Quantification of *Helicobacter pylori* in the viable but nonculturable state by quantitative PCR (qPCR) in water disinfected with ozone

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ABSTRACT

Helicobacter pylori is a Gram-negative spiral-shaped bacterium that colonizes the gastric mucosa and is associated with gastric diseases. It may present morphological adaptations when it is placed out of its natural environment, such as in water. The morphological adaptation is a coccoid form, which is a viable but non-culturable state (VNC), where the DNA remains active and therefore infective. Due to the incapacity of culture with traditional methods in the VNC state, we developed a methodology that includes a molecular technique, like quantitative PCR (qPCR), which is capable of measuring the bacteria in both forms (bacillary and coccoidal) and therefore is able to measure a disinfection process, and to estimate the resistance of the bacteria to ozone. The methodology developed measures the efficiency of the ozone disinfection when bacteria are in a VNC state only. Bacterial culture at 9×10^8 CFU/mL diluted in 40 mL reaction volumes were exposed to a wide range of CT values (0.11–15 mg-min/L). The results show a 3.92-log reduction when treated with 15 mg-min/L. Our results demonstrate the feasibility of using qPCR for the quantification and detection of *H. pylori*, in coccoid form, in water systems treated with an ozone disinfection process.

Key words | *Helicobacter pylori*, ozone disinfection, qPCR, VNC state

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INTRODUCTION

Helicobacter pylori is a leading cause of gastrointestinal disease in humans, including duodenal and gastric ulcers, and gastric cancer, among other diseases (Kusters *et al.* 2006). It has been suggested that some possible routes of transmission are fecal–oral, gastric–oral, and oral–oral; however, drinking water may be an additional source (Hulten *et al.* 1996; Mazari-Hiriart *et al.* 2001; Watson *et al.* 2004). It has been recognized that *H. pylori* may present morphological adaptations when it is placed out of its natural environment, such as in water (Azevedo *et al.* 2007). Nevertheless, only recently has it been accepted that these modified forms of the bacteria retain their pili, which in turn allows them to preserve their mobility properties for up to one year in such environments (She *et al.* 2003). It is well known that *H. pylori* is able to form biofilms and stay in viable but non-culturable (VNC) forms for up to one year while being in a water environment (Sasaki *et al.* 1999; Kusters *et al.* 2006; Linke *et al.* 2010). Moreover, this bacterium presents more resistance to chlorine and ozone than *Escherichia coli* at concentrations normally found within distribution systems and when it is in a bacillary state (Baker *et al.* 2002), and this may be attributed to the resistance of the bacteria or the method of culture preparation (Johnson *et al.* 1997).

Due to the lack of effective water treatment systems, especially in developing countries, the natural sources of water for human consumption, and the water distribution systems, have also become some of the main routes for the transmission of gastrointestinal diseases (Ezzati *et al.* 2002). There are several pathogenic microorganisms (*H. pylori*, *E. coli*, *Salmonella typhimurium* and *Vibrio* sp.) which when left for long periods of time in aquatic environments, may develop morphological adaptations that allow them to acquire resistance to certain disinfecting agents (Bitton 2011).

One of the morphological adaptations that these types of microorganisms may go through is the conversion to a

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