

Detection of *Helicobacter pylori* in biofilms by using real-time PCR

Linke, S., Gebel, J., Büttgen, S., Exner, M.

Institute for Hygiene and Public Health, University of Bonn

Introduction: *Helicobacter pylori*, a cause of the peptic ulcer disease, and agent of gastric cancer is discussed as possible water-borne organism. The primary mode of transmission remains undetermined though fecal-oral and oral-oral transmissions are widely accepted. *H. pylori* enters the viable but nonculturable state (vbnc) at unfavorable conditions and has therefore only rarely been cultured from water-samples and biofilms.

The hygienic aspects of biofilms are of increasing importance since it became widely accepted that they are natural habitats for the superior number of microorganisms. Especially in health-care facilities the control of water-supplies and the surveillance and treatment of plumbing systems became a new challenge for the prevention of nosocomial infections.

H. pylori forms biofilms and like many other (opportunistic) pathogens and biofilm associated bacteria they cannot be detected by conventional drinking-water analysis methods like cultivation on agar plates for several reasons. The main focus lies on coliform bacteria and although *H. pylori* can be transmitted by fecal-contaminated water there exists no correlation between the presence of coliform bacteria and *H. pylori*. Due to the vbnc-state *H. pylori* can not be cultivated on R2A-agar additionally. Therefore it is necessary to develop new strategies for the prevention and control of pathogens in drinking water.

Aim of the study: The aim of this study was to establish an exact and reliable detection- and quantification- method for *H. pylori* by real-time PCR in biofilms. The method was established for untreated water and freshwater-samples and adjusted for the detection of *H. pylori* within biofilms.

Additionally the influence of the biofilm on the sensitivity of the real-time PCR and the resulting loss of detectable cells was investigated.

The investigations should give evidence about a possible reservoir for *H. pylori* in drinking water and drinking water-biofilms. The results should be a basis for a risk assessment concerning the possible acquisition of *H. pylori* by drinking water.

Method and Results: The biofilms were generated in a silicone-tube model at the Institute for Hygiene and Public Health, University of Bonn.

The selected target gene for the urease subunit ureA is highly specific and conserved and it has previously been reported to show no crossreactivity to other closely related bacteria or subspecies of *Helicobacter spp.* which predisposed it for PCR and especially real-time PCR.

The investigations included experiments to examine the formation of *H. pylori* into the viable but nonculturable status. The relationship between morphology and culturability was examined by plate counts. The relationship between morphology and viability was examined using an Live/Dead BacLight assay. Additionally the morphology was examined by scanning electron microscopy. Our results confirmed a possible existence of *H. pylori* in drinking-water biofilms.

Acknowledgement: This work was financially supported by the German Federal Ministry of Education and Research (grant number 02WT0836)