Clarithromycin resistance by *Helicobater pylori*: a review

Helicobacter pylori is a gram-negative bacteria that is a significant pathogen in humans. It commonly causes stomach ulcers, among other gastrointestinal diseases, and when left untreated can lead to stomach cancer. Concerningly, *H. pylori* exhibits high levels of resistance to antibiotics, including clarithromycin, making it a difficult pathogen to treat. Currently, to combat potential resistance to common antibiotics a cocktail of antibiotics is prescribed. However, this is only a short term solution as resistance of pathogens continues to evolve. The mechanism of resistance must be properly understood to find better treatments while avoiding further antibiotic resistance. Current research indicates that efflux pumps found in high levels in biofilm producing *H. pylori* play a key role in this issue.

An abundance of research supports that there is a correlation between clarithromycin antibiotic resistance and biofilm production. For instance, out of a group of antibiotics, resistance to clarithromycin was the highest among biofilm producing cells (Kryzyzek et al., 2022). Similarly, *H. pylori* biofilm producing cells were more resistant to clarithromycin than planktonic cells (Yonezawa et al., 2013). Furthermore, Fauzia et al. quantified biofilm production as strong, medium, or weak. They observed that the strong biofilm producing cells were overwhelmingly more resistant to clarithromycin than the weak biofilm producing cells. Increased biofilm density was correlated with increased resistance to clarithromycin (2020). Overall, the research is in agreement that biofilm production contributes to the resistance of *H. pylori* to clarithromycin.

Various lines of evidence indicate that the increase in resistance to clarithromycin is due to efflux pumps within biofilm cells. However, it must first be established that efflux pumps are present at higher levels in biofilm cells. Expression of efflux pump genes *hefA* and *hp1165* was found to be higher in biofilm cells after evaluation with real time PCR (Attaran et al. 2017). Hashemi et al., also found that expression of *hefA* upregulated efflux pump genes (2019). Similarly, by using real-time PCR to study CDNA and broths of cell culture, Yonezawa et al. found that there were high levels of efflux pump gene expression in *H. pylori* biofilm cells. Interestingly, out of all the antibiotics studied, this result was specific to clarithromycin (2019). Overall, across the research it has been established that efflux pump genes are upregulated in biofilm cells.

With the understanding that efflux pumps are upregulated in biofilm cells, the possibility that efflux pumps are responsible for *H. pylori* resistance must be considered. By applying varying concentrations of an efflux pump inhibitor drug, Hirata et al. found that high efflux pump activity was tied to high tolerance to clarithromycin (2010). Additional research indicates that efflux pumps do contribute to resistance, but that they are just one possible component of a complex system. For example, Fasafi et al. cultured resistant *H. pylori* from hospital patients and observed that 43% of them had efflux pump activity that contributed to resistance (2009). Therefore, efflux pumps do contribute to resistance, but they are not the only mechanism at work. One study found that *H. pylori* resistance was due to cytotoxic genes rather than efflux pump genes (Bachir et al. 2018). Matta et al. found an additional source of resistance in *H. pylori.* A decrease in binding affinity was the mechanism of resistance, rather than efflux pump production (2023). In combination, this research depicts that efflux pumps are a

mechanism of antibiotic resistance in *H. pylori*, in addition to other factors which are strain, condition, and antibiotic dependent.

In sum, the research establishes three main points. First, biofilm production is correlated with increased antibiotic resistance to clarithromycin in *H. pylori*. Second, biofilm cells of *H. pylori* have a higher level of efflux pump production than non-biofilm producing vegetative cells. Third, efflux pumps are one component that contribute to the complex system of clarithromycin resistance in *H. pylori*. In combination with each other, these points suggest that the increased efflux pump activity in biofilm cells is what causes them to exhibit higher levels of resistance. A study used biofilm assays, plating with antibiotics, and sequencing of resistant colonies to confirm the relationship between biofilm production, efflux pumps, and resistance (Hou et al. 2022).

Moving forward, it is important to continue to improve our understanding of the mechanism of antibiotic resistance in *Helicobacter pylori*. In doing so we can improve upon and create new ways to treat this common, and serious, infection without it developing into stomach cancer. Further research on the development of efflux pump inhibitor drugs, as an alternative to antibiotics is particularly useful. Efflux pump inhibitors, like the ones used by Hirata et al. (2010) could be used as a line of defense that decreases the amount of different antibiotics prescribed for *H. pylori*. With further research, antibiotics may even be able to be replaced completely. Researching the efficacy and side effects of efflux pump inhibitors compared to that of an antibiotic cocktail would be an appropriate next step.

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