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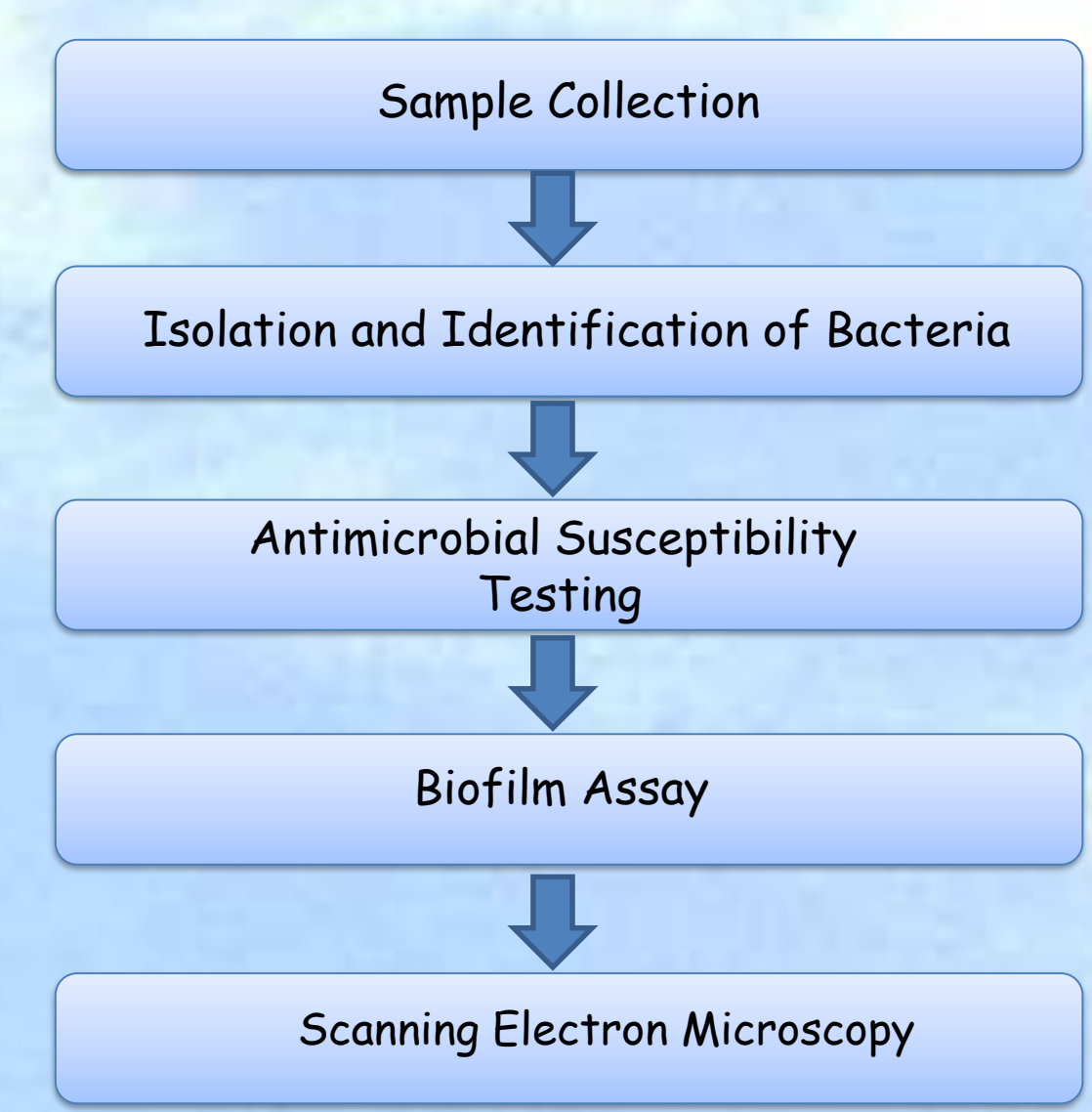
Introduction

Artificial lakes, ornamental fountains and other water courses are present in most city parks and green areas, enabling people to practice sports or simply interact with nature and relax. Water is essential for life but, the presence of infectious agents, toxic chemicals, and radiological hazards could represent a threat for human health. Several studies have provided evidence for the presence of potential human pathogens in waters containing ornamental fish. The water in these city parks might be dangerous, so this study aims to characterize the microbiota from different ornamental lakes and fountains located in Lisboa, Portugal. A special attention was given to the ability of environmental bacteria to persist within biofilms and the antibiotic susceptibility profiles of clinically relevant members of the ornamental waters microbiota.

Materials and Methods

Water and biofilm samples were collected, in 2 lakes (L1-L2) and ornamental fountains (L3-L4) in February/2015. In May/2015 and monthly during a year (starting March/2016) samples from L4 were collected. The samples were collected within a park with a total area of 26 hectares, located in the northern area of Lisboa, Portugal. Biofilm assembly was monitored by crystal violet assay and SEM and antibiotic susceptibility was performed by conventional methods.

Diagram below explain the process:



Results:

Table 1. L4 microbiota (March 2016 to February 2017).

Month	March 16	April 16	May 16	June 16	July 16	Aug 16	Sep 16	Oct 16	Nov 16	Dec 16	Jan 17	Feb 17
Actinobacter lwoffii												*
Aeromonas hydrophila/caviae												*
Aeromonas sobria	*	*	*	*	*	*	*	*	*	*	*	*
Aeromonas spp												*
Aeromonas veronii												*
Alcaligenes faecalis spp faecalis												*
Bacillus spp												*
Bacillus subtilis			*									
Bacilo éneg												*
Comamonas testasteni/ P oleovorans									*	*	*	*
E coli									*	*	*	*
Enterobacter cloacae complex				*	*	*	*	*	*	*	*	*
Escherichia fergusonii												*
K. oxytoca									*	*	*	*
K. pneumoniae									*	*	*	*
K. pneumoniae spp pneumoniae				*	*	*	*	*	*	*	*	*
Klebsiella oxytoca									*	*	*	*
Lecteria adecarboxylata									*	*	*	*
Pantoea spp												*
Pseudomonas fluorescens												*
Pseudomonas oryzae												*
Pseudomonas putida									*	*	*	*
Pseudomonas spp									*	*	*	*
Raoultella planticola	*											*
Raoultella ornithinolytica				*								*
Raoultella planticola (K. planticola)									*	*	*	*
Serratia marcescens						*	*	*	*	*	*	*
Serratia fonticola						*	*	*	*	*	*	*
Serratia marcescens						*	*	*	*	*	*	*
Serratia plymuthica									*	*	*	*
Shewanella putrefaciens									*	*	*	*
Shingomonas paucimobilis		*		*					*	*	*	*

Table 2. Antibiotic susceptibility of bacterial isolates identified from ornamental waters in February/2015.

ID	Bacteria	Antibiotic Susceptibility *						
		AMC	FOX	CAZ	CTX	IPM	GM	CIP
L1	<i>Klebsiella oxytoca</i>	R	S	S	S	S	S	S
	<i>Klebsiella pneumoniae</i>	R	S	S	S	S	S	S
	<i>Serratia marcescens</i>	R	S	S	S	S	S	S
	<i>Serratia odorifera</i>	R	S	S	S	S	S	S
	<i>Serratia rubidea</i>	S	S	S	S	S	S	S
	<i>Vibrio metschnikovii</i>	R	R	S	S	S	S	S
L2	<i>Elisabethkingia meningoseptica</i>	R	R	S	R	R	S	S
	<i>Enterobacter spp.</i>	R	S	S	S	S	S	S
L3	<i>Stenotrophomonas maltophilia</i>	R	R	R	R	R	S	S
	<i>Serratia rubidea</i>	R	S	S	S	S	S	S
L4	<i>Klebsiella pneumoniae ozonae 1</i>	S	S	S	S	S	S	S
	<i>Klebsiella pneumoniae ozonae 2</i>	R	S	S	S	S	S	S
	<i>Pastorella, Shigella</i>	S	S	S	S	S	S	S

* AMC: Amoxicillin/clavulanic acid, FOX: Cefoxitin (FOX), CAZ: Ceftazidime, CTX: Cefotaxime; IMP: Imipenem; GM: Gentamicin; CIP: Ciprofloxacin. R: Resistant, S: Susceptible; I: Intermediate.

Table 3. Bacteria ability to assemble biofilms *in vitro* at different temperatures.

Biofilm Recovered Bacteria ID	OD 570 nm (AU)	
	25 °C	37 °C
<i>K. pneumoniae</i>	1.159 ± 0.09	0.285 ± 0.01
<i>A. sobria</i>	0.284 ± 0.06	0.155 ± 0.03
<i>A. veroni</i>	0.761 ± 0.11	0.185 ± 0.004
<i>C. violaceum</i>	0.017 ± 0.01	0.096 ± 0.05

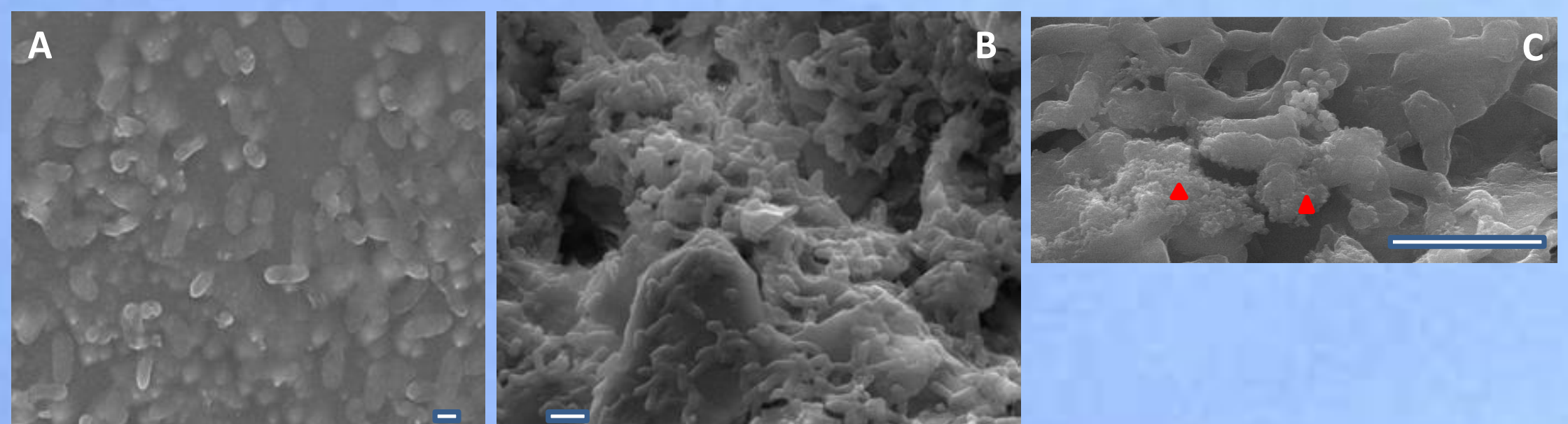


Figure 1. Biofilms assembled on cement at 25 °C (A) by *A. sobria* isolated from biofilms present in L4 (May/2015); (B) and *K. pneumoniae* isolated from biofilms present in L1 (February/2015); (C) A detail of *K. pneumoniae* biofilm highlighting the extracellular matrix (red triangles). Scale bar 1 μm.

Conclusions

- In this study potential human pathogens were identified in samples from a typical urban park.
- These pathogens were found in both planktonic and biofilm forms.
- Waterborne pathogens such as *Aeromonas* species are a significant cause of acute bacterial gastroenteritis in young children.
- The obtained results support a periodic control of ornamental water microbiota as simple preventive measure to avoid potential health issues.



L3; L4 ornamental fountains Lake (L1) Lakes (L2)