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The cellular and flagella morphologies of ulcerogenic *Helicobacter pylori* paediatric strains.

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Helicobacter pylori is a pathogenic spiral-shaped, microaerophilic, gram-negative bacterium, that inhabits the human stomach. Infection is usually acquired during childhood and always elicits an acute immune response that is, however, inefficient in bacteria clearance. Therefore, in the absence of effective treatment, infection and gastritis (non ulcer dyspepsia, NUD) persist throughout the patient's life. Depending on its severity and pattern, in about 15% of infected adults, this silent destruction of the gastric mucosa may further progress to peptic ulcer disease (PUD) (gastric and duodenal ulcers, GU and DU respectively) and/or gastric cancer [1]. Infection with *H. pylori* is also the major cause for the development of paediatric PUD, a rare event that may occur shortly after infection. In addition to the still undisclosed genetic susceptibility of these young patients, the virulence of the implicated *H. pylori* strain plays a crucial role in the paediatric PUD pathogenesis. Recently, we proved by *in vitro* infection assays that, compared with paediatric NUD-associated isolates, a group of paediatric ulcerogenic-strains present a greater ability to induce a marked decrease in the gastric cells viability and to cause them severe cytoskeleton damage and mucins' production/secretion impairment [2]. Moreover, we showed that their enhanced virulence result from a synergy between the ability to better adapt to the hostility of their niche and the expression of *cagA*, *vacAs1*, *oipA* ''on'' status, *homB* and *jhp562* virulence factors. Accordingly, these ulcerogenic strains share a particular proteome profile, providing them with better antioxidant defences, a metabolism favouring the biosynthesis of aromatic amino acids and higher motility [2].

We are now characterizing/comparing the cellular and flagella morphologies of *H. pylori* strains isolated from Portuguese children, associated with DU, GU or NUD, belonging to the vast and multiethnic collection of the Instituto Nacional de Saúde Dr. Ricardo Jorge (Portugal). For that, bacteria were grown in *H. pylori* selective medium (Biogerm, Maia, Portugal) at 37ºC in a microaerobic environment (Anoxomat®, MART Microbiology BV, Drachten, The Netherlands) for 24 h. For Leifson staining analysis, a drop of each bacterial suspension (in PBS) was spread in cleaned microscope slides, stained with the Leifson dye solution until a golden film developed on the dye surface and a precipitate appeared throughout the sample, and analysed by optical microscopy. For Transmission-Electronic-Microscopy (TEM) studies bacterial pellets were fixed sequentially in glutaraldehyde, osmium tetroxide and uranyl acetate, dehydrated in ethanol and embedded in Epon-Araldite. Thin sections contrasted with uranyl acetate and lead citrate were observed with a JEOL 100-SX electron microscope.

Corroborating the better swimming abilities of the PUD strains, as previously shown by motility assays [2], optical microscopy analysis of Leifson stained slides demonstrated marked differences in the morphology of the studied strains (Figure 1). The *H. pylori* strain associated with DU (*Hp* 1152/04) seem longer than all the others and, in contrast, that associated with GU (*Hp* 499/02) is the shortest one and presents a, more pronounced, spiral

morphology. Moreover, our preliminary data on TEM analysis indicate the presence of more abundant and apparently more organized flagella in the GU-associated strain *Hp* 499/02, in contrast to the NUD control strain, *Hp* 655/99 (Figure 2).

References

1. Ubukata H., *et al.*, Gastric Cancer, 14:4, 2011.
2. Vitoriano I., *et al.*, PLoS ONE, 6:e26265, 2011.

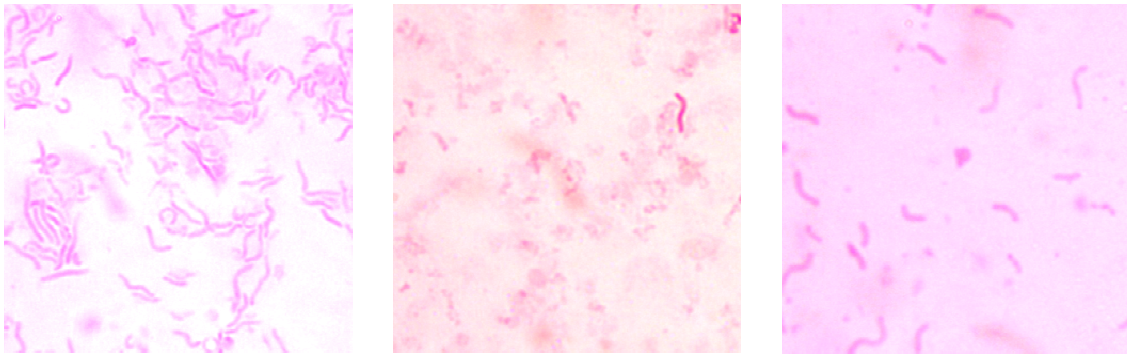

Figure 1. Leifson staining of the most representative paediatric *H. pylori* strains associated with DU (strain 1152/04), GU (strain 499/02), and gastritis (strain 655/99), showing marked morphological differences between strains.

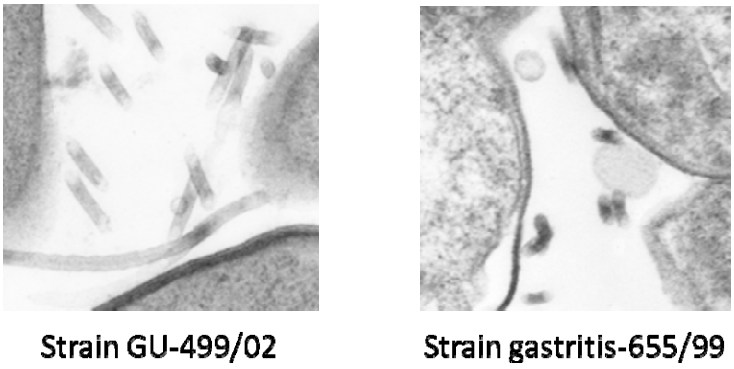

Figure 2 - Preliminary data on TEM analysis of paediatric *H. pylori* strains associated with GU (strain 499/02) and gastritis (strain 655/99), showing a marked difference between strains regarding the number of flagella. Images captured with a JEOL 100SX electron microscope.

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