Study of surface characteristics of water-exposed *Helicobacter pylori* and plumbing materials

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Abstract

Adhesion of *Helicobacter pylori* to six materials of different hydrophobicity and commonly used in drinking water distribution systems was investigated using epifluorescence microscopy. The values obtained were subsequently compared with theoretical adhesion values, based on surface tension components and hydrophobicity, calculated through contact angle measurements. Bacteria were found to have a hydrophobic surface with a water contact angle of 62.4 ± 4.7 degrees as determined by the sessile drop method. All the test surfaces examined were also hydrophobic except for glass. *H pylori* was able to adhere to all materials, but glass appeared to support lower numbers, which can be explained by the hydrophilic character of this material.

Keywords

Helicobacter pylori; contact angles; water; adhesion; DAPI; fluorescence microscopy

INTRODUCTION

The route of transmission of *Helicobacter pylori* between the human population has been linked with water (Mazari-Hiriart et al. 2001, Azevedo et al. 2004) and water biofilms (Park et al. 2001). Fluorescence *in situ* hybridization experiments have shown that *H. pylori* is able to attach directly to stainless steel surfaces in diverse species biofilms (Azevedo et al. 2003). It is therefore important to study the adhesion processes between the bacterium and typical plumbing materials.

The aim of this work is to study the phenomena involved in the bacterial adhesion on plumbing materials in order to understand the possibility of *H. pylori* to adhere to pipe materials and therefore be transmitted through the water. For that, we have applied the Lifshitz –van der Waals acid–base (LWAB) approach (Van Oss et al. 1988), using three different liquids (2 polar and 1 apolar) to calculate the free energy of adhesion. This parameter was subsequently compared with the experimental values of adhered cells obtained by fluorescence microscopy.

MATERIALS AND METHODS

Culture maintenance

H. pylori NCTC 11637 was maintained as described in Azevedo et al. (2004).

Test surfaces preparation

Coupons measuring 2 by 2 cm were prepared at the Centro de Engenharia Biológica from stainless steel 304 and 316, copper, PVC, PP and glass. Copper coupons were polished with an alumina suspension. All materials were immersed in a solution of commercial detergent and pre-warmed distilled water for 30 min while gently mixed. To remove rests of detergent coupons were rinsed five times in ultrapure water and air dried. They were subsequently immersed in 90% ethanol for 30 min, except PVC and PP that were only immersed for 10s. After being rinsed with ultra-pure water, air dried and wrapped in foil, SS304, SS316, copper and glass coupons were autoclaved for 15 min at 121 °C, whereas PP and PVC were autoclaved for 20 min at 80 °C. Coupons were finally placed in wells of a 6-well tissue culture plate or used for contact angle measurements.

Adhesion of *H. pylori* to the test surfaces and microscopy quantification

Cells from 2 days old cultures were harvested from Columbia Agar plates, suspended in 10 ml of autoclaved tap water and vortexed for 30s. This inoculum was then transferred to a sterile bioreactor containing 1000 ml of autoclaved distilled water, with a final concentration of 3×10^6 CFU/ml. The bioreactor was maintained at room temperature (approx. 23±2°C) and continuously stirred (120 rpm) using a magnetic bar. After 10 min, 5mL of the suspension were dispensed in to each of the wells containing the coupons. After 48 hours, coupons were removed from the wells, rinsed three times in autoclaved distilled water, and left to air dry. Total cell counts of adhered bacteria were obtained by applying 80 µL of a solution containing 100 µg/mL of 4,6-diamino-2-phenylindole (DAPI) for 5 min directly to the coupons which were then covered with a coverslip. Cells were visualized under an epifluorescence microscope equipped with a filter sensitive to DAPI fluorescence. A total of 20 fields were counted using an ocular grid and the average was used to calculate total cells per cm^2 of the test surface.

Contact angles measurement

Contact angles were measured by the sessile drop technique on the test surfaces prepared as described in the test surface preparation section, using a contact angle measurements apparatus. The measurements were performed at room temperature, using three different liquids: water, formamide and 1-bromonaphtalene. Each assay was performed in triplicate and at least 10 contact angles, per sample were measured. For *H. pylori* contact angles measurements, 150 mL of the suspension from the bioreactor were filtered through 0.2 µm pore size membranes. Membranes were subsequently sliced in four parts and left to dry in a petri dish containing glycerol agar. It had been previously determined that the time needed for the contact angle to reach a plateau in such conditions to be 2.5 hours.

RESULTS AND DISCUSSION

The use of fluorescence microscopy showed that *H pylori* appears to be able to adhere to all materials, but glass supports lower numbers of the bacteria (Figure 1). The DAPI method allowed a very good discrimination of the adhered bacteria in all materials, with the exception of copper, where the intensity of fluorescence was not very strong.

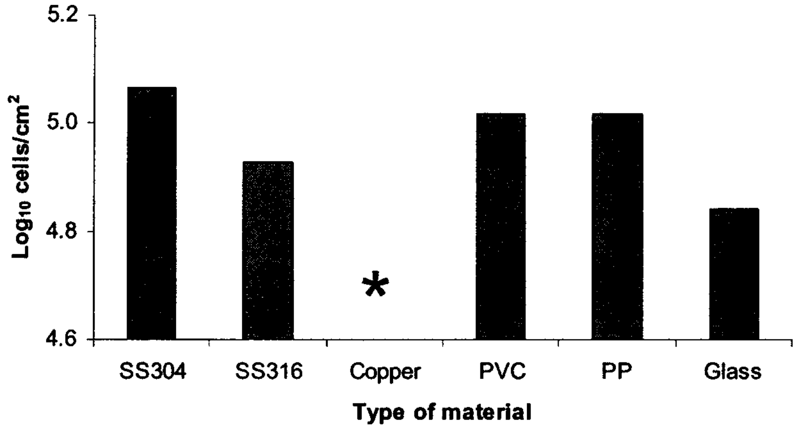

Figure 1. Comparison between the adhesion values obtained by the DAPI method in four of the materials tested.* Copper cell counts are not presented because of the reason already mentioned.

In terms of surface characteristics and observing Table 1, it appears that most materials were hydrophobic, based on the threshold of 60° of the contact angle between the surface and water described in Reid *et al.* (1992). Based on the same value, *H. pylori* cells can also be considered hydrophobic. The percentage of deviation of contact angles from surfaces without preparation to surfaces with preparation ranged from 5 to more than 100%, with the largest deviations being observed in the measurements with 1-bromonaphatelene. The comparison between the experimental values obtained by the DAPI method and the theoretical values based on the LWAB theory is yet to be assessed. However, it was observed by microscopy that for some materials, a large fraction of the bacteria predominantly adhered in certain parts of the surface. This observation is not accounted for in the LWAB theory, and might influence the efficiency of the comparison.

Table 1. Values of contact angles measured with water (θ_w), formamide (θ_f) and 1-bromonaphatelene (θ_b) for lawns of *H. pylori* NCTC 11637 and different test surfaces. Results for the test surfaces were obtained before and after preparation.

Test surface		θ_w (± SD)	θ_f (± SD)	θ_b (± SD)
Before treatment	SS304	72.2 (± 7.5)	60.5 (± 6.2)	21.2 (± 3.1)
	SS316	73.4 (± 5.1)	62.6 (± 3.4)	22.2 (± 3.5)
	Copper	90.6 (± 2.8)	74.1 (± 3.5)	22.0 (± 2.5)
	PVC	79.0 (± 5.8)	50.6 (± 3.7)	18.7 (± 3.2)
	PP	n. p.	n. p.	n. p.
	Glass	54.4 (± 2.2)	24.9 (± 5.4)	34.9 (± 4.0)
After treatment	SS304	76.3 (± 6.9)	67.0 (± 9.3)	48.4 (± 6.3)
	SS316	63.6 (± 10.8)	60.7 (± 7.4)	32.9 (± 4.2)

Copper	112.1 (± 2.7)	67.0 (± 9.3)	48.8 (± 9.4)
PVC	86.0 (± 6.4)	68.6 (± 4.8)	25.0 (± 3.4)
PP	98.1 (± 5.7)	81.0 (± 5.5)	44.5 (± 6.1)
Glass	38.8 (± 5.1)	34.3 (± 6.7)	38.1 (± 7.2)
H. pylori NCTC 11637	62.4 (± 4.7)	72.5 (± 6.8)	62.5 (± 4.6)

n.p. – not performed

CONCLUSIONS

The following conclusions were reached:

- *H. pylori* cell surface appears to be hydrophobic, as well as most materials tested. Glass appears to be hydrophilic which is in agreement with values from the literature.
- Treatment of surfaces affects the contact angle measurements, specially the ones performed with 1-bromonaphatelene.
- Of the materials tested, *H. pylori* adheres in lower numbers to glass, which is also the material with the lower contact angle formed with water.

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