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Comment on "Effect of biofilm formation by clinical isolates of *Helicobacter pylori* on the efflux-mediated resistance to commonly used antibiotics"

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Abstract

Attaran *et al*^[1] have recently shown that decreased susceptibility of established *Helicobacter pylori* (*H. pylori*) biofilms to specific antibiotics, was associated with the overtly enhanced transcription of two efflux pump genes, *hp1165* and *hefA*, involved in specific resistance to tetracycline and multiple antibiotics, respectively. Apart from antibiotic exposure, secretion of multiple antimicrobial peptides, such as human β -defensins ($h\beta$ Ds), by the gastric epithelium upon *Hp* challenge, may act as early triggering events that positively impact biofilm formation and thus, antibiotic resistance. In this regard, we undertook genomic transcriptional studies using *Hp* 26695 strain following exposure to sublethal, similar to those present in the gastric niche, concentrations of $h\beta$ Ds in an attempt to provide preliminary data regarding possible mechanisms of immune evasion and selective sensitivity of *Hp*. Our preliminary results indicate that $h\beta$ D exposure ignites a rapid response that is largely due to the activation of several, possibly interconnected transcriptional regulatory networks – orignons - that ultimately coordinate cellular processes needed to maintain homeostasis and successful adaptation of the bacterium in the gastric environment. In addition, we have shown that both antibiotic and $h\beta$ D resistance are mediated by dedicated periplasmic transporters, including the aforementioned efflux pump genes *hp1165* and *hefA*, involved in active export of antibiotics from the cell membrane and/or, as recently suggested, substrate sensing and signalling. Furthermore, it

appears that sublethal doses of h β Ds may enhance biofilm formation by the sustained expression of, mainly, quorum sensing-related genes. In conclusion, we provide additional data regarding the role of specific innate immune molecules in antibiotic cross-resistance mechanisms that may deepen our understanding in the context of the development of novel eradication regimens.

Key words: *Helicobacter pylori*; Human β -defensins; Biofilm; Antimicrobial resistance

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Core tip: In the course of *Helicobacter pylori* infection, epithelium-derived human β -defensins may act as early triggering signals that induce biofilm formation and enhanced expression of antibiotic resistance genes, regardless of prior antibiotic exposure.

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TO THE EDITOR

Attaran *et al*^[1] concluded that, in biofilm-forming populations, overexpression of two efflux pump genes, *hp1165* and *hefA*, conferring resistance to tetracycline and multiple antibiotics respectively, may favor reduced antibiotic susceptibility of *Helicobacter pylori* (*H. pylori*) *in vivo*.

Further to antibiotic exposure, additional, epithelial-derived molecules may function as triggering signals during the dynamic *H. pylori* interaction with the gastric mucosa, provoking overexpression of efflux pumps that in turn, regulate the bacterium's biofilm-producing capacity and promote its virulence. Several studies have unraveled the role of constitutive and/or induced expression of human β -defensins (h β Ds)1 - 4 in the bacterium's adaptation in the human stomach and *H. pylori* -related pathologies^[2,3].

In this respect, we performed whole genome transcriptome analyses (competitive genomic RNA/RNA hybridisations) using *H. pylori* -specific microarrays based on the *Hp* 26695 and J99 genome sequences and annotation available at the time. Briefly, *H. pylori* 26695 strain was exposed to sublethal, similar to those encountered at the gastric epithelium concentrations of h β Ds, in an attempt to identify possible mechanisms of *H. pylori* immune escape and clarify their role in biofilm development *in vitro*. Our preliminary results have identified profound changes in the transcriptional profile

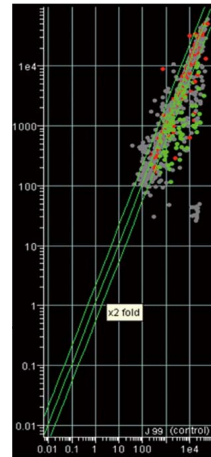


Figure 1 Representative scatter analysis of the general patterns of *H. pylori* genomic response to human β -defensin 3 (h β D3) revealed by transcriptional profiling. Scatter plots (log₂ ratio) of average normalized intensities representing Cy5-red channel versus Cy3-green channel are shown for experiments in the presence of sublethal concentrations of h β D3 compared with “control” conditions (no h β D3). Differential expression of a given gene is reflected by deviation from the central diagonal line. The upper diagonal defines ≥ 2 -fold up-regulation and the lower one defines ≥ 2 -fold down-regulation.

of *H. pylori* 26695 demonstrated by the induction or suppression of multiple gene components of distinct regulatory and signaling cascades activated as a result of environmental stress (Figure 1, unpublished data). Overall, the vast majority of genes affected, encoded components of the cell wall stimulon, possibly as means to prevent h β D-specific binding and proper immune recognition, or could be further assigned to certain origins, essential for colonisation of the gastric niche and long-term adaptation, intracellular metal homeostasis and urease activation that largely determine *H. pylori* pathogenicity. Apart from the marked induction of *hp1165* and *hefA*, also reported by the authors^[1], several other genes coding for transmembrane ABC transporters (*glnP*, *dppF*, *hp1458*, *hp1486*), efflux proteins (*hp0656*, *hp0946*), multidrug and toxic extrusion proteins were found to be significantly up-regulated, thereby indicating their prominent role in the cellular response to h β Ds challenge, membrane detoxification and maintenance of osmotic balance.

Interestingly, enhanced biofilm production by *Hp* 26695, observed in our studies upon exposure to sublethal concentrations of h β D1 and h β D3, was primarily attributed to the down-regulation of *metK* and *luxS* genes, involved in synthesis of quorum-sensing autoinducer-2, in accordance to previously published data^[4,5].

Collectively, our results indicate that sublethal doses of epithelial-secreted antimicrobial peptides such as h β Ds, may select co-resistance to antibiotics commonly used in *Hp* eradication therapies and *vice versa*, considering that they provoke the activation of shared, contact-dependent signaling networks, including efflux pumps. Furthermore, it appears that h β Ds may independently act as triggering stimuli

promoting biofilm formation *in vivo* which in turn, accounts, at least partly, for the observed failure of eradication regimens and the establishment of *H. pylori* -related chronic inflammation.

Given the complexity of *H. pylori* -host epithelial crosstalk aforementioned data warrant further investigation to achieve the development of successful anti-biofilm strategies that will ultimately re-enforce our therapeutic options mainly towards eradication of *H. pylori* -related resistance. Furthermore, future research focus on the polymorphic variability of the human genome that directly affects epithelial dynamics of h β Ds expression may reveal important correlation patterns between *H. pylori* pathogenesis, including biofilm formation, and individual disease susceptibility.

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