

From water resistome to human pathobiome: a new look on water-related infectious diseases

Almakki A.¹, Laurens C.^{1,2}, Jean-Pierre H.^{1,2}, Héry M.³, Jumas-Bilak E.^{1,4}, Licznar-Fajardo P.¹

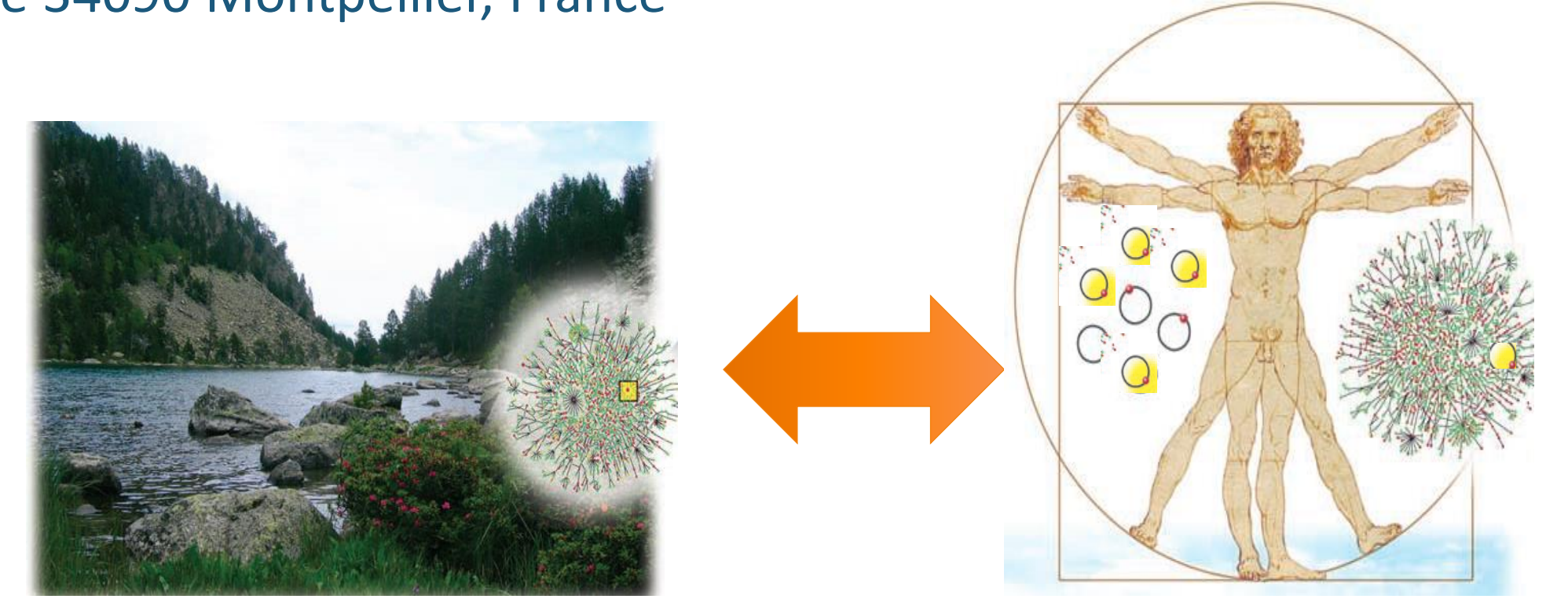
¹ UMR 5569 Laboratoire HSM, Equipe Pathogènes Hydriques Santé Environnements, UFR Sciences Pharmaceutiques et Biologiques, 15 Av Ch. Flahault 34095 Montpellier, France

² Laboratoire de Bactériologie-Virologie, CHRU Montpellier, Hôpital Arnaud de Villeneuve, 371 Av du Doyen Gaston Giraud 34295 Montpellier Cedex 5, France

³ UMR 5569 Laboratoire HSM, Equipe Pollutions minières Environnement et Santé, Place Eugène Bataillon, CCMSE, 34095 Montpellier Cedex 5, France

⁴ Laboratoire d'hygiène hospitalière, CHRU Montpellier, 80 Av Augustin Fliche 34090 Montpellier, France

Water-related infectious diseases remain a leading cause of morbidity and mortality worldwide. Aquatic ecosystems are favourable environments for microbial interactions. Indeed, water is a matrix that ensures a continuum among hydrogeological (underground and superficial systems), technological (domestic, hospital, industrial and waste waters) and living compartments (vital resource for all living beings). Aquatic ecosystems submitted to biotic and abiotic constraints, can be considered as amplifiers/reactors in which microbial communities could evolve towards pathobiome and therefore could participate to human infectious diseases.



Clinical case in a near-drowning patient

- Respiratory tract colonized with *Aeromonas veronii* and *Pseudomonas fluorescens* group
- Bacteremia due to *Enterobacter asburiae*
- The 3 bacteria display resistance to carbapenems including *Enterobacter asburiae* producing the IMI-2 carbapénémase.

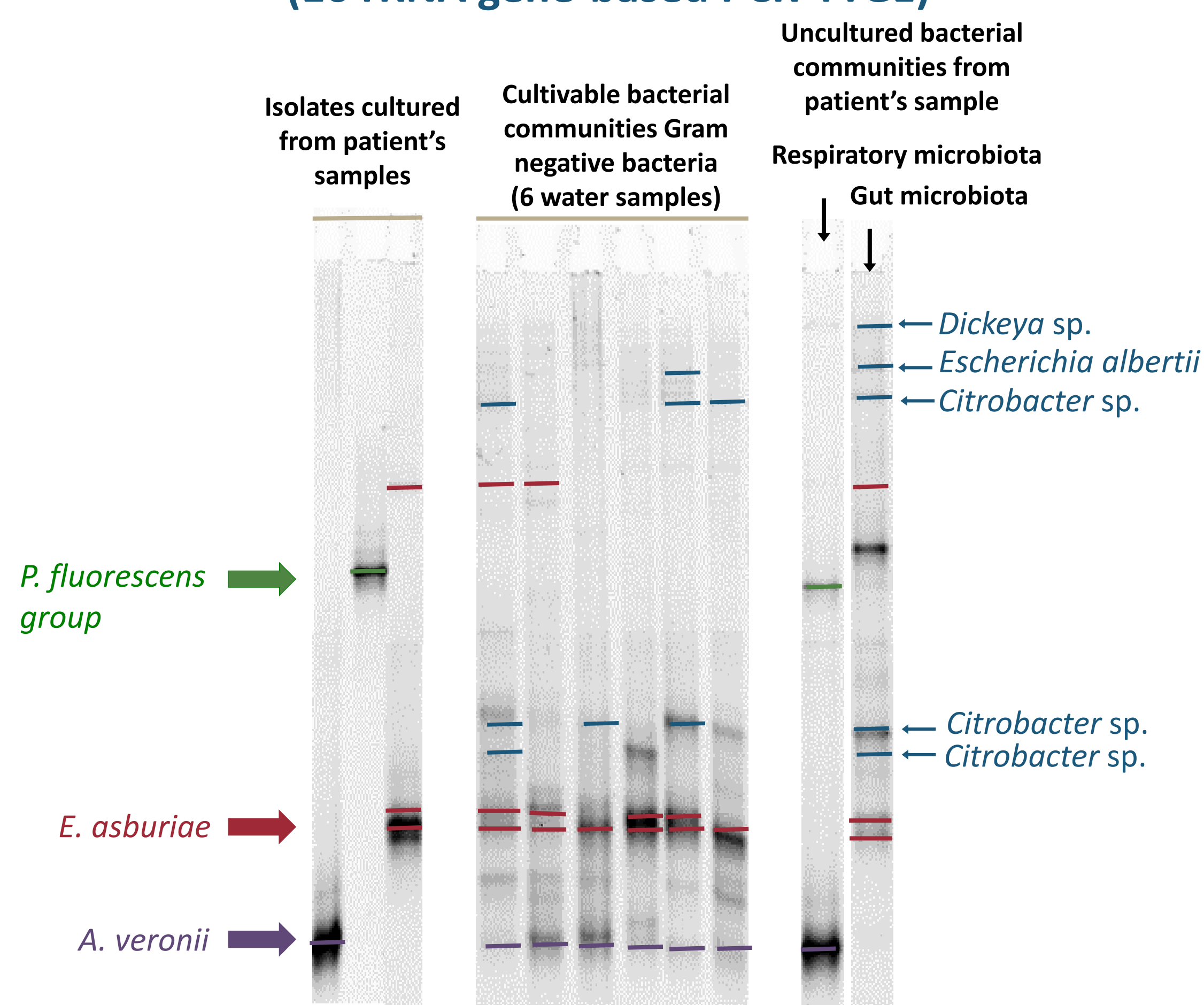
Environmental investigations

To elucidate the relation between environmental bacterial community and human infection and/or human microbiota, water samples were collected from the river at the site of patient's near-drowning.



To compare the patient's respiratory and digestive microbiota with aquatic cultivable bacterial communities, we used 16S rRNA gene-based PCR-TTGE (Temporal Temperature Gradient Gel Electrophoresis) fingerprint.

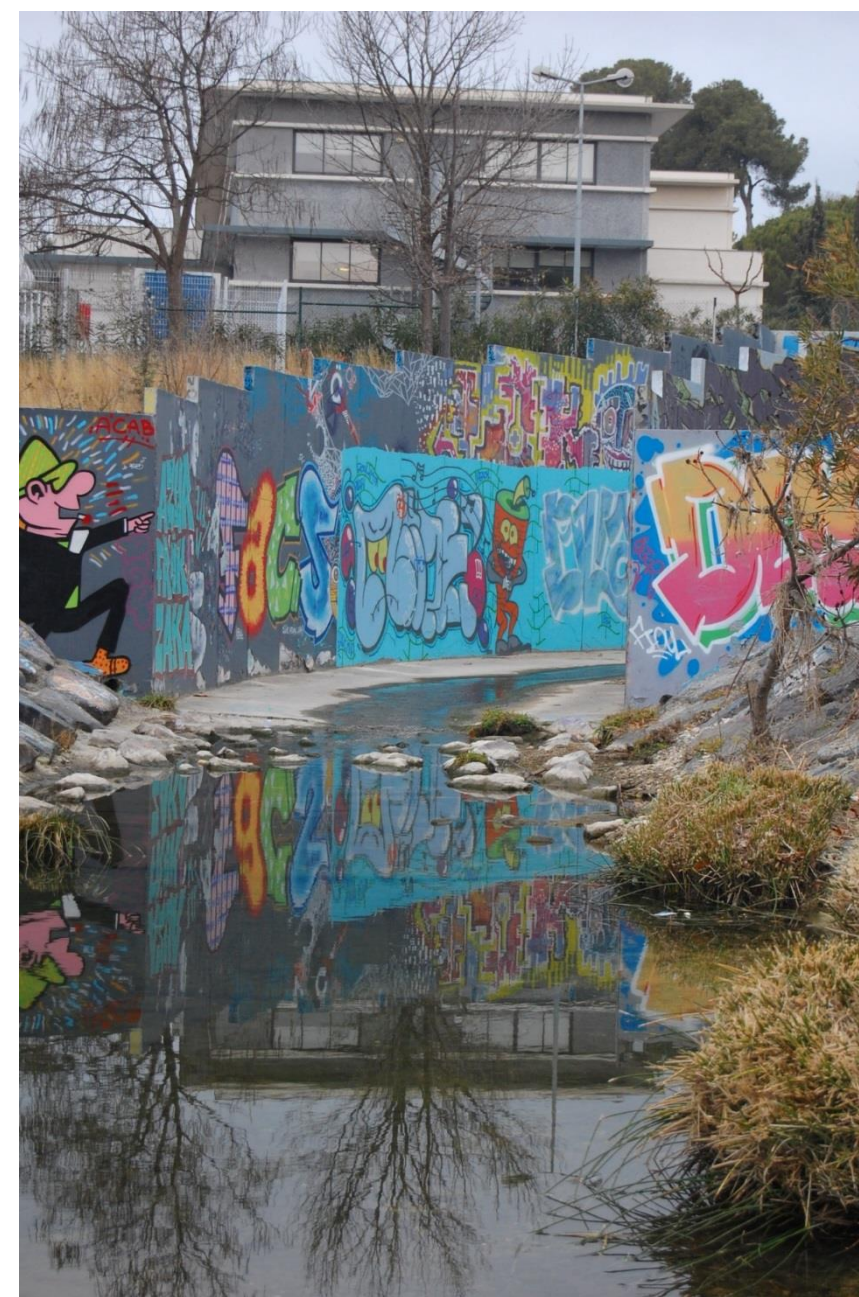
Transmission of bacteria from environmental reservoir (16 rRNA gene-based PCR-TTGE)



=> the river bacterial community partially replaced the respiratory and gut microbiota;
=> the more prevalent bacteria in the patient's pathological microbiota were mild opportunistic human pathogens displaying resistance to carbapenems including *Enterobacter asburiae*, *Aeromonas veronii* and *Pseudomonas fluorescens*;
=> the partial replacement of the gut microbiota by an environmental microbial community led to the bacteremia. Indeed, *E. asburiae* isolated from patient's blood and from river samples had the same PFGE (pulsed-field gel electrophoresis) pattern.

Inventories of antibiotic resistant bacterial communities in natural waters in Montpellier region

Water samples were collected under contrasted hydrogeological conditions and anthropogenic influence : karstic waters, related urban waters.

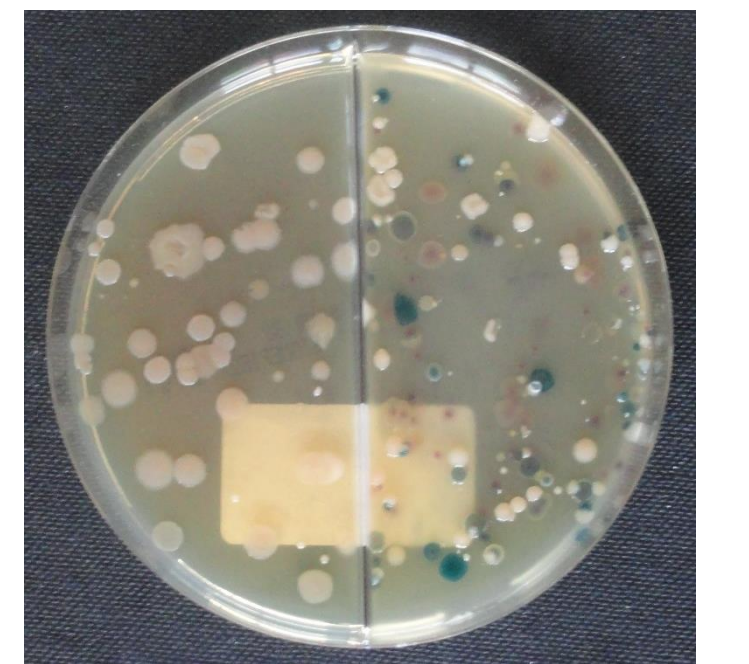


To evaluate i) the antibiotic (ATB) resistance level and ii) the diversity of resistant bacterial communities, we developed a method associating selective culture (with ATB or MDR (multi-drug resistant) selective media) and molecular tools using TTGE and ARISA (Automated Ribosomal Intergenic Spacer Analysis).

Taxonomic diversity in antibiotic-resistant bacterial communities

With culture methods, we observed:

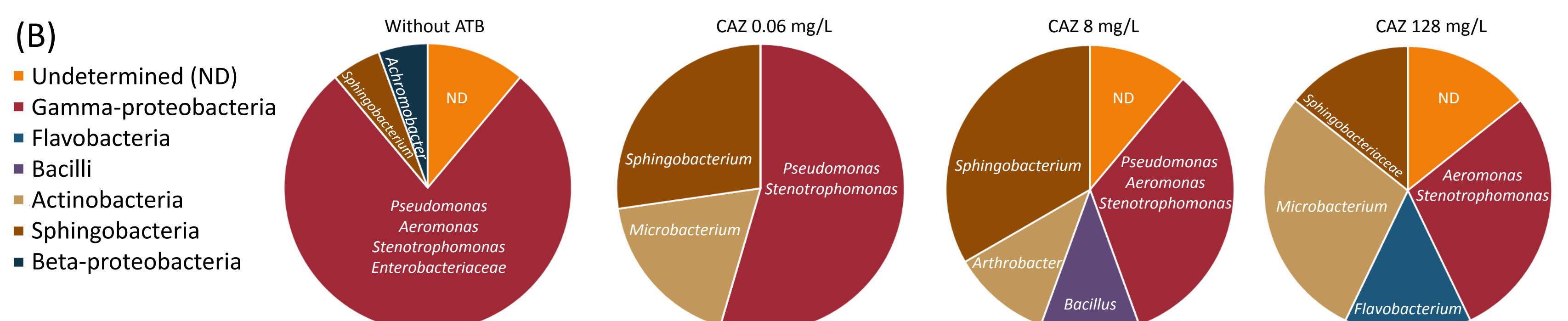
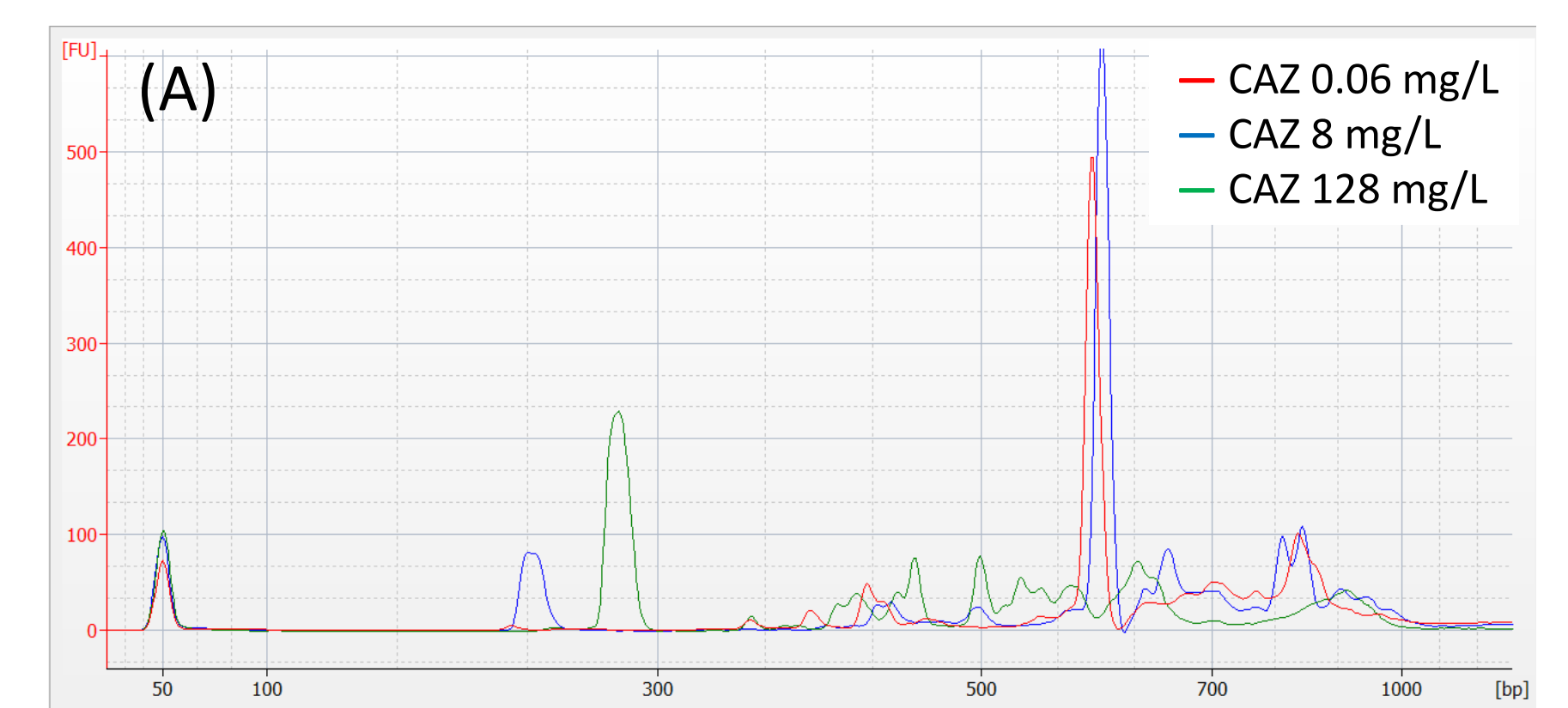
- that resistance level to antibiotics (Amoxicilline, Ceftazidime (CAZ) and Cefotaxime) varies according to both the hydrogeological conditions and the sampling period.
- that we can select bacterial communities i) in the conditions classically used in clinical context to isolate MDR bacteria and ii) growing on CARBA SMART (Biomérieux) media that is a chromogenic media used to specifically isolate carbapenemases-producing and metallo-beta-lactamases-producing bacteria.



With molecular methods, we showed:

- a clear impact of low ATB concentrations (0.03 mg/L) on the bacterial community taxonomic composition;
- that in presence of high ATB concentrations, potentially human pathogens like *Aeromonas* sp. can be selected because of their phenotypic resistance;
- that high ATB concentrations select populations not detected without ATB or with lower concentrations.

ARISA (A) and TTGE (B) results of water sampled from a poorly anthropized site, during spring:



Water-related infectious diseases involving recognized pathogens remain a leading cause of morbidity and mortality worldwide. Moreover, the spectrum of these diseases is expanding towards emerging infectious diseases, opportunistic infections and antimicrobial resistance. We showed how environmental and clinical resistant communities are interlinked and, through a clinical situation, how environmental resistome can modify patient's microbiota and provoke a human infection with a resistant emerging pathogen. This observations fit with the pathobiome concept⁽¹⁾.

The follow-up of communities and the determination of the level of antimicrobial resistance in anthropized environments is a first step for further characterization of these pathobiomes.

It is important to take into consideration :

- how diversity and dynamics of water microbial communities are linked with hydrogeology and with human practices.
- the role of aquatic bacterial communities in the emergence of host infections at both epidemiological and individual scales
- the role of local constraints on resistome design.

(1) Vayssier-Taussat et al., Shifting the paradigm from pathogens to pathobiome: new concepts in the light of meta-omics, Front Cell Infect Microbiol. 2014 Mar 5;4:29.