Biogeochemical characterization of sediments under the anthropogenic influence at mouth of Rio de Minas, Punta Arenas, Chile

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INTRODUCTION

The lack of the purification of urban wastewaters before its discharge into the natural recipient could represent the epidemiological route for spread of pathogenic bacteria. Bacteria resistant to carbapenems have become a worldwide problem, because carbapenems are used as last-resort antibiotics to treat human infections caused by antibiotic-resistant bacteria[1]. Recently, dissemination of viable carbapenem-resistant *Acinetobacter baumannii* of clinical significance via the hospital and urban wastewater to river was described in Croatia[2]. The type of urban wastewater and its consequent influence on the natural recipient may vary according to the life style of inhabitants. The aim of this investigation was to characterize the anthropogenic influence of untreated urban wastewaters on the river and sea sediments in South America.

MATERIALS AND METHODS

Three surface sediment samples were collected in Punta Arenas, Chile, South America (Population: ±125,000) during autumn (April 2018) at the mouth of the river Rio de las (Fig. 1). Two sediments were from river flow (1, 2) and one from the Pacific sea (3). The pH value of sediment was measured after suspension (1:2.5) of sediment in distilled water. The mineralogical composition of sediments was determined by X-ray diffraction.

Bacteriological analyses were performed in triplicate after the suspension and dilution of sediment in sterile peptone water. Aerobically grown total heterotrophic bacteria were determined on Nutrient agar (Biolife) after incubation at 22ºC/72 h[3]. Carbapenem-resistant bacteria were determined on CHROMagar Acinetobacter supplemented with CR102 (CHROMagar), intended for the cultivation of clinically relevat carbapenem-resistant bacteria, after incubation at 35ºC/72 h. The numbers of bacteria were determined as Colony Forming Units (CFU), logarithmically transformed, and expressed as log CFU per 1 mℓ of wet sediment. The prevalence of carbapenem-resistant bacteria among total heterotrophic bacteria was calculated as (log CFU/mℓ carbapenem-resistant / log CFU/mℓ heterotrophic) x 100. The colonies of carbapenem-resistant bacteria were analysed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The sediment samples were placed in 2% glutaraldehyde whereafter standard processing procedures was followed for scanning electron microscopy (SEM). The samples were viewed with the Zeiss Ultra PLUS FEG SEM.

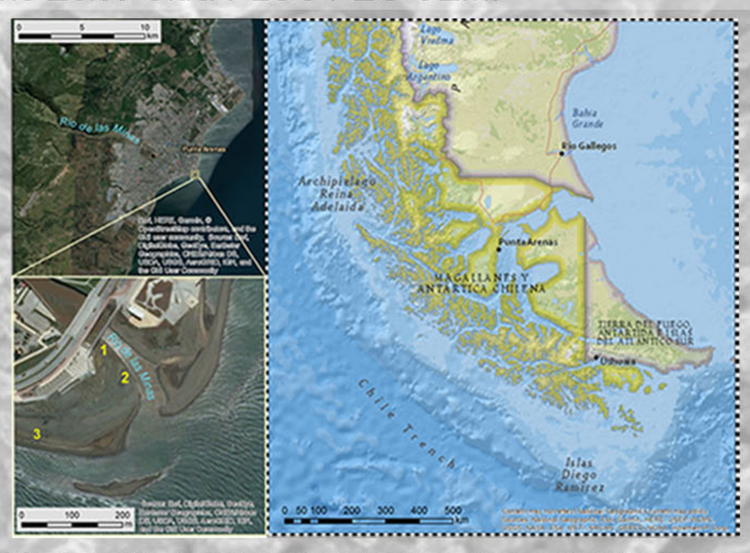

Figure 1: Sampling stations at the mouth of Rio de las Minas (1,2) and Pacific sea (3).

RESULTS

Fine river sediments were dark brown to black in colour, without any smell. X-ray diffraction allowed the identification of mixture of montmorillonite and kaolinite with quartz and albite, while ilmenite was also identified in one sample. All sediment samples displayed slight alkaline reaction, with the sea sediment having the highest pH value (Table 1).

Heterotrophic bacteria were found in abundance of 6.2-6.6 log CFU/mℓ in all three sediment samples (Table 1) and as much as 1.9 and 2.7 log CFU/mℓ of carbapenem-resistant bacteria (Fig. 2) were found in the river sediments, but were absent in seawater sediment. The prevalence of carbapenem-resistant bacteria among total heterotrophic bacteria was 29% and 42% log CFU (Tabel 1). The results are comparable to the prevalence of carbapenem-resistant bacteria (49% log CFU) reported in landfill leachate from Croatia[4].

Table 1: The pH values and number of bacteria in sediment samples

Parameter	River 1	River 2	Sea 3
pH	8.16	7.62	8.91
Heterotrophic bacteria (log CFU/mℓ)	6.4±0.1	6.6±0.2	6.2±0.2
Carbapenem-resistant bacteria (log CFU/mℓ)	1.9±0.0	2.7±0.0	0
Prevalence of carbapenem-resistant bacteria (%)	29	42	0

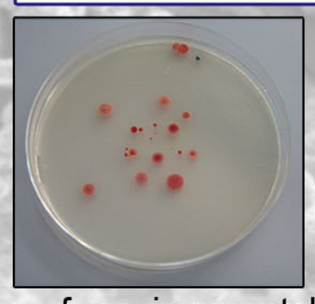

Figure 2: Carbapenem-resistant bacteria grown on selective medium CHROMagar Acinetobacter supplemented with CR102.

A variety of environmental bacterial species possess intrinsic resistance to carbapenem. However, the intrinsic resistance to carbapenems is not common among clinically important bacteria[1]. Clinical isolates of carbapenem-resistant bacteria possess the acquired resistance and represent a global healthcare problem[1]. As confirmed by MALDI-TOF MS analysis, the carbapenem-resistant bacteria from analysed river sediments were represented by species of the genus *Pseudomonas*, which are human pathogens with acquired resistance to carbapenems[5]. SEM confirmed the presence of randomly distributed bacteria in the sediment samples (Fig. 3). Bacteria were strongly attached to the sediment particles with signs of biofilm formation.

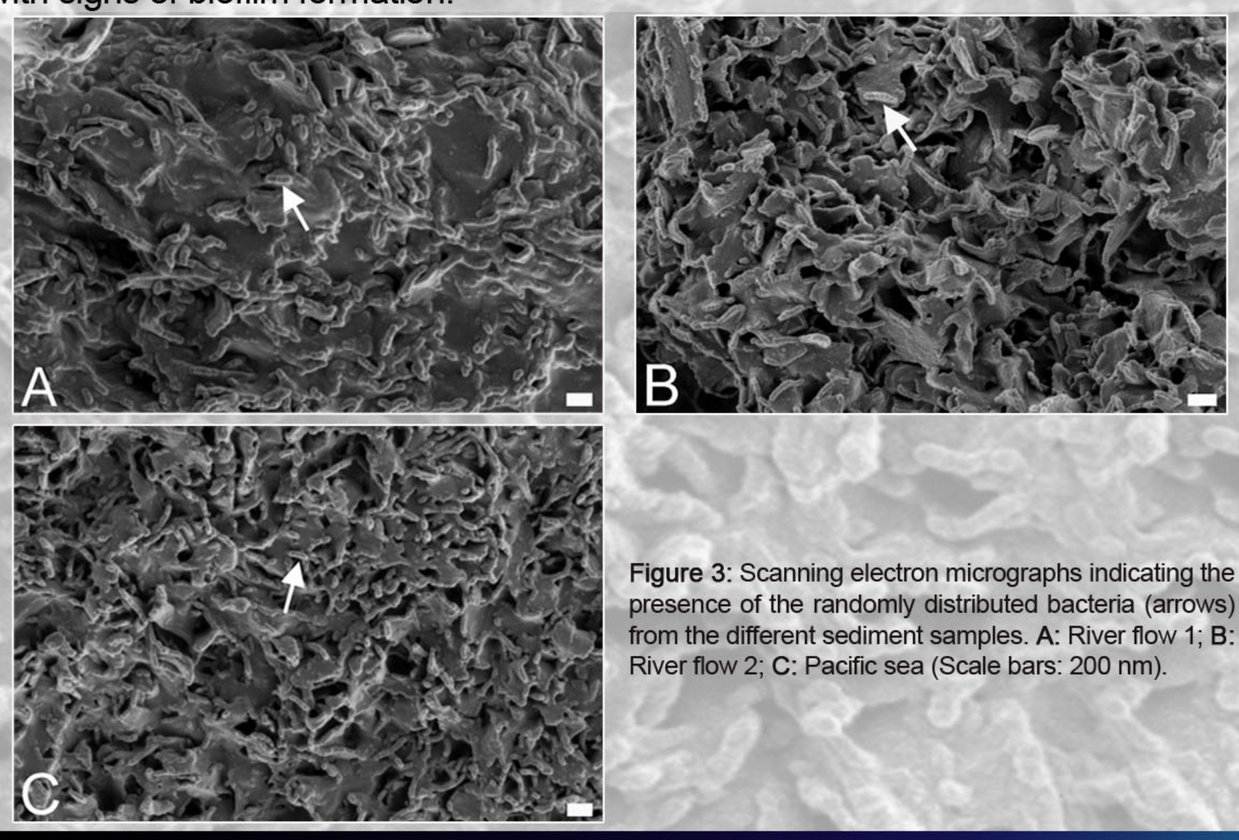

Figure 3: Scanning electron micrographs indicating the presence of the randomly distributed bacteria (arrows) from the different sediment samples. **A:** River flow 1; **B:** River flow 2; **C:** Pacific sea (Scale bars: 200 nm).

CONCLUDING DISCUSSION

The river sediments under the anthropogenic influence of untreated urban wastewaters reveal a significant source of potentially pathogenic carbapenem-resistant bacteria. However, no anthropogenic influence was detected in the nearby seashore.

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