

## ***Helicobacter* is preserved in yeast vacuoles! Does Koch's postulates confirm it?**

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### **Abstract**

The manuscript titled "Vacuoles of *Candida* yeast behave as a specialized niche for *Helicobacter pylori* (*H.*

*pylori*)" not only has not been prepared in a scientific manner but the methodology used was not adequate, and therefore the conclusion reached was not correct. First of all, "yeast" is a broad terminology covering a great number of genera and species of unicellular micro-organisms. The authors should have defined the organism with its binary scientific name. This measure would allow experiment reproduction by the scientific community. Moreover, the criteria established by Robert Koch to identify a specific microorganism or pathogen was not adopted in the methodology used. Regarding the methodology applied, use of the chicken egg-yolk (IgY) antibody and PCR of the apparently tainted yeast population to prove *H. pylori* existence in the yeast vacuoles might be main factors for their wrong conclusions. Bacterial tropism toward yeast extract is a known phenomenon, and yeast extract is one of the main ingredients in culture media. Their internalization through phagocytosis or similar pathways does not seem possible or practical because of the thick and cellulosic yeast wall. While the small size of yeast cells does not support their ability in harboring several *H. pylori*, other observations such as inefficiency of anti-fungal therapy as anti-*Helicobacter* therapy strongly reject the conclusion reached by the above-mentioned article.

**Key words:** *Helicobacter pylori*; Yeast; *Acanthamoeba castellanii*; Koch's postulates

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**Core tip:** An article titled "Vacuoles of *Candida* yeast behave as a specialized niche for *Helicobacter pylori*," was published in the *World Journal of Gastroenterology* (2014; 20: 5263-5273). This "letter to the editor" is intended to demonstrate the shortcomings of that article related to the methodologies applied, the conclusion reached and the outcomes presented.

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## TO THE EDITOR

We read with interest the review article titled "Vacuoles of *Candida* yeast behave as a specialized niche for *Helicobacter pylori* (*H. pylori*)" by Siavoshi *et al*<sup>[1]</sup>. Based on the other research articles by the same authors, this review article concludes that: *H. pylori* are able to penetrate into the "*Candida*" yeast, multiply inside its vacuoles, and potentially transfer into the daughter cells when the yeast cells are dividing. They hypothesized that the yeast can act as the vehicle in transferring *Helicobacter* into human. They have included figures demonstrating the presence of several *Helicobacter* in *Candida* yeast cells. For the following reasons, we do not agree with their methodology and conclusion.

Yeast is a general word that describes a great amount of genera and species of unicellular micro-organisms, including beverage yeasts, baker's yeasts, fruit yeasts, food yeasts, industrial yeasts, environmental yeasts, pathogenic yeasts, *etc.* A binary scientific name must be indicated in scientific manuscripts (*e.g.*, *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Candida albicans*, *Candida tropicalis*, *Candida krusei* *etc.*). They can be differentiated from each other by simple biochemical tests. Without their identifications, other scientists will not be able to reproduce these outcomes in their own laboratories.

Koch's postulates requires relying on the defined standard methods and criteria. Culturing and sub-culturing micro-organisms in the required culture media is one of these criteria. The authors have not practiced these postulates due to the fastidious nature of *H. pylori*.

Authors have used IgY "chicken egg-yolk antibodies" against *H. pylori*, to demonstrate the presence of *H. pylori* in yeast cells. However, IgY is not accurate enough for such an experiment. Human serum IgG antibodies of *H. pylori* from positive duodenal ulcer patients could be a more reliable tool than chicken IgY. *Campylobacter* which share similar antigenic cross reaction with *H. pylori* is present in the normal flora of poultry and chicken gut. So, antibodies produced in chicken eggs cannot be accurate enough.

Bacterial tropism toward yeast extract is a known phenomenon and has been reported by others<sup>[2,3]</sup>. This is a natural tropism of living bacteria toward food, and is not a novel finding. Similarly, to *Acanthamoeba* protozoa<sup>[4,5]</sup> histological smears from different body fluids demonstrate their co-existence. Such observation would not be possible if the *Helicobacter*

was inside vacuoles embedded in the yeast cells! Yeast extract is one of the main ingredients in the culture media.

Internalization of food particles or bacteria into a eukaryotic cell may adopt different pathways such as phagocytosis and receptor- or transporter-mediated transportation. Bacteria mostly enter larger cells with soft and flexible membranes, such as the white blood cells (through phagocytosis). The thick and cellulosic nature of the yeast cell wall limits its phagocytic ability and the direct entrance of large particles. On the other hand, such ability can be easily acceptable in the case of protozoa and amoeba with their pseudopods and softer membranes. Cells that are specialized in bacteria internalization and ingestion are known as "Bacterivores". *Helicobacter* must have magic ability in passing through the thick cellulosic cell wall of the yeast (like the internationally-known magician "David Cooperfield" who appeared to pass through the Great Wall of China only to have the reality demonstrated to be a trick of the camera in the show). As the larger size of *Acanthamoeba* indicates, these cells can internalize several *Helicobacter*. Therefore, ingestion of *Helicobacter* by *Acanthamoeba* seems more logical than their ingestion by yeast cells.

Antibiotics are very slow in their entry into the yeast cells compared to their entrance into the *Acanthamoeba*. Therefore, it is very hard to imagine complete eradication of the *Helicobacter* by antibiotics if they are internalized into the *Candida* cells. Such eradication will not be difficult, as it happens in infected patient treatments, if the *Helicobacter* are located on the surface of the yeast cells.

Moreover, the prevalence of *Helicobacter* infection should be higher in females than males and patients with human immunodeficiency virus, due to the higher yeast infection rates in these two groups. In fact, the situation is the other way around<sup>[5-7]</sup>.

Interestingly, several articles in the literature have shown similarity in prevalence of *Acanthamoeba* in drinking water sampled from different geographical locations and the prevalence of *H. pylori* in patients<sup>[8-19]</sup>. While we cannot observe such overlap between yeast and *H. pylori* incidences, it is more logical to believe that yeast cannot be a reservoir of *H. pylori* but that *Acanthamoeba* can play such a role.

Moreover, anti-*Helicobacter* therapies, including anti-fungal drug usage, have not shown statistically significant difference upon comparison with no treatment<sup>[17,20]</sup>.

The above arguments reject the idea of yeast harboring *H. pylori* in its vacuole. In theoretical analysis, internalization of *H. pylori* by yeast cells can be out of two possibilities: The *Helicobacter* should cross the yeast external wall and then cross the specific vacuole membrane where they will be trapped even if they could multiply, or they should be internalized *via* phagocytosis to end up in a digestive vacuole and be digested. If we imagine that *H. pylori*

may infect the yeast cells in a way comparable to viral infection, it will be an exception for bacterial pathogenesis and the propagation mechanism. Such an idea needs an accurate and reliable study, however. The positive PCR reaction stated in Siavoshi *et al.*<sup>[1]</sup>'s article, ought to have stemmed from the *Helicobacter* located on the surface of the yeast cells.

In conclusion, with all respect to the authors of the above-mentioned review article, we believe that the interpretation of their observation is totally wrong. While being aware of the symbiotic nature of *H. pylori* and *Candida* yeast and the close relationship between these two organisms, they went wrong on internalization and survival of bacterial colonies inside yeast vacuoles.

Our initial response to the Siavoshi *et al.*<sup>[1]</sup> article was published as an independent manuscript<sup>[18]</sup>. We ought to admit that this was not an appropriate approach to make our thought and beliefs known. We were directed to express our comments in the form of the present "Letter to the Editor". We hope this manuscript will clarify the issue and set the record straight.

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