RESISTANCE OF *LEGIONELLA PNEUMOPHILA* AND *HELICOBACTER PYLORI* TO CHLORINATION IN DRINKING WATER BIOFILMS

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Under stressful conditions bacteria can adhere to surfaces to form complex biofilms where they are protected. Biofilms formed in drinking water distribution systems have been widely studied due their relationship with the protection of pathogens, such as *Legionella pneumophila* and *Helicobacter pylori*. The aim of this work is to study the influence of chlorine on the inclusion and survival of *L. pneumophila* and *H. pylori* when associated with heterotrophic biofilms.

Biofilm studies on PVC coupons were carried out using a two-stage chemostat system with different concentrations of chlorine (0.0; 0.2 and 1.2 mg Cl_2 L^{-1}). The coupons were removed at different times (up to 32 days) and scraped with sterile glass beads. Planktonic and sessile cells were quantified by standard cultivation techniques (R2A for heterotrophic bacteria, BCYE for *L. pneumophila* and HP medium for *H. pylori*) and SYTO9 staining. In addition, two specific 16S rRNA peptide nucleic acid (PNA) probes were used to quantify the total numbers of *L. pneumophila* and *H. pylori* in the biofilm, tested in separate experiments.

Even though both *L. pneumophila* and *H. pylori* were not recovered by plating procedures, both pathogens were identified in biofilms by their strong PNA probe fluorescence, demonstrating a high rRNA content indicative of viability and indicating that these pathogens can persist in chlorinated biofilms for at least 32 days even at the highest concentration of chlorine (1.2 mg L^{-1}).

This work demonstrates the extended resistance of these two pathogens to chlorine and is of public health concern.