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# A new methodology to assess antimicrobial resistance of bacteria in coastal waters; pilot study in a Mediterranean hydrosystem





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## ABSTRACT

The global resistome of coastal waters has been less studied than that of other waters, including marine ones. Here we develop an original method for characterizing the antimicrobial resistance of bacterial communities in coastal waters. The method combines the determination of a new parameter, the community Inhibitory Concentration (c-IC) of antibiotics (ATBs), and the description of the taxonomic richness of the resistant bacteria. We test the method in a Mediterranean hydrosystem, in the Montpellier region, France. Three types of waters are analyzed: near coastal river waters (Lez), lagoon brackish waters (Mauguio), and lake freshwaters (Salagou). Bacterial communities are grown in vitro in various conditions of temperature, salinity, and ATB concentrations. From these experiments, we determine the concentrations of ATB that decrease the bacterial community abundance by 50% (c-IC<sub>50</sub>) and by 90% (c-IC<sub>90</sub>). In parallel, we determine the taxonomic repertory of the resistant growing bacteria communities (repertory of Operational Taxonomic Units [OTU]). Temperature and salinity influence the abundance of the cultivable bacteria in presence of ATBs and hence the c-ICs. Very low ATB concentrations can decrease the bacterial abundance significantly. Beside a few ubiquitous genera (Bacillus, Pseudomonas, Shewanella, Vibrio), most resistant OTUs are specific of a type of water. In brackish water, resistant OTUs are more diverse and their community structure less vulnerable to ATBs than those in freshwater. We anticipate that c-IC measurement combined with taxonomic description can be applied to any littoral region to characterize the resistant bacterial communities in the coastal waters. This would help us to evaluate the vulnerability of aquatic ecosystems to antimicrobial pressure.

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<sup>1</sup> In memoriam.

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# 1. Introduction

The human population inhabiting the coastal areas expands continuously (Cravo et al., 2015; Dorsey et al., 2013) and creates environmental stress on adjacent ecosystems. In this context, coastal ecosystems are particularly vulnerable. It is well known that human activities can influence the proliferation and distribution of fecal bacteria including potential pathogens (Cravo et al., 2015; Dorsey et al., 2013). Beside fecal indicators, the abundance and richness of antibiotic (ATB)-resistant bacteria in ecosystems are of major concern in the current era of global resistance burden (Laxminarayan et al., 2013).

At the "omics" era, metagenomic methods approach the stock of genes encoding ATB resistance in ecosystems (Amos et al., 2014; Kristiansson et al., 2011; Marti et al., 2014; Port et al., 2014; Schlüter et al., 2008; Szczepanowski et al., 2008). These culture-independent tools have demonstrated the huge diversity of resistance genes and encoded mechanisms, which form the environmental resistome. However, resistance to ATBs is a bacterial behavior observed *in vitro* as a resistant phenotype, which is predictive, in medicine, of patient treatment failure. Clinically resistant phenotypes are defined by comparison of Minimal Inhibitory Concentrations (MIC) values (concentrations that inhibit the growth of a bacterial strain) with thresholds called clinical breakpoints edited by expert committees for each ATB and bacterial type. When MIC is over the clinical breakpoint value, the strain is categorized as resistant to the tested ATB. Similarly, for environmental bacteria, in vitro studies of ATB-resistant phenotypes are generally performed on isolated strains of well-known pathogenic species (Maravić et al., 2012; Okoh and Igbinosa, 2010; Ramírez Castillo et al., 2013). Studies on isolated environmental autochthonous nonpathogenic bacteria are scarcer (Schreiber and Kistemann, 2013; Varela et al., 2016; Vaz-Moreira et al., 2011), and antimicrobial resistance of the whole cultivable communities is even less studied (Varela et al., 2014; Weber et al., 2011). Moreover, indicators are needed to evaluate both the vulnerability of ecosystems facing antimicrobial constraints and the potential risks for humans to be exposed to environmental ATB-resistant bacteria. In this context, aquatic environments are particularly interesting because they are involved in the dispersal and mix of antibiotics (and more generally xenobiotics) and antibiotic/xenobiotic-resistant bacteria from diverse origins (Baquero et al., 2008; Gaze et al., 2013).

While antibiotic resistance is monitored in human or animal medicine by standardized methods, there is still a need for harmonization to monitor antibiotic resistance in environment (Brauner et al., 2016; Rizzo et al., 2013). This study intends to propose such a method. We introduce an original approach to evaluate the resistance to antibiotics of bacterial communities in water systems. We report this approach through a pilot study of Mediterranean hydrosystems in the Montpellier region, France, where we analyzed both freshwater and brackish waters subjected to different levels of anthropization.

#### 2. Materials and methods

### 2.1. Study area, water sampling and processing

We selected three sites with contrasted environments (Fig. 1). Two sites are in the Montpellier urban area (about 400,000 inhabitants) located in the Mediterranean coastal basins of the Gulf of Lion, France. Montpellier city is located in a small watershed that flows towards the Lez River. This coastal 13-km-long river runs through the city where it is channelized, and emerges in lagoons connected to the Mediterranean Sea such as the Mauguio Lagoon. The Lez catchment covers 709 km<sup>2</sup> and contains the densely urbanized area of Montpellier. The first sampling site is located in the Lez River downstream the city (freshwater)



Fig. 1. Study areas and sampling stations in the Montpellier region (South of France) (géoportail: https://www.geoportail.gouv.fr/).

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