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Original Article

# Characterization of genetic determinants involved in antibiotic resistance in *Aeromonas* spp. and fecal coliforms isolated from different aquatic environments

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**Abstract**

*Aeromonas* spp. and fecal coliforms, two abundant and cultivable bacterial populations that can be found in water ecosystems, might substantially contribute to the spread of antibiotic resistance. We investigated the presence and spread of transposons (elements that can move from one location to another in the genome), integrons (structures able to capture and incorporate gene cassettes) and resistance plasmids in strains isolated from polluted and unpolluted water. We recovered 231 *Aeromonas* and 250 fecal coliforms from water samplings with different degrees of pollution (hospital sewage, activated sludge of a wastewater treatment plant, river water before and after treatment and water from an alpine lake). Sixteen *Aeromonas* spp. and 22 fecal coliforms carried *intI*, coding for the site-specific integrase of class 1 integrons, while 22 *Aeromonas* spp. and 14 fecal coliforms carried *tnpA*, the transposase gene of the Tn*3*-family of replicative transposons. The majority of *intI* and *tnpA*-positive strains were phenotypically resistant to at least four antibiotics. Integrons and transposons were mainly located on mobilizable plasmids.

Our results did not detect common mobile structures in the two populations and therefore relativize the role played by *Aeromonas* spp. as vectors of antimicrobial resistance determinants between water and commensal gut bacteria.


*Keywords:* Class 1 integrons; Tn*3* family transposons; Resistance plasmids; Aquatic environment

## 1. Introduction

Antibiotic-resistant bacteria and antibiotic resistance genes are emerging biological contaminants of the environment. They originate mainly from hospital and community wastewater, animal farms, agricultural lands and wastewater treatment plants. In the aquatic environment, antibiotic resistance determinants may become part of the environmental gene pool, spread horizontally, and may be transferred to humans and animals colonizing bacteria through food and drinking water [1].

The genetic elements most frequently involved in horizontal spread of resistance genes are class 1 integrons, transposons (particularly those of the Tn*3* family) and transferable antibiotic resistance plasmids [2–4]. Integrons consist of a gene encoding for a site-specific integrase (*intI*), a recombination site (*attI*, recognized by the integrase) at which gene cassettes may be inserted, and a promoter that directs transcription of cassette-encoded genes. Integrons may be found on mobile elements such as plasmids and transposons, or on chromosomes. Transposons, genetic elements that can move from one location to another in the genome, are basically composed of a transposase (*tnpA* gene product), a resolvase (*tnpR* gene product) and additional, often antibiotic resistance genes. The Tn*3*-family of replicative transposons is one of the main families responsible for dissemination of antibiotic

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resistance genes in Gram-negative bacteria [4,5]. Resistance plasmids carry one or more antibiotic resistance genes, often associated with transposons and integrons, and are involved in the emergence and dissemination of multiresistance in human pathogens [2]. Many resistance plasmids are conjugative and able to transfer from one bacterium to another via sex pili, whereas other are mobilizable, encoding functions needed for transfer of their own DNA, but lacking the genes needed to initiate self transfer [6]. Mobilizable and conjugative plasmids can be classified according to their set of mobility (MOB) genes, each encompassing several incompatibility groups [7].

*Aeromonas* are Gram-negative, oxidase-positive bacilli and are ubiquitous in aquatic environments where they are exposed to the actions of micropollutants such as residual antibiotic compounds that may possibly be present [8]. Aeromonads are commonly associated with fish diseases, but they also play a role as opportunistic pathogens of cold- and warm-blooded animals, including humans, in whom they can cause life-threatening infections [9,10]. Fecal coliforms are Gram-negative, non-sporulating aerobic or facultative anaerobic, oxidase-negative bacteria, and include bacterial genera that originate mainly from human and animal feces [11]. *Escherichia coli*, for instance, represents the most specific indicator of fecal contamination in the aquatic environment [12]. Fecal coliforms are exposed to the selective pressure of antibiotics, particularly as members of human and animal microbiota. In water, *Aeromonas* spp. and fecal coliforms may reach considerable concentrations ($10^9$ and $10^7$ CFU in 100 mL, respectively) [13]. Many authors consider Aeromonads as reservoirs of mobile DNA elements conferring drug resistance in water environments and fecal coliforms as carriers of drug resistance elements selected in clinical settings. They therefore represent two major populations of cultivable bacteria, differently submitted to the selective pressure of antibiotics, that may interact with each other in water environments, interchanging and spreading antibiotic resistance [14,15].

In order to gain insight into the role of these two populations in the spread of antibiotic resistance in natural aquatic environments, we studied the presence and distribution of transposons, integrons and resistance plasmids in *Aeromonas* spp. and fecal coliforms isolated from polluted and unpolluted waters. Our aims were to identify any commonalities of the detected mobile genetic elements and to determine whether they were transferable among strains isolated from the water environments investigated.

## 2. Materials and methods

### 2.1. Samplings and strains

Water samples (1 L) were collected in 2011 from a river before (b-WWTP) and after treatment at a wastewater treatment plant (a-WWTP), from the activated sludge of the wastewater treatment plant (WWTP), from hospital wastewater (HWW) and from an alpine lake (SW) located 2000 m above the sea level in Ticino (southern part of Switzerland). Sampling areas are shown in Fig. 1.

Logarithmic dilutions (from 100 mL to 100 μL) of the water samples were prepared in 0.1% peptone water and filtered through 0.45 μm-pore size, 47 mm sterile filters (Millipore). Filters were placed on two commercial media (Biolife): m-*Aeromonas* selective agar base supplemented with ampicillin, added as a selective agent (since aeromonads are generally resistant to it), for isolation of *Aeromonas* spp., and C-EC agar to isolate fecal coliforms. *Aeromonas* spp. selective plates were incubated at 30 °C and fecal coliforms C-EC agar plates at 44 °C for 24 h. Fifty colonies of *Aeromonas* spp. and fifty colonies of fecal coliforms from each sample were randomly selected, plated onto blood agar (Columbia agar with 5% Sheep Blood, BD BBL) and identified to the species level using MALDI-TOF MS (matrix assisted laser desorption ionization/time of flight, mass spectrometry) [16].

*E. coli* J53, a derivative of *E. coli* K-12, which is resistant to sodium azide, widely used as a general recipient strain, was used in conjugation experiments.

### 2.2. Total DNA and plasmid extraction

Total DNA was extracted from single colonies after 24 h of growth on blood agar using the Instagene Matrix (BioRad), while plasmids were extracted from overnight cultures in LB broth (Luria–Bertani broth: 10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) with the plasmid DNA purification kit NucleoBond® PC100 (Machery-Nagel), according to the manufacturer's instructions.

### 2.3. Dot-blot and southern blot hybridization

Dot-blot experiments and immunological detection were carried out with the DIG High Prime DNA labelling and detection starter kit I (Roche Applied Science). Integrase, transposase and relaxase genes were amplified by PCR, purified and labelled with digoxigenin (Table 1). Samples of total DNA or plasmid extract were spotted onto a nylon membrane, cross-linked at 100 °C for 1 h and hybridized overnight with the specific probes. To determine the putative plasmid location of the genetic determinants investigated, plasmid extracts were separated by electrophoresis and blotted onto a positively charged nylon membrane following standard procedures [19]. Labelling and detection were performed as described above.

### 2.4. Integron gene cassette identification

Gene cassette identification was made by PCR amplification and sequencing of the $P_{ant}$-qacEΔ1 region of class 1 integrons as described by Sandvang [20].

### 2.5. Antibiotic susceptibility test

Strains were tested for antibiotic susceptibility by the disk diffusion method according to the EUCAST [21]. The antibiotics tested were cefazolin (30 μg), cefuroxime (30 μg), ceftriaxone (30 μg), cefoxitin (30 μg), gentamicin (10 μg), bactrim (23.75/1.25 μg), ciprofloxacin (5 μg), meropenem