoroquinolones, aminoglycosides, and tigecycline.

I1

IDENTIFYING THE DIVERS OF ANTIMICROBIAL RESISTANCE THOUGH THE "EYES" OF *AEROMONAS*: A GLOBAL ONE HEALTH PERSPECTIVE

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According to the World Health Organization, antimicrobial resistance (AMR) continues to pose one of the top 10 greatest threats to global health, already claiming 1,270,000 lives a year [1]. One approach to combating resistance is to better understand the interconnectedness of human, animal and environmental habitats on the emergence and evolution of AMR. Considering the ubiquitous presence of *Aeromonas* spp. in aquatic environments, as well as ability to colonize and cause disease in both warm- and cold-blooded animals [2], we analyzed AMR among *Aeromonas* populations. To justify the use of *Aeromonas* as an indicator species to study AMR from a One Health perspective, we compared its prevalence to the more commonly studied *Escherichia coli* from 10,229 rRNA sequences acquired from the integrated microbial next generation sequencing database (https://www.imngs.org/) spanning a variety of environments. *Aeromonas* spp. was identified in all sectors – human, wastewater, drinking water, surface water and throughout agriculture with a significant abundance over *E. coli* in wastewater, surface water, and aquaculture. We next performed a systematic review and meta-analysis of all AMR data associated with *Aeromonas* spp. from 2000 to 2020 in all major sectors to analyze AMR from a Global One Health approach. This involved screening 7,382 articles and extracting AMR data from 221 published articles (14 agricultural, 82 seafood, 87 clinical, 8 drinking water, 19 surface water, and 11 wastewater studies) that met our inclusion criteria resulting in data from 15,891 isolates spanning 57 countries [3]. From these data, we were able to compare resistance levels against 21 different antibiotics across six major sectors: clinical, wastewater, surface water, drinking water, seafood, and agriculture. We identified similar resistance levels across sectors for most antibiotics, though wastewater populations were significantly more resistant to the critically important antibiotics aztreonam and cefepime. Within sectors, we identified that treated wastewater contained more susceptible populations compared to untreated. We also identified increased prevalence of AMR to ciprofloxacin and tetracycline associated with isolates coming from farm-raised seafood compared to wild-caught. One of the WHO strategies to combat AMR was to categorize therapeutic antibiotics against common childhood diseases into either Access, Watch, or Reserve based upon the risk of inducing or disseminating AMR. The mitigation strategy was to promote increased use of Access to Watch drugs within each country. Our findings supported that countries that used more Watch to Access drugs in 2015 compared to 2000 correlated with *Aeromonas* isolates exhibiting more resistance. We also recognized an inverse association of AMR with socioeconomic status and environmental performance indices. Together, our findings stress the need for global antimicrobial stewardship programs to address socioeconomic and environmental policies, specifically advocating for global support of basic infrastructure to help combat AMR. Lastly, our findings highlight the pros and cons of wastewater treatment and provide support for its use as wastewater-based epidemiology to track AMR patterns.

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I2

CHEMOTYPING OF BACTERIAL GLYCANS USING NMR SPECTROSCOPY. HIGH-RESOLUTION MAGIC ANGLE SPINNING NMR IN THE ANALYSIS OF *PLESIOMONAS SHIGELLOIDES* O-ANTIGENS

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Bacteria expose on the surface complex glycans that are essential for structural integrity and interactions with hosts. Gram-negative bacteria produce lipopolysaccharides (LPS), which are the major component of the outer cell membrane - essential for physical organization and function. They constitute the most exposed antigens in non-encapsulated bacteria and targets for specific antibodies. The antigenic schemes of *P. shigelloides* have been extensively studied with serological methods. The serotyping scheme of *P. shigelloides* points to at least 102 O-serotypes[1-6]. However, the knowledge about the unique structures of the O-antigens remains rather limited.

High-resolution magic angle spinning NMR spectroscopy (HR-MAS NMR) is a unique technique, that allows for the investigation and validation of glycans directly on the surface of intact bacteria[7]. The structural identities of the O-antigens on the surface of bacteria, but also in the isolated LPS and O-specific polysaccharides[8] can be assessed. When combined with the structure reporter group concept[9] the technique is suitable for detection of chemical differences between strains, without a prior knowladge of the glycan structures, as the changes in the HR-MAS NMR spectra provide immediate distinction between differing structures. This approach allows to chemotype and classify bacteria according to the NMR detected structural features of the surface glycans. The possible applications of the HR-MAS NMR technique In chemotyping of bacterial surface carbohydrates, including the benefits and downsides as well as prospects will be presented and discussed.

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I3

AFTER THIRTY YEARS OF APPLYING PHYLOGENETIC ANALYSIS TO *AEROMONAS* TAXONOMY, THE PARADIGM PERSISTS: HOW DISTANT IS DISTANT? (OR HOW CLOSE IS CLOSE)

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Pioneer phylogenetic analysis of the genus *Aeromonas* based on the gene sequences coding for the 16S rRNA (16S rDNA), first achieved by us thirty years ago, evidenced a complex scenario in the taxonomy of this genus. The sequences were extremely conserved when compared to overall rates observed in other bacteria, in some cases they were almost identical, and therefore, species cannot be delineated according to the standards currently established based on 16S rDNA sequence similarities. The presence of 'microheterogeneities' (i.e., nucleotide diversity at homologous positions of the rRNA multigene family) and some chronometric distortions (i.e., convergent evolutionary changes) are additional drawbacks. Despite all this, genus-specific signature moieties have been discerned, allowing cost effective mass identification of *Aeromonas* colonies on rich media cultures not bypassed by the use of selective antibiotics. Sequences of housekeeping genes, mainly coding for DNA processing proteins (i.e., replication, transcription, translation, etc.), are suitable high-resolution phylogenetic markers. Since they are subjected to a degenerative code, the nucleotide changes may spread along the gene without consequences in the protein primary structure. Evolution mode differs to this of rRNAs (that follows a mosaic of discrete variation located at signature regions), making the protein-coding genes very powerful to discriminate highly related strains. The *gyrB* gene was the first housekeeping protein-coding gene used for a phylogenetic analysis of the genus *Aeromonas*, published by us 20 years ago, and evidenced an extraordinarily resolution to split closely related *Aeromonas* species. The use of a single protein-coding gene phylogeny is not recommended as not all of them show the same phylogenetic resolution (depending on function relevancy) or they may be subjected to a possible horizontal gene transfer/recombination process. Consequently, robust taxonomy and identification should incorporate concatenated multigene phylogenies, as currently recommended in bacterial systematics. The strategy, although first named "multi-locus sequence analysis" (MLSA), was then re-named by us "multi-locus phylogenetic analysis" (MLPA) as it was considered a more appropriate term (the analysis of data is intrinsically phylogenetic). The approach is based on a hypothetically synchronized mode of evolution of genomes, i.e., genes evolve in concert and, consequently, concatenated multigene phylogeny may be 'the mirror' of the overall relationships of the entire genomic content. Currently, Whole Genome Sequencing (WGS) provides a new chance to assess total *in silico* DNA similarities. Indexes from pairwise genome comparisons have been suggested as the next gold standard in taxonomy for species definition. Of course, in addition, WGS makes available many (if not all) housekeeping gene sequences which can be used to perform MLPA. Nevertheless, a number of difficulties in handling this huge amount of data makes the full genome analysis a tedious approach for standard laboratories of microbial control. We are globally promoting a more pragmatic and affordable MLPA approach for the screening of isolates (single colonies), previously identified as *Aeromonas* by conventional PCR. Results from recent MLPA identification of some *Aeromonas* species isolated from psychrophilic environments (Antarctic and Patagonia) will be shown. Although the contribution of the MLPA approach to *Aeromonas* taxonomy has already surpassed any expectation, everything seems to indicate that an explosive description of *Aeromonas* diversity is yet to come.

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I4

PREVALENCE OF AEROMONAS IN PATIENTS FROM GULF COAST AREA: *IN VIVO* EXPRESSION OF VIRULENCE GENES FROM FATAL CASES

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A 14-year retrospective review of medical records from patients with culture-positive *Aeromonas* infections was conducted, and clinical presentations, antibiotic resistance patterns as well as comparison of *in vitro* versus *in vivo* expression of virulence genes by transcriptome analysis from two fatal cases were assessed. The study revealed sequalae of clinical manifestations in patients, multiple-antibiotic resistance patterns, and identification of known and of potentially new virulence gene(s) that could be associated with pathogenesis of *Aeromonas* infections.

O1

DESCRIPTION AND PATHOGENIC CHARACTERIZATION OF TWO NEW SPECIES, *AEROMONAS ENTERICA* AND *AEROMONAS INTESTINALIS,* ISOLATED FROM CLINICAL SAMPLES

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The genus *Aeromonas* belongs to the Family *Aeromonadaceae* and embraces oxidase- positive, facultatively anaerobic, Gram-Negative bacilli. Currently, 32 *Aeromonas* species have been recognized, however, from several studies in our laboratory, two strains could not be assigned to any of the known *Aeromonas* species based on the *rpod* gene. Subsequently, Figueras et al. [1] performed an announcement showed that both strains 1178C^T and 113634^T of clinical origin belonged to new species of *Aeromonas* based on the MLPA and *is*DDH, and proposed calling them *Aeromonas intestinalis* and *Aeromonas enterica*, respectively. Despite this, these two species have not been described so far, so this study aims to describe both species, as well as to characterize their pathogenic potential. For the description, according to the Committee of Systematic of Prokaryotes, the phenotypic characteristics were studied to compare with the other species within the genus. Also, to describe the nearest *Aeromonas* species the MLPA was constructed with all strains in the database (137 *Aeromonas* strains), and the genome comparison with the genomic indexes ANI and *is*DDH was performed using four platforms: ANI calculator, JSpecies, OrthoANI, and Genome-to Genome Distance Calculator. The size and presence of flagella were determined by electron microscopy. [2] For the pathogenic characterization, an *in vitro* study was performed using the mouse macrophage BALB/c cell line J774A.1. To determine the impact of the infection with both strains on the macrophages, the intracellular survival and phagocytosis were studied, cell damage was measured by the release of lactate dehydrogenase (LDH) to the cell culture supernatant, and the expression of a variety of genes related to the immune system was analyzed by RT-qPCR. [3] Based on the MLPA and the genome indexes, the nearest species to the clinical strain 1178C^T were *A. aquatica* and *A. encheleia*. In the case of the other clinical strain 113634^T, the species most closely related corresponded to the species *A. bestiarum*. The inability of strains 1178C^T and 113634^T to produce acid from D-sucrose is a useful test for their recognition. The size of *A. intestinalis* was 1.3–2.3 µm long and 0.5–1.0 µm wide (average: 1.5 µm long and 0.7 µm wide), while the size of *A. enterica* was 1.0–2.5 µm long and 0.5–0.9 µm wide (average: 1.7 µm long and 0.7 µm wide). Both species induced greater phagocytosis by murine macrophages, tripling the result of the more prevalent species *A. caviae*. However, both cell damage and intracellular survival were lower compared to the most prevalent species in the clinic. The expression results were similar to those obtained with A*. salmonicida*, a species that is not very prevalent in the clinical sample. This study provides the description of two new species for science, *A. enterica* and *A. intestinalis*. Both show a level of pathogenicity similar to less prevalent species in the clinic, so this could be an explanation for their low presence.

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O2

EFFECT OF SUB-INHIBITORY WASTEWATER STRESSORS ON MUTATION FREQUENCIES BETWEEN CLINICALLY RELEVANT AEROMONAS SPECIES

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Antimicrobial resistance among pathogens are on a continual rise resulting in untreatable infections and increased mortality rates, with an estimation of 10 million deaths worldwide by 2050 [1]. A common factor accelerating resistance is the presence of sub-inhibitory microbial stressors (e.g. antimicrobials, heavy metals, and disinfectants), which can drive horizontal gene transfer and mutagenesis in various environments [2]. Improper disposal of pharmaceuticals, excretion of antimicrobial byproducts from humans and livestock, as well as excess storm runoff are common sources of these pollutants [3]. An environmental reservoir rich in stressors and bacterial populations, including the emerging pathogen Aeromonas, is wastewater [2]. Our objective in this study was to determine the impact of sub-lethal concentrations of wastewater, antimicrobial contaminants and wastewater disinfectants on their mutagenic effect amongst residential wastewater bacterial populations. Fluctuation assays were performed to quantify mutation frequencies in environments with these pollutants. Briefly, clinically-relevant bacterial cultures of A. hydrophila and A. caviae were incubated with various stressors for 24-hours with subsequent plating on tryptic soy agar containing eight times the MIC value of rifampin. For each treatment group evaluated, sixteen or more independent experiments were included. Mutation frequencies were determined by dividing resistant colonies by total colonies on tryptic soy agar without antibiotic. In all, sub-lethal concentrations of wastewater influent, four antibiotics (i.e. ciprofloxacin, tetracycline, trimethoprim, and ceftazidime) each with different molecular targets, and common wastewater disinfectants (i.e. ultraviolet light and calcium hypochlorite) were assessed for their role on accelerating mutagenesis. Our findings identified that filtered influent wastewater increased mutagenic evolution by 3-fold within A. caviae. When looking at the impact of sub-inihibitory concentrations of antibiotics, the strongest impact on mutagenesis was tetracycline, trimethoprim, and ceftazidime among A. hydrophila. Whereas, ciprofloxacin and trimethoprim were most influential to mutation frequencies of A. caviae populations. Although, most antimicrobials appear to lose their effect at 0.25X of the MIC value, trimethoprim impacted mutation frequencies as low as 0.156 µg/mL (0.0078X MIC). Common wastewater disinfectants also accelerated the presence of specific mutations in A. caviae. Together, although wastewater treatment is instrumental in reducing microbial populations, as well as resistant populations [4], our findings stress the potential impact wastewater and its disinfectants have on the evolution of antimicrobial resistance and virulence.

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O3

A GLOBAL SURVEY AND DISEASE CONTROL OF HYPERVIRULENT *AEROMONAS HYDROPHILA* IN FISH

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Aeromonas hydrophila is an important opportunistic pathogen of fish and other aquatic species. Over the past decade vast mortalities of farmed fish due to Motile *Aeromonas* septicemia (MAS) have occurred in channel catfish (*Ictalurus punctatus*) farmed in the United States and grass carp (*Ctenopharyngodon idella*) and other carp species farmed in China. While typically regarded as a secondary pathogen, the emergence of *A. hydrophila* J-1, isolated from farmed carp with MAS disease in China indicated that this hypervirulent *A. hydrophila* (vAh) corresponds to a unique sequence type (ST251) that acts as a primary pathogen causing high fish mortality. In the US, vAh has been isolated from farmed catfish in Alabama, Mississippi, and Arkansas, resulting in the cumulative loss of over 35 million pounds of channel catfish in AL alone. Due to the significant threat of vAh to aquaculture-farmed fish, a global survey of vAh was conducted [1] to investigate its geographical distribution, phylogeny, and potential transmission. Isolates from the fish with MAS symptoms from Southeast Asia, South Asia, Europe, Northern America, and Africa were tested for *myo*-inositol utilization which is unique to vAh strains among known *A. hydrophila* strains. The phylogenetic affiliation of *myo*-inositol catabolizing strains was determined based on *gyrB* sequences. Five strains isolated from Cambodian pangas catfish (*Pangasius pangasius*) and Vietnamese striped catfish (*Pangasianodon hypophthalmus*) were identified as vAh, based on the results of phylogenetic analysis, pairwise comparison of average nucleotide identity and predicted virulence factors. To control vAh and other fish pathogens, *Bacillus velezensis* AP193 was identified as a potential biocontrol agent due to its ability to inhibit the growth of multiple aquatic pathogens including vAh. The bioactive metabolite responsible for vAh inhibition was found to be the translation inhibiting compound difficidin. *B. velezensis* AP193 was applied onto feed at 10^7 CFU/g and fed to tilapia (*Oreochromis niloticus*) for 8 weeks, which significantly reduced mortality caused by vAh ML09-119 from 71% to 27% ($P < 0.05$). To overexpress difficidin in *B. velezensis* AP193, a streptomycin-resistant mutant was and found to have 394% difficidin production compared to the wild type strain. Future studies will evaluate the role of enhanced production of bioactive metabolites like difficidin in preventing disease due to vAh and other fish pathogens.

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O4

ANTIMICROBIAL SUSCEPTIBILITY OF ISOLATES OF AEROMONAS SPP COLLECTED IN A FRENCH RIVER IMPACTED BY TERRESTRIAL AND AQUATIC FARMING ACTIVITIES: A 17 MONTHS FOLLOWING UP

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The “Resist3A” project aimed to determine the occurrence of AMR upstream and downstream of two trout fish farms, located respectively at the source (FF1) and at the mouth (FF2) of a river influenced by agriculture, terrestrial farming and a wastewater treatment plant. Every two weeks for 17 months, water samples were collected upstream and downstream of the two fish farms and biofilm samples were collected in the same pond per farm. In total, of 144 water samples and 72 biofilm samples were collected. In addition, raw and treated wastewater samples were collected from three wastewater treatment plants connected to the river (13 raw wastewater and 16 treated effluent samples).

Antimicrobial susceptibility testing of a selection of 355 *Aeromonas* (water samples n=172; biofilm n=183), was performed by agar diffusion according to CLSI. Antimicrobial agents tested included (i) eight antibiotics which are critical for human health (WHO list and those used to treat human infections): piperacillin, amoxicillin-clavulanic acid, ceftazidime, cefepime, ciprofloxacin, gentamicin, amikacin and tobramycin and (ii) six agents of interest in aquaculture according to CLSI: chloramphenicol, florfenicol, enrofloxacin, oxolinic acid, oxytetracycline and trimethoprim-sulfamethoxazole. In addition, integrons known to be associated with anthropic pressure were detected by qPCR in the 355 isolates from the river and from the 252 isolates collected from raw wastewater (n=127) and treated wastewater (n=125).

From source to the mouth in water samples, no decrease in susceptibility was observed for oxolinic acid, florfenicol, oxytetracycline, ceftazidime. On the contrary, a slight decrease in susceptibility was observed for enrofloxacin. Isolates collected in the biofilm of the FF1, showed a reduced susceptibility to oxytetracycline and oxolinic acid. No reduction in susceptibility was observed for isolates collected in the FF2 biofilm.

Integrons (esp. class 1 integron) were detected in 18.5% (n=693) of the tested isolates. Three isolates recovered from raw wastewater harbored both class1 and 2 integrons. In raw wastewater the frequency of integron detection was almost three-times higher than in treated effluents (14.5% vs 6.4%), representing the acquisition of genetic material from the environment or other bacteria (horizontal gene transfer). Integron detection was significantly higher in isolates of waters collected at the mouth of the river than those collected at its source, 27.9% (n=27/86) and 1.2% (n=1/82), respectively, leading to the assumption that mobile genetic elements were steadily incorporated into isolate genomes as a consequence of natural or artificially associated evolution processes. In biofilms FF1 (24.8%, n=30/121) and FF2 (30.3%, n=46/152), no significant differences were observed ($p< 0.31$). The notified results are in agreement with the increasing anthropic pressure from the source to the month of the river.

While the AMR situation in the investigated ecosystem currently seemed to be only slightly influences by the used antimicrobials, the tolerance of bacteria against them may increase steadily over time of exposure. Furthermore, artificial pollutants may also influence the natural competence and the rate of the evolution of the bacteria potentially leading to a development of emerging pathotypes.

O5

STRUCTURAL DIVERSITY AMONG *AEROMONAS SALMONICIDA* O-POLYSACCHARIDES ISOLATED FROM ILL AND HEALTHY FISH AS AN EPIDEMIOLOGICAL TOOL

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Aeromonas salmonicida is an important pathogen of fish, producing the systemic disease furunculosis. Since the annual worldwide losses of farmed fish due to diseases involve millions of dollars, this pathogen has been subjected to considerable investigation. One of the principal virulence factors of this pathogen is an S-layer (named the A-layer) that consists principally of a 2-dimensional crystalline tetragonal protein (A-protein) array, which is tethered to the cell by lipopolysaccharide (LPS). The A-layer appears to cover most of the surface of virulent *A. salmonicida*, although some LPS may also be exposed. This structure has been shown to protect this bacterium from killing by serum in a manner that somehow requires both LPS and the A-layer. The LPS is one of the major structural and immunodominant molecules of the outer membrane. It consists of three domains: lipid A, core oligosaccharide, and O-specific polysaccharide (O-antigen).

During studies the answers for two important questions were expected: 1. Is there a correlation between *A. salmonicida* O-polysaccharide structures and antimicrobial resistance of the strains? and 2. Which *A. salmonicida* O-polysaccharides are isolated from ill and which from healthy fish (structural differences)?

The 41 of *A. salmonicida* strains from ill and healthy has been isolated. The data have shown the differences between *A. salmonicida* strains in term of antimicrobial susceptibily and presence of mobile genetic element like for example integron. For each isolate the susceptibility to eight antibiotic agents (the one used in aquaculture and some of critical importance for human health) were determined by the microbroth dilution method. In a recent study, it was observed a higher frequency of resistant bacteria isolated from ill fish. The O-polysaccharides from *A. salmonicida* strains (pathogenic and not pathogenic) were compared by using ^{1}H,^{13}C high-resolution magic angle spinning (HR-MAS) NMR spectroscopy. The structural differences between *A. salmonicida* O-polysacchaides have been identified. Additionally, the first database of *A. salmonicida* O-polysaccharides will be created.

Identification of *A. salmonicida* O-polysaccharides (different LPS chemotypes) presented in ill fish could be very useful for veterinarian either to confirm the etiologic agent of disease and either in improving biosecurity of fish farms: by having a quick tool to detect the presence of pathogenic *A. salmonicida* O-polysaccharides before the level of the pathogen reach density and causes disease.

O6

DETECTION AND ANTIMICROBIAL SUSPECTIBILITY OF *PLESIOMONAS SHIGELLOIDES* IN FRENCH RIVER

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As part of a research project to investigate the spread of antimicrobial resistance (AMR), bacteriological monitoring survey was carried out along a river, where two fish farms are located one near the source the other at the mouth. For 17 months, every two weeks, water samples were collected upstream and downstream the two fish farms and biofilm samples were collected in a same fishpond per farm. 144 water and the 72 biofilm samples were cultured on TBX and TSA at 37°C for 48°h. The collection is composed of 3,088 isolates, 957 coming from biofilm (248 from TBX, 709 from TSA) and 2,131 from water samples (691 from TBX, 1,440 from TSA).
A total of 71 isolates of *Plesiomonas shighelloides* were identified by Maldi-Tof and confirmed by PCR. *Plesiomonas* was detected in 41.6% (15/36) of the sampling campaigns in the water samples and/or in the biofilm samples. Detection of *Plesiomonas* was unsuccessful in samples collected between October and May. Most of the isolates were collected from the source of the river (n=50) and from TBX (86%, n=61). Among the isolates collected on TBX, the prevalence of *Plesiomonas* is higher in biofilm than in water, 21.4% and 1.15% respectively.
On a selection of 36 isolates (one per sample, per date and per culture media) genetic diversity by ERIC-PCR, and antimicrobial susceptibility by agar diffusion were performed. A panel of 31 agents including nine classes was tested. Clinical breakpoint defined by CLSI for Enterobacterial (M100 ed 31) were applied.
Based on the ERIC-PCR, the isolates are divided into four groups with one profile (profil A) representing 75% of isolates. 25 out of 26 isolates of this profile were isolated from TBX. Thus, we can suppose that the media of culture affected the diversity of the isolates. Among the ten other isolates, all collected from TSA, the profile B is dominant with seven isolates. No link between geographical or origin of the sample and profile was observed. Nevertheless, ERIC-PCR is a methodology for a first screening; PGFE will allowed a better discrimination.
Using available clinical breakpoint, the 36 isolates tested were all susceptible to quinolone. Among beta-lactam class, all isolates were susceptible to third generation cephalosporins and carbapenems. At the opposite all isolates were categorized as resistant (n=35) or intermediate (n=1) to ampicillin. We could suspected an initiate resistance to this molecule but not to the penicillin class. Indeed, only one isolate was categorized as intermediate to piperacillin. 88.9% of the isolates were intermediate (n=31) or resistant (n=1) to kanamycin, three of them were intermediate to amikacin too; and one isolate is also intermediate to gentamycin and resistant to amikacin. But all isolates are susceptible to netilmicin. Twenty-one isolates presented the same multidrug resistance profile: tetracycline – SXT – Trimethoprim – ampicillin and intermediate to kanamycin. These isolates excepted one belong to ERIC-PCR profile A and were collected from TBX.
Detection of resistance gene by qPCR system (i) was performed on ten of them. They all harbored resistance gene associated with resistance to sulfonamide (*sul1*, *sul2*), to trimethoprim (*dfrA*12). This in agreement with phenotypic results and could be linked to the presence of integron class 1.
These first results raise the question of the possible role of *Plesiomonas* in the dissemination of antibiotic resistance in water and therefore in fish. Thanks to the complementarity of the research teams, an in-depth study of this collection (serotyping, genetic diversity, etc.) will be able to continue.

O7

STRUCTURAL STUDIES OF *PLESIOMONAS SHIGELLOIDES* CNCTC 70/89 LIPOPOLYSACCHARIDE

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Plesiomonas shigelloides is a Gram-negative rod belonging to the Enterobacteriaceae family. P. shigelloides is associated with episodes of intestinal infections and outbreaks of diarrhoea in humans. Foreign travel, particularly to Latin America, the Caribbean, and South and Southeast Asia, is a second major risk factor associated with *Plesiomonas* infections in humans [1]. The extra-intestinal infections caused by this bacterium, e.g. meningitidis, bacteraemia and septicaemia, usually have gastrointestinal origin and serious course. Lipopolysaccharide (LPS, endotoxin), the main component of the outer membrane of the cell envelope of Gram-negative bacteria, is built of an O-specific polysaccharide and core oligosaccharide covalently linked to lipid A. Despite the rising knowledge of *P. shigelloides* LPS structures over the past two decades, complete or partial LPS structures have been elucidated only for 15 strains out of 102 identified O-serotypes [2].

The structure of P. shigelloides O5 LPS was determined by chemical analysis, mass spectrometry and NMR spectroscopy. The O-specific polysaccharide of *P. shigelloides* O5 has the following structure: →4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*NAc-(1→. The same structure was identified before in the O-specific polysaccharide of Hafnia alvei strain 38 [3]. Furthermore, a new core oligosaccharide was described, which shares P. shigelloides common feature, that is the presence of uronic acids. The lipid A of *P. shigelloides* O5 LPS is identical with lipid A of the *P. shigelloides* serotype O74 [4].

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O8

IMMUNOCHEMICAL STUDIES OF *PLESIOMONAS SHIGELLOIDES* CNCTC 5112 LIPOPOLYSACCHARIDE

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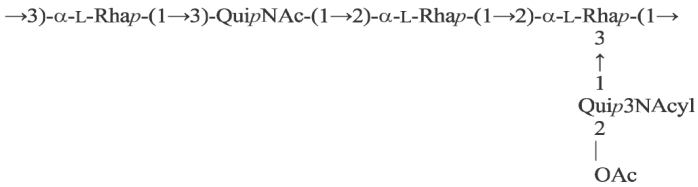
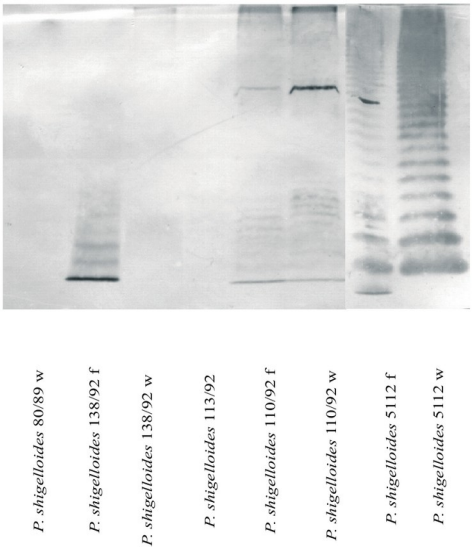
Plesiomonas shigelloides is a species of gram-negative, rod-shaped bacteria. *P. shigelloides* was formerly classified in the *Vibrionaceae* family, but is now a part of the *Enterobacteriaceae* family. They are able to grow at salt concentrations of 0-5%, at a pH of 4.0-8.0, and at temperatures of 8-44^{o}C. The primary reservoirs of *P. shigelloides* are aquatic environments, including freshwater, estuarine water, and seawater. *P. shigelloides m*ay result in gastroenteritis or extraintestinal infections. Clinical symptoms of gastroenteritis include fever, secretory or dysenteric diarrhea, abdominal pain, vomiting, nausea, chills, arthralgia, and headache[1-3]. As the lipopolysaccharide is the major unique component of the bacterial membrane and the structure of this molecule was not known for the *CNCTC* 5112 strain we decided to establish the build of this molecule as well as undertake serological similarities analysis. The structure of the *P. shigelloides CNCTC 5112 O antigen repeating unit was established:*





Cross reactivity of the antiserum against *P. shigelloides CNCTC 5112* bacterial cells suggests structural similarities of the 5112 strain to the structure of the LPS 110/92 and 138/92 strains, this being most likely due to the -3)-βQuipN and d-hydroxybutyric acid structural motifs present. Lack of crossreactivity of the monospecific serum recognizing the core oligosaccaride structure of the 113/92 (anti-OS-BSA conjugate serum) strongly suggests of differences in core structure of the *CNCTC 5112 strain.*

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O9

BEAGLE IN A FISH POND: INSIGHTS INTO THE EVOLUTION OF THE PSYCHROPHILIC FISH PATHOGEN *AEROMONAS SALMONICIDA* FOLLOWING INTRODUCTION TO A MEDITERRANEAN CLIMATE

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The fish pathogen *Aeromonas salmonicida,* infecting multiple aquacultured fish species worldwide, is considered to be mostly psychrophilic, with optimal growth around 20^{o}C. In Israeli aquaculture, *A. salmonicida* first emerged in the 1980's as a winter pathogen infecting Goldfish, and has since persisted as a stable pathogen of cyprinid species. A collection of *A. salmonicida* strains, isolated from cyprinids during furunculusis outbreaks, has been assembled over the past decade. Significantly, close to 20% of outbreaks were reported during spring and summer months, with typical pond temperatures of 25-30^{o}C, suggesting adaptation of *A. salmonicida* to local climate conditions. Phylogenetic analysis of this collection revealed the presence of two clades within the collection, one infecting koi and common carp and the other preferentially infecting goldfish. Here I will describe results from genomic analysis of 10 *A. salmonicida* strains, representing the two clades, isolated between 2013 and 2018. Genomes within each clade were found to be highly conserved, suggesting a clonal origin, possibly following a single introduction event. Detailed bioinformatic analysis provided several insights into genome evolution of each clade, and possible differences in evolutionary trajectories between the two clades. The system described here provides a unique opportunity to study the evolution of *A. salmonicida* as it adapts to a Mediterranean climate, highlighting the potential of these important fish pathogens to adapt to future climate conditions in aquaculture systems worlwide.

O10

AEROMONAS SP. *GENOMOSPECIE PARAMEDIA* BEARING A CLASS 4 CHROMOSOMAL INTEGRON ISOLATED FROM HUMAN FEACES

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Integrons are genetic elements that exchange and express gene cassettes. These elements are characterized by containing an *int*I gene coding for an integrase, a cassette integration site (*att*I) and a Pc promoter followed by a variable region constituted by cassettes. In contrast to class 1 and 2 integrons, class 4 show a rather limited distribution among bacteria. In this work, a functional class 4 integron found in an *Aeromonas* strain is described. This element is located on the chromosome and contain ten ORFs with unknown function and only one gene that confers resistance to streptomycin (*aad*A1). *Aeromonas* sp. 3925 was isolated from diarrheal stools of a patient who came to the medical service suffering an episode of self-limited gastrointestinal disease. This strain was subjected to a concatenated multilocus sequence analysis (MLSA) using the *gyr*B, *gyr*A, *rpo*D, *rec*A, *dna*J and *dna*X genes. The phylogenetic analysis grouped it into a different clade from the species already reported, and was impossible to assign it to a given species. Therefore, the complete genome was sequenced and a phylogenomic analysis was carried out. The strain was related to *A. media* and *A. rivipollensis* clusters, but clearly different from these species. Based on in silico DNA-DNA hybridization (isDDH) and average nucleotide hybridization (ANI) analyzes we conclude that this strain corresponds with high probability to the genomospecies *Aeromonas paramedia.*

O11

HORIZONTAL GENE TRANSFER IN *AEROMONAS* SPECIES

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Given the low capability of the genus *Aeromonas* to incorporate exogenous DNA, this work aimed to determine the transformability in *Aeromonas* strains isolated from environmental and clinical sources. We selected a group of *Aeromonas* strains (N=30) (*A. trota*, *A. hydrophila*, *A. caviae. A. dakhensis, A. taiwanwensis* and *A. rivipollensis*) susceptible to most antimicrobials, which might result from the inability of this species to acquire DNA. These strains were analyzed to determine their ability to incorporate DNA through conjugation, electroporation, natural transformation, and vesiduction. In conjugation, two mobilizable plasmids were tested pBAMD1-2 and pBBR1MCS-3, a suicide and an autonomously replicating plasmid respectively. Fifty percent of *Aeromonas* strains were capable of conjugating and receiving both plasmids. Interestingly in some pBBR1MCS-3 transconjugants several plasmid isoforms were observed. In all instances, the conjugation frequency was low. *Aeromonas* strains were also subjected to electroporation with pRANGER-BTB3 plasmid but only two strains of *A. caviae* and *A. hydrophila* were transformed with very low efficiency. As an alternative, transfer of chromosomal resistance markes via natural transformation was attempted, but no antibiotic-resistant transformants were found. Finally, strains were tested for horizontal gene transfer through vesiduction with outer membrane vesicles carrying pRANGER-BTB3. As before, none of the strains could get the plasmid. These results confirm that *Aeromonas* species are extremely refractory to horizontal gene transfer. Further investigations to understand the possible barriers to HGT by *Aeromonas* are needed.

FC1

STUDY OF THE DIVERSITY OF STRAINS OF *AEROMONAS SALMONICIDA* ISOLATED IN THE FISH FARMING CONTEXT IN FRANCE

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The diversity of *Aeromonas salmonicida* strains is increasingly highlighted in the literature. This calls into question the existence of only a few subspecies in this bacterial species. Recent studies suggest the potential existence of, at least, ten additional subspecies; many of which exhibit mesophilic rather than psychrophilic behavior as classically described for this bacterial species. In the present study, thanks to a large collection of more than 150 strains of *A. salmonicida* isolated over a period of more than 15 years in France in various types of fish farms [1], we focused on strains with resistance to antibiotics or a mesophilic lifestyle. Thus, a number of 34 strains were analyzed in more detail at the phenotypic and genetic level with five strains having their whole genome sequenced. Our preliminary results confirm that the diversity of *A. salmonicida* is also present in France with at least two mesophilic strains. In addition, psychrophilic strains with characteristics not previously observed are also part of the 34 analysed strains. Some have a unique small plasmid content, a capacity for growth at intermediate temperatures and an alteration of their pAsa5 plasmid usually necessary for their virulence. The next steps in this project will involve a more detailed analysis of the genome of several of these strains and a robust phylogeny to understand the relationship of these French bacterial strains with the *A. salmonicida* subspecies.

[1] Bull. Acad. Vét. France — 2016 - Tome 169 - N°3 http://www.academie-veterinaire-defrance.org

FC2

EPIDEMIOLOGY AND GENOTYPING OF 438 CLINICAL ISOLATES OF *AEROMONAS* RECOVERED FROM 74 CASES OF GASTROENTERITIS

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Background: *Aeromonas* species are considered emerging pathogens that cause a wide spectrum of diseases, being gastroenteritis affecting young children and elderly people the most frequent presentation [1,2]. Co-infections with other enteropathogens, especially *Campylobacter* spp. and *Salmonella,* and co-isolation of two or more distinct *Aeromonas* strains in the same sample have been reported [1,3-5]. However, studies that investigate the incidence of the latter do not exist. Therefore, the aim of this study was to analyze several isolates recovered in cefsulodin irgasan novobiocin (CIN) agar media from the feces of 74 patients suffering from *Aeromonas* gastroenteritis to determine if they belong or not to the same or distinct strains. This data will enable to establish the incidence of monomicrobial or polymicrobial infections involving different clones and/or species of *Aeromonas* alone or in combination with other enteropathogens.
Methods: Between June 2017 and April 2018, 74 patients were diagnoses with *Aeromonas* gastroenteritis, at the University Hospital Joan XXII from Tarragona (Spain), after screening their stools for the presence of enteropathogens. A representative number of colonies (6 on average) showing typical *Aeromonas* morphology in Yersinia CIN Agar (Biomerieux®, Marc l'Etoile, France) were identified by MALDI-TOF MS (Bruker Daltonics®, Bremen, Germany) and genotyped, after DNA extraction, with the Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR using the primers and conditions described by Versalovic et al. [6]. After analyzing the obtained banding patterns and when different clones were found in the same sample, isolates were identified to the species-level using the sequences of the housekeeping *rpoD* gene using primers and conditions described by Soler et al.[7].
Results: Out of 74 patients, of the 438 genotyped presumptive *Aeromonas* isolates, 111 (23%) represented different clones. In 51 out of 74 patients (68.9%), *Aeromonas* was the only pathogen, and in 41 of these 51 cases (80.4%) only a single clone was found by ERIC PCR. However, two or more distinct *Aeromonas* clones from the same or different species were detected in the remaining 10 patients (19.6%). In 23 of 74 patients (31.1%) *Aeromonas* was isolated alongside other enteropathogens, mainly *Campylobacter jejuni* (43.5%) and *Salmonella enteritica* (34.8%). In 16 of the 23 cases (69.6%) only one clone of *Aeromonas* was detected by ERIC-PCR, while the remaining seven cases (7/23, 30.4%) showed distinct clones of *Aeromonas caviae* as well as co-infection with either *Aeromonas veronii, C. jejuni* or *S. enteritidis*. Of the total of 17 (21.6%) patients with more than one clone of *Aeromonas,* 14 (82.3%) involved two or three distinct clones of *A. caviae* (12 and 2 patients, respectively) and the remaining three showed clones of more than one *Aeromonas* spp.
Conclusions: *Aeromonas* was the only isolated pathogen in most patients with gastroenteritis (68.9%), supporting the importance of this pathogen. Co-infections with other enteropathogens were mostly associated with *C. jejuni* (43.5%) and *S. enterica* (34.8%) as described in previous studies. In 21.6% of the patients with gastroenteritis, more than one clone of *Aeromonas* was identified, being the most frequent co-infection the isolation of two distinct clones of *A.caviae*. This is the first study that establishes the prevalence of different clones of the same or different species of *Aeromonas* involved in gastrointestinal infections.

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FC3

IN SILICO ANALYSIS OF *Aeromonas* MCR PROTEINS

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Antimicrobial resistance is a problem of global importance, due to the complexity of the problem, the dissemination of resistance mechanisms from the environment and the emergence of strains resistant to first-line antimicrobial treatment. The latest treatment alternatives are antimicrobials such as colistin, for which resistance mechanisms have been described in various bacterial genera, including *Aeromonas*, a human pathogen present in the environment. Therefore, in the present work we proposed the *in silico* analysis of the MCR proteins of *Aeromonas*.
For comparative analysis and phylogeny of MCR proteins, MCR protein sequences of *Aeromonas* strains were downloaded from the NCBI Protein database. The MCR protein files in fasta format were analysed by multiple alignment by using ClustalW algorithms. Subsequently, a phylogenetic tree was built, for which the HKY85 model, the MAFFT and PhyML programs were used; the results were visualized in the Jalview program (V2.11.2.2). After the construction and comparison of the phylogenetic trees, the MEME program was used to detect motifs in the MCR protein sequences that were downloaded from the NCBI.
The sequences of *Aeromonas* MCR-1, MCR-3, and MCR-5 proteins downloaded from NCBI were analysed and two phylogenetic trees were calculated, one with PhyML and the other with MAFFT, and the MEME tool was used to detect motifs. The phylogeny, a grouping of the MCR-3 proteins in a clade was demonstrated and of the MCR-1 and MCR-5 proteins that formed a clade together, moreover, it was determined that the presence of motifs could be target sequences to the MCR proteins modification.
In silico analysis with MEME software, several motif sequences were detected in each of the MCR proteins, and consensus sequences were obtained for each of them, which allowed us to visualise possible sites of inhibition of enzyme activity at the cell wall level of colistin-resistant gram-negative bacteria mediated by MCR proteins. Through the consensus sequences of the motifs, the logo was obtained with the WebLogo application, for the graphic display of the active sites in each sequence. Those sites that are suggested as possible conserved regions in the sequence, which could be used as a target of modification to alter the function of the MCR protein, are observed in larger letters. The MCR-1 and MCR-5 proteins are phylogenetically related to each other.

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FC4

INTERACTION OF pAsa5 AND pAsa8 PLASMIDS IN *AEROMONAS SALMONICIDA* SUBSPECIES *SALMONICIDA*

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The bacterium *Aeromonas salmonicida* subsp. *salmonicida* (*A. sal.*) is the etiological agent of furunculosis in salmonids. The virulence of this pathogen is characterized by the presence of a needle-like protein structure named the type three secretion system (TTSS). The TTSS locus which is known to be lost when the bacterium is grown at temperatures of 25 °C and above is located on the large plasmid pAsa5. The loss of the locus is due to the recombination of the insertion sequences flanking the TTSS region. These recombination events seem to be linked to a chromosomic gene cluster still not characterized (Marcoux, 2020). The plasticity of pAsa5 also involves other plasmids found in the bacterium: a fusion between pAsa9 and pAsa5 was observed after heat stress in strain 01-B526 (Tanaka et al., 2017) and the insertion of the pAsa8 transposon in pAsa5 was also described in strain SHY16-3432 (Massicotte et al., 2019). pAsa8 is a large plasmid bearing antibiotic resistant genes. In the recent years, we isolated additional strains bearing pAsa8. Further analyses on these strains revealed that the fusion between pAsa5 and the complete version of pAsa8 occurs frequently. The pAsa8 transposon insertion in pAsa5 seen in SHY16-3432 is likely an aberrant event compared to the fusion of the two full-length plasmids. Many fusion scenarios seem to be possible with at least one of them involving an insertion sequence common to both plasmids. This study demonstrates a new aspect of the impact of insertion sequences on the biology of the pAsa5 plasmid and an additional risk for the propagation of antibiotic resistance genes in *A. sal.*

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FC5

IMPROVEMENT OF MALDI-TOF FOR THE IDENTIFICATION OF AEROMONAS SALMONICIDA AND AEROMONAS BESTIARUM: TWO IMPORTANT FISH PATHOGEN

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The identification of *Aeromonas* isolates to species level remains a challenge. Sequencing of housekeeping genes (gyrB, rpoB...) is considered the gold standard. However, these methods are costly and time-consuming. Pérez-Sancho et al. (2018) demonstrated the utility of MALDI-TOF MS (MT) for the identification of *Aeromonas* at the genus level, but accurate identification of the main species involved in fish diseases is not possible. The quality of the identification performed by MT depends on the associated database. Currently, the MT database proposed by Bruker contains reference spectra for only 22 of the 34 described species. Furthermore, the reference spectra are mainly performed for a single isolate per species, which reduces the consideration of intra-species diversity. Finally, the culture conditions used to create the spectra are not the same for all species and may be different from those used for the isolation of pathogenic species collected from fish for example.
The objective of this study was to generate a new MT database including all 34 Aeromonas species, using culture conditions at 22°C for 48h and to test its accuracy on different species of interest. For each species, three reference spectra were generated to assess repeatability and reproducibility.
A collection of 196 *Aeromonas* isolates (*A. salmonicida* n=78, *A. bestiarum* n=68, *A. sobria* n=27, *A. popoffii* n=20 and *A. encheleia* n=3) collected from fish and water samples and identified by sequencing of *gyrB* and *rpoB* genes were used to test the new database and also Bruker. Two sample preparation methods (direct deposit and after simple acid extraction using formic acid deposit) have been evaluated.
Thanks to the new database, the identification of A. salmonicida and A. bestiarum species is improved in comparison with the Bruker database. These results provided depend on the deposit used. For *A. popoffii*, the result was less effective. For *A. sobria* and *A. encheleia* no mismatch were recorded.
These first results confirm the possibility of using TM for the identification of Aeromonas species, provided that a suitable database is available.

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FC6

STRUCTURE OF THE LIPOPOLYSACCHARIDE O ANTIGEN OF *PLESIOMONAS SHIGELLOIDES* O82 (STRAIN CNCTC 5119)

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Gram-negative bacteria synthesize various surface antigens, including carbohydrate-containing molecules. Among them, lipopolysaccharides (LPS, endotoxin), capsular polysaccharide (CPS, antigen K), enterobacterial common antigen (ECA), and exopolysaccharides (EPS) are important factors influencing virulence or modulating host immune response. *Plesiomonas shigelloides* is a Gram-negative rod belonging to the *Enterobacteriaceae* family. This ubiquitous and facultatively anaerobic organism has been isolated from water and many wild and domestic animals. It is causative agent of travelers' diarrhea. The pathogenicity of *P. shigelloides* is not yet fully understood. Among the cholera-like toxin, 3 thermostable and thermolabile toxins, and β-haemolysin, LPS is important virulence factor of this species. It is built of lipid A, core oligosaccharide and O-specific polysaccharide (O-PS, O-antigen). The latter region determines O-serotype. Since first publications considering *P. shigelloides* O-PS [1,2], number of structural studies of its O antigen have been published, including core OS and lipid A structures. Herein O-PS structure of the *P. shigelloides* O82 was elucidated. The O-PS was isolated from LPS by mild acid hydrolysis followed by purification and analyzed using chemical methods, MALDI-TOF mass spectrometry, and 1D and 2D NMR spectroscopy techniques. The following structure of the pentasaccharide repeating unit of the *P. shigelloides* O82 O-antigen was elucidated:
[→4)-α-L-Gal*p*NAcA-(1→3)-α-D-Qui*p*NAc-(1→3)-β-D-Qui*p*4N**X**-(1→3)-α-D-Gal*p*NAcA-(1→]$_n$, where X stands for rare amino acid L-*allo*-Threonine (L-*allo*-Thr) and n≥1. The O-PS structure is similar to the O-PS identified for the LPS of *Vibrio cholerae* O43:[→4)-α-D-GalpNAc-(1→3)-α-D-QuipNAc-(1→3)-β-D-Quip4NX-(1→3)-α-D-GalpNAcA-(1→]$_n$ also containing L-*allo*-Thr.

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FC7

UNUSUAL STRUCTURAL ELEMENTS OF O-ANTIGEN FROM *PLESIOMONAS SHIGELLOIDES* O68 (STRAIN CNCTC 138/92) INVESTIGATED BY HR-MAS NMR SPECTROSCOPY

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Plesiomonas shigelloides is a potential human and animal pathogen that has been implicated in outbreaks of food poisoning with acute gastroenteritis. With the gradual increase in antibiotic-resistant strains of *P. shigelloides* development of vaccine therapy is of urgent interest.
The carbohydrate antigens expressed on the surfaces of *P. shigelloides* bacterial cells have been recognized and classified to 102 O-antigens and 51 H-antigens. To date, structures of the O-specific polysaccharide (O-PS) component of the lipopolysaccharide (LPS) have been determined only for the serotypes O1, O12, O17, O22, O24, O33, O36, O37, O51, O54, O74 [1]. However, the number of the known *P. shigelloides* O-PS structures is growing since the presence of unique sugar residues and rare substituents such as D-bacillosamine unit, L-pneumosamin, pseudaminic acid and 3-hydroxy-2,3-dimethyl-5-oxoproline were reported.
We have investigated the structures of O-antigens from a set of *P. shigelloides* strains. The high-resolution magic angle spinning (HR-MAS) NMR spectroscopy was applied for the studies of the O-antigens directly on bacteria to ensure that the native unmodified structures are identified. The screening of the *P. shigelloides* surface components indicates chemical differences in the O-specific side chains as well as shared elements among studied *P. shigelloides* strains. The comparative studies have revealed similarities between the ^{1}H HR-MAS NMR profile of *P. shigelloides* O74 (strain CNCTC 144/92)[2] and that of *P. shigelloides* O68 (strain CNCTC 138/92). The ^{1}H HR-MAS NMR spectra of the *P. shigelloides* O68 and O74 show similarities in the regions of the acetyls, methylene and methyl groups suggesting the common structural elements in their O-PS chains.
In-depth studies by ^{1}H and ^{13}C NMR spectroscopy, complementary mass spectrometry and chemical methods confirmed that the *P. shigelloides* O68 O-PS is composed of a trisaccharide repeating unit with the →4)-α-D-Glc*p*6OAc-(1→4)-β-D-Glc*p*NAcyl3NAc-(1→3)-β-Fuc*p*NAc4N-(1→ structure, in which β-D-Glc*p*NAcyl3NAc is a 3-acetamido-2,3-dideoxy-glucosamine acylated with D-3-hydroxybutyric acid. The presence of the substituent, O- and N-acetyls make the *P. shigelloides* O68 O-PS more hydrophobic than typical O-polysaccharide. The structural elements of similar nature to these present in *P. shigelloides* O74 may influence the biological and physicochemical properties of their LPS. The unique structure of *P. shigelloides* O68 O-PS indicate a need for further studies of biological activities that would be essential for development a potential antigen of multicomponent vaccine against the *P. shigelloides*.

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FC8

STRUCTURE OF THE CORE OLIGOSACCHARIDE OF *PLESIOMONAS SHIGELLOIDES* SEROTYPE O68. CAN WE IDENTIFY THE "MINIMAL COMMON STRUCTURE" AMONG *PLESIOMONAS* CORES?

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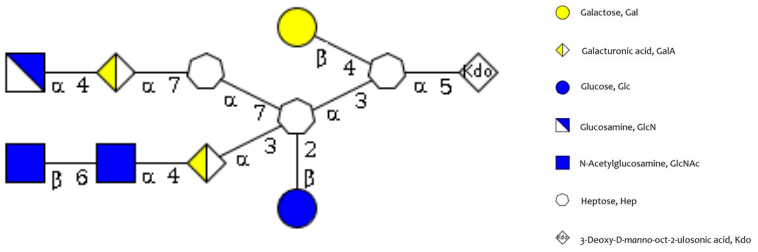
Bacteria expose on the cell surface a variety of complex carbohydrate molecules that are essential for the structural integrity and interactions with hosts. Gram-negative bacteria produce lipopolysaccharides (LPS), which are the main components of the outer membrane of bacterial envelopes and play a major role in the pathogen interactions with the immune system of the host and manifest endotoxic activities similar to those of enteric bacteria. *Plesiomonas shigelloides* has been reported as the most common etiological agent in outbreaks of travellers' diarrhea. 102 serotypes have been identified for *P. shigelloides,* but only for 15 strains complete or partial LPS structures have been elucidated. Despite the rising knowledge of *P. shigelloides* LPS structures over the past three decades, this virulence factor is still poorly characterized. The core oligosaccharides were described for strains assigned to serotypes O1,[1,2] O12,[3] O13,[4] O17[5,6] and O36,[7] O22,[8] O24,[9] O33,[10] O37,[11] O54,[12] O74.[13] We now report on structural studies of the core oligosaccharides isolated from *P. shigelloides* strain CNCTC 138/92 lipopolysaccharide [serotype O68]. NMR spectroscopy and mass spectrometry were the principal methods used. It was concluded that the main core oligosaccharide of the strain is composed of eleven monosaccharide residues having the following structure:



The core is not substituted by phosphate, but instead by glycine, both being rather unusual features. This structure represents a novel core oligosaccharide among *P. shigelloides* O-antigens. Additionally, we have now also compared the structures of all *P. shigelloides* described to date in an attempt to identify the common conserved structural elements of the core OS and indicate the variability of this segment of *P. shigelloides* LPS.

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FC9

ANTIBIOTIC RESISTANCE AND VIRULENCE PROFILES OF *AEROMONAS* IN CLINICAL, BEACH, AND POST-CHLORINATED WASTEWATER ISOLATES IN MILWAUKEE, WISCONSIN, USA

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Traditionally an aquatic bacterium, Aeromonas is now recognized as an emerging human pathogen with a growing arsenal of antibiotic resistance genes and virulence factors. Considering its ubiquitous presence amongst aquatic environments, characterizing their populations amongst recreational beaches is needed to better understand its potential health risk predisposing humans to possible infection and disease. The purpose of our study was to identify Aeromonas species along with their antibiotic resistance and virulence factor profiles among recreational beaches and post-chlorinated wastewater (POC) compared to clinical samples acquired from the same city - Milwaukee, Wisconsin, USA. Beach and POC Aeromonas isolates were acquired from plating water samples on ampicillin dextrin agar with vancomycin and irgasan (ADA-VI). Antibiotic resistance was determined utilizing Kirby-Bauer disk diffusion against eleven different antibiotics. Overall, similar resistance profiles were evident between POC and clinical isolates; however, nalidixic acid resistance was significantly more prevalent amongst POC (10.5% to 42.9% respectively). Sulfamethoxazole-trimethoprim (SXT) was significantly different among the sites with clinical isolates (26%) > POC (18%) > beach (4%). Additionally, tetracycline was significantly different among the sites with POC having the most resistance (9%), then clinic (5%), followed by beach (1%). Multi-drug resistance (3 or more classes) was most prevalent in POC (38%) followed by clinical isolates (16%) and beach (0.01%) isolates. Considering similar resistance profiles existed between clinical and POC, we wanted to determine if the mechanism of resistance was shared. Both POC and clinical resistant isolates encoded sul1 and tetE, while sul2 presented in the clinic, beach, and POC isolates. To assess the clinical relevance of environmental isolates, we determined the species and virulence potential. Subsequent species identification was done using gyrB PCR amplification and sequencing to identify all clinical isolates, antibiotic resistant isolates, and random subpopulations of beach and POC isolates. Thirteen species were identified among all sources, with the most prominent clinical isolates being A. hydrophila, A.caviae, and A. veronii. Among beach samples, A. veronii was most common, whereby A. hydrophila and A. caviae predominated among POC. After analyzing for the prevalence of ten virulence genes, we identified minimal difference between the total number of virulence genes in clinical (6.7), POC (5.8) and beach (6.2) populations. The most prominent virulence genes amongst all three sectors detected were serine protease (ser), aerolysin (aero), lipase (lip), and nuclease (nuc) with almost all clinical strains (95%) encoding them. This virulence profile was also seen in most environmental isolates (beach=83%, POC=82%). In conclusion, strong similarities in ARGs and virulence factors, as well as being similar species, between environmental and clinical isolates, suggest beaches are a potential source of infection.

FC10

COMPARATIVE GENOMICS IN SHIGA-TYPE TOXIN PRODUCING STRAINS OF *Aeromonas* spp.

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The genus *Aeromonas* has a mechanism of multifactorial pathogenicity. Strains of *Aeromonas* have been isolated from patients treated at the National Institute of Pediatrics (*Instituto Nacional de Pediatría*, INP) in Mexico City, involved as the only causal agent of intestinal and extraintestinal infections. In previous studies, it has been shown that these strains have the genes encoding the Shiga-like toxin (Stx), which causes a cytotoxic damage, similar to the damage caused by *Escherichia coli* O157:H7. [1]

Nowadays, the Next Generation Sequencing (NGS) is a tool that allows to detect various biological data. Therefore, in this work, NGS was used in order to carry out the comparative genomics and proteomics of strains of *Aeromonas* spp., to detect the differences and/or similarities between the strains, as well as the presence of prophages in these strains.

13 *Aeromonas* spp. strains were obtained from clinical samples of patients hospitalised at the INP. Sequencing was performed on the *Illumina Next Seq 500* platform, in paired-end type. The obtained readings were filtered by quality with *Trimmomatic* 0.36; *de novo* assembly and annotation of the genomes was carried out with the *SPAdes, BASyS* and *RAST* servers, respectively. Comparative genomics and proteomics were performed with the *Gview* and *SEED RAST* servers, respectively. The search for families in the *PATRIC* server was carried out, then a *BLAST* and comparative proteomics were carried out to search for proteins suggestive of prophages.

The assemblies with the best quality were obtained with *SPAdes;* these were annotated, and proteins related to prophages were detected, such as endonuclease proteins, replication, packaging machinery, DNA synthesis, tail proteins, capsid proteins, and tail fibre proteins. Additionally, proteins related to multi-resistance to biciclomycin, bleomycin, acriflavine, fosmidomycin, tetracycline, and quaternary ammonium salts were detected. Furthermore, the AexT protein was identified in strains of *A. hydrophila;* this toxin has only been reported in strains of *A. salmonicida,* which causes furunculosis in fish.

Both genomics and proteomics indicate four sites interrupted in the sequence, suggestive of areas of incorporation of mobile genetic elements, such as prophages; these areas are characteristic of containing high GC content, and they are shared by the 13 strains. Additionally, the identification of families of proteins reveals that the accessory genome of the strains contains families of proteins belonging to prophages. Moreover, the alignment in BLAST reveals that they are proteins belonging to prophages encoding for Stx. Therefore, a comparative genomics analysis was carried out between the genomes and the prophages database, revealing the presence of phage proteins in the previously identified areas with high GC content, although no complete prophage was detected.

Consequently, the strains of *Aeromonas* contain a large number of putative virulence factors, resistance, and secretion systems, which gives them the ability to be virulent. Likewise, due to these strains producing Stx, it is proposed that the *stx* gene is in the prophages as it happens in *Escherichia coli* O157: H7, detecting four probable sites to insert them.

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FC11

REPURPOSING OF FENOFIBRATE AS THERAPEUTIC ALTERNATIVE FOR *AEROMONAS* INFECTION

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Fenofibrate is a fibric acid derivative used as antihyperlipidemic drug in humans. Its active metabolite, fenofibric acid, acts as an agonist to the peroxisome proliferator activated receptor alpha (PPARα), a transcription factor involved in different metabolic pathways. Some studies have reported the potential protective role of this drug mainly in *Yersinia* infections. [1] The aim of this work was to study the *in vitro* effect of fenofibrate in the macrophage cell line J744A.1 against infections produced by *Aeromonas*, a pathogen for humans whose resistance to antibiotics has increased in recent decades. [2] The macrophages were infected at MOI 10 with 4 strains of *A.caviae* and *A. hydrophila*, isolated from human clinical samples and subsequently treated with fenofibrate for 16 hours as a treatment. Subsequently, the cell damage levels measured by the release of lactate dehydrogenase (LDH) to the cell culture supernatant, as well as the percentages of intracellular survival were analyzed. The gene expression of PPARα and 4 immune-related genes (TNF-α, CCL3, CCL20, and IL-1β) was assessed by RT-qPCR, using GAPDH as a housekeeping gene. [3] The results showed a decrease both in cell damage in macrophages and in the intracellular survival of the bacteria after 16 h treatment with fenofibrate. Transcriptional analysis by the RT-qPCR revealed significant differences in the expression of PPARα and the immune-related genes in fenofibrate-treated macrophages in relation to the cells without treatment and in comparison with the non-infected cells, being the TNF-α the most expressed gene. In this work, we provide evidence that fenofibrate offered some protection *in vitro* in macrophages against *Aeromonas* infection. However, further studies are needed with other bacteria to determine their potential antibacterial effect and the route by which this protection is achieved.

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FC12

THE CRISPR-CAS SYSTEM IN STRAINS OF *AEROMONAS*: AN IN SILICO ANALYSIS

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The identification and characterisation of new CRISPR-Cas systems in bacteria has made it possible to expand their usefulness for genetic editing in various models, which is prior to the specificity of the new discovered Cas proteins. Members of the genus *Aeromonas* are highly susceptible to infection by ex-trachromosomal DNA, which could suggest that they have CRISPR-Cas systems with greater com-plexity due to the high rates of infection by bacteriophages, plasmids, and transposons. [1]
In the present work, the genomes of 121 *Aeromonas* spp. strains isolated from clinical environments, water and fish samples were analysed. The genome assembly was performed from sequences obtained by using the Illumina NextSeq 500/550 platform. A total of 121 genomes of the genus *Aeromonas* were characterised. A total of 10 species, 2 subspecies and one biovariety were defined. In order to define the presence of CRISPR-Cas systems, as well as their types and subtypes, the assembled genomes at scaffold level were employed for an initial characterisation using the CRISPR Disco v1.3 a CLI (command-line interface) software, by using the nucleotide and aminoacidic FASTA filesas a screening method. Subsequently, the matching results were analysed with the CLI software CRISPR Detect v2.2, which allows the generation of a gene-finding format (.gff) file containing the spacers in the CRISPR array. This format is in turn used by the CRISPR Studio v1.0 program to generate the spacer profiles. Finally, the genomes were analysed by using the CLI software CRISPRCasTyper v1.2.0 to confirm the system locus or loci of each strain and for the construction of the CRISPR-Cas arrays of the detected loci.
The CRISPR system was canonically detected in 35 of the genomes (considering the reference sequences) and non-canonically in 53 (*i.e.*, an “incomplete” system). In the universe of genomes analysed (CRISPR locus present or absent), types IV (181 loci), V-U (24 loci) and I (22 loci) prevailed. While the subtypes mostly detected were VU-4 (24), VU-2 (22), I-F (9), I-E (8), I-C (5), and III-C (1). Out of a total of 727 genomes (65 initial reference + 121 assembled genomes + 541 GenBank genomes) a CRISPR system was detected in 99 genomes (13.62% 99/727). Only 4 genomes had no Cas protein at all (0.55% 4/727) and 110 of all genomes analysed harboured only the DinG helicase. The variety of types and subtypes is particular to the genus *Aeromonas,* which allows to evaluate in depth the biological role that this system plays in this group of bacteria. However, the results obtained from the spacer profile rule out the use of this profiles as typing method in the genus. This feature lies particularly in the type of system that the genus has, because it is necessary to establish that various strains contained more than one CRISPR loci. Some of these loci have the ability to add spacers to their system, while others do not, as they are not dependent on an adaptive immune memory due to the biochemical nature of their effector proteins.

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