

## Risk assessment for public health from human interaction with ornamental waters

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Water is essential to life; nevertheless ingestion of contaminated water could result in death caused by waterborne diseases such as cholera [1]. Pathogens present in the water can cause diseases, other than those resulting from water ingestion, being registered an increase in the number of case reports in recent years. It is not clear if this increase is due either to a better case reporting system or to an increase in microorganism's virulence. The generalized use of antibiotics in agriculture and animal farming contributed to their dissemination in the environment which promotes microorganism selection and emergence of resistant strains. This phenomenon can be enhanced by the ability of microorganism to persist within complex communities known as biofilms.

In the present work we aim to characterize the microbial population present in ornamental waters and perform a risk assessment for public health resulting from human interaction with it.

Samples were collected in the Lisboa area between December 2014 and February 2015. Bacteria were identified either by growth on non-selective/ selective media incubated at different temperatures or VITEC after filtration through a 0.45µm membrane. Antibiotic susceptibility tests were performed and analyzed for planktonic bacteria according to EUCAST. Biofilm assembly, sample preparation for scanning electron microscopy (SEM) and minimal inhibitory concentration for amoxicillin (AMX) were performed as described previously [2].

In all water samples more than 300 colony forming units (CFU) per 10 mL were found on plate count agar. The majority of the identified microorganisms were members of Enterobacteriaceae family namely, *Klebsiella pneumoniae* (Kp), *K. pneumoniae ozaenae*, *Enterobacter spp.*, *Serratia marcescens*, *S. rubidea* e *S. odorifera*. Non fermentative, oxidase positive bacteria such as *Elizabethkingia meningoseptica*, *Stenotrophomonas maltophilia* and *Vibrio metschnikovii* were also identified.

The presence of a main etiological agent of healthcare associated infections (HAI) with high rates of antibiotic resistance among the isolates is an interesting and concerning result [3]. Since Kp resistance to antibiotics is linked to biofilm assembly [2] we decided to investigate the presence of Kp biofilms within the lakes. Kp biofilms were present only in lake 2. Further analysis of the Kp isolates rendered the results showed in table 1.

All the bacteria were able to assemble biofilms in conditions mimicking those present in the environment (25°C). This ability is decreased at 37°C or even absent (Kp2). Bacteria fitness to environmental conditions might suggest a diminish ability to colonize human beings. The environmental fitness was further

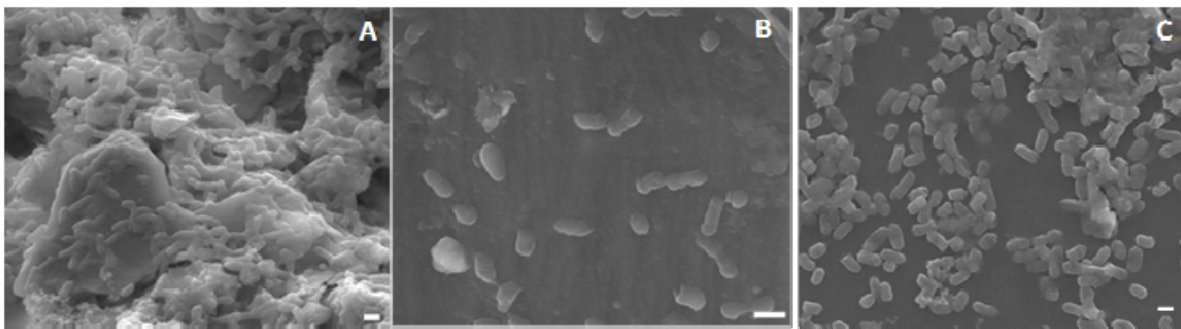
documented by analysis of biofilms using SEM. The best organized biofilms were assembled on cement at 25°C (Fig. 1.A). Both temperature (Fig. 1.B) and surface (Fig. 1.C) changes lead to a decreased biofilm assembly.

In contrast to clinical isolates environmental Kp were susceptible to the 3 main groups of antibiotics:  $\beta$ -lactam, aminoglycosides and quinolones. In other words environmental Kp do not meet the criteria to be classified as multi-resistant bacteria. For this reason only biofilm susceptibility to amoxicillin was assayed. Biofilm assembly increased antibiotic resistance of Kp. Two isolates (Kp3 and 4) exhibited the same MIC value (500  $\mu\text{g}/\text{mL}$ ) which is significantly higher than the MICs founds for Kp2 (0.98  $\mu\text{g}/\text{mL}$ ) and Kp3 (7.81  $\mu\text{g}/\text{mL}$ ). This result together with the fact that Kp3 and 4 were isolated from the same lake lead us to hypothesize that the two isolates might be part of the same biofilm being the presence of Kp3 in the water explained by biofilm dispersion.

The low risk of environmental Kp for human health and the universal role played by biofilm assembly on antibiotic resistance are the main conclusions arising from this study.

#### References:

- [1] J.P.S. Cabral, *Int. J. Enviro Res Public Health*, **7** (2010) p. 3657.  
 [2] M. Bandeira, *et al.*, *Pathogens*, **3** (2014) p. 720.  
 [3] ECDC Europe, (2013), <http://www.ecdc.europa.eu/en/publications/publications/annual-epidemiological-report-2013.pdf>



**Figure 1.** *Klebsiella pneumoniae* biofilms. The ability of Kp4 to assemble biofilms *in vitro* on cement at 25°C (A) and 37°C (B) was evaluated. In order to evaluate the role played by surface on biofilm assembly glass was also used (C). All the biofilms were 48h old. Scale bar = 1 $\mu\text{m}$ .

**Table 1.** Characterization of *Klebsiella pneumoniae* isolates.

ID	Source	Biofilm assay (OD570nm)		Antibiogram (mm)							MIC AMX ( $\mu\text{g}/\text{ml}$ )	
		25°C	37°C	AMC	FOX	CAZ	CTX	IPM	GM	CIP	Plant	Biof
Kp1	Lake 1	0.240	0.153	18	28	>30	>30	>30	24	>30	7,81	62,5
Kp2	Lake 2	0.076	---	6	20	25	30	25	18	28	0,98	125
Kp3	Lake2	0.740	0.256	6	24	>30	>30	22	18	>30	500	>500
Kp4	Biof Lake 2	1.159	0.285	6	6	25	29	25	18	28	500	>500

Plant: Planktonic bacteria, Biof: biofilm; AMC: combination of amoxicillin and clavulanic acid; AMX: amoxicillin; FOX: cefoxitin; CAZ: ceftazidime; CTX: cefotaxime; IPM: imipenem; GM: gentamicin and CIP: ciprofloxacin.