Sequence analysis of levofloxacin resistance-associated genes – *gyrA* and *gyrB* in treatment-naïve *Helicobacter pylori* patients from Malaysia

Heng Kang Ng[1], Khean Lee Goh[2], Boon Pin Kee[1], Kek Heng Chua[1] and Suat Moi Puah[1*]

[1]*Department of Biomedical Science, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.*

[2]*Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.*

Background

Levofloxacin is a fluoroquinolone antibiotic used in a salvage therapy to treat the infection of gastric pathogen *Helicobacter pylori* when the first-line therapy antibiotic, clarithromycin failed. The overall prevalence of primary levofloxacin-resistance of *H. pylori* was reported as 14% worldwide [1]. Mutations detected in the *gyrA* and *gyrB* genes, especially the quinolone resistance-determining region were reported to have an association with the resistance of levofloxacin [2].

Objective

This study aimed to identify variants in the levofloxacin-resistance associated genes – *gyrA* and *gyrB* of *Helicobacter pylori* in Malaysian patients via sequencing.

Methodology

Genomic DNA was extracted from *H. pylori* positive biopsy samples (n=50).

↓

Full-length amplification of *gyrA* and *gyrB* genes using polymerase chain reaction and subject to Sanger sequencing.

↓

ClustalW alignment was performed among DNA sequences with *H. pylori* reference strain (ATCC 26695) to search for DNA variants.

↓

DNA variants were translated into amino acid sequences and followed by *in silico* docking using HPEPDOCK webserver to predict their relative binding affinity towards levofloxacin (PDB ID: 3rae) [3].

↓

Comparison between the docking scores of wild type and mutant was analysed.

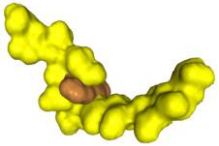

Figure 1. Surface structure between the binding of amino acid sequences and levofloxacin predicted by *in-silico* blind protein-peptide molecular docking using HPEPDOCK webserver.

Results & Discussion

Table 1. Variants and their respective frequencies detected in *gyrA* and *gyrB* genes.

Variants	*gyrA*	*gyrB*
Reported mutations	G468E (80%) P484Q (76%) A594D (16%)	R484K (26%) D481E (20%)
Novel polymorphisms	V741I (80%) S492A (62%)	S240A (16%)

Footnote: Reported mutations were variants reported by previous literatures. Novel polymorphisms were variants detected in current study and were not reported by previous literatures.

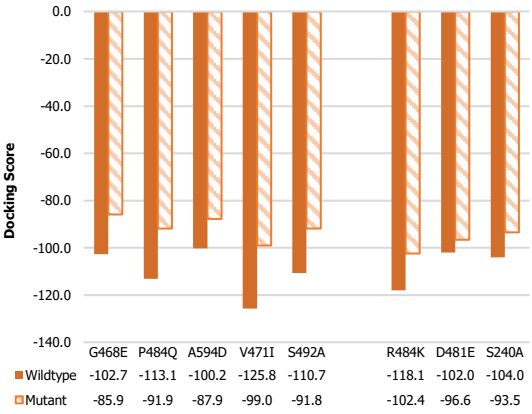

Figure 2. Comparison of docking scores predicted by HPEPDOCK between mutants detected in *gyrA* and *gyrB* genes and their respective wild types.

In *gyrA* gene, 3 mutations **(G468E, P484Q, A594D)** and 2 novel polymorphisms **(V471I, S492A)** docking scores decreased from 16.36% to 21.25%.

In *gyrB* gene, 2 mutations **(R484K, D481E)** and 1 novel polymorphism **(S240A)** docking scores decreased from 5.23% to 13.23%.

↓docking scores can signify ↓ binding affinities between the levofloxacin binding sites on the gyrase protein hence affecting their efficiency.

Conclusion

The novel variants identified in the *gyrA* and *gyrB* genes might be attributed to levofloxacin resistance in *H. pylori*, therefore, warrant further investigation.

Acknowledgment

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References

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