SINGHEALTH · DUKE-NUS SCIENTIFIC CONGRESS
17 - 18 SEPTEMBER 2021

REVOLUTIONISING MEDICINE - THE NEW FRONTIER

BSTR-00054

Development of a real-time PCR assay for simultaneous detection of Helicobacter pylori and genetic mutations associated with clarithromycin resistance

Jiang Boran,[1] Lee Hui Zi,[1] Heng Ying Xuan,[1] Koo Seok Hwee,[2] Lily Ng Siew Yong,[1] Tan Thean Yen[1]

1. Microbiology Laboratory, Changi General Hospital
2. Clinical Trials & Research Unit, Changi General Hospital

Aims

Helicobacter pylori (HP) has been associated with symptoms ranging from dyspepsia, peptic ulcer disease, to gastric cancer. Due to the potential severe consequences of this infection, accurate diagnosis, including susceptibility testing is important for successful disease management. The aim of this study is to develop a real-time PCR assay that can simultaneously detect H. pylori infection and screen for point mutation in the 23S rRNA gene, which is responsible for clarithromycin (CLA) resistance.

Methodology

A Taqman probe based real-time PCR protocol, followed by melting curve analysis, was developed and evaluated in this study. Primers flanking a 173bp region of the 23S rRNA gene were used for the target amplification. Taqman probe was designed to cross over the mutation hotspots, e.g. A2142G, A2143G, to detect point mutations in the melting stage. (Figure 1a) Melting temperature (Tm) of double strands of normal HP strain and taqman probe is higher than Tm of double strands of mutant HP strain and taqman probe, as the taqman probe was designed as perfect match with normal strain. (Figure 1b)

Figure 1a

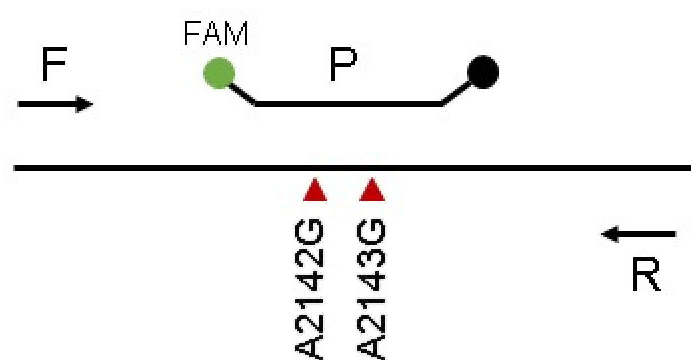
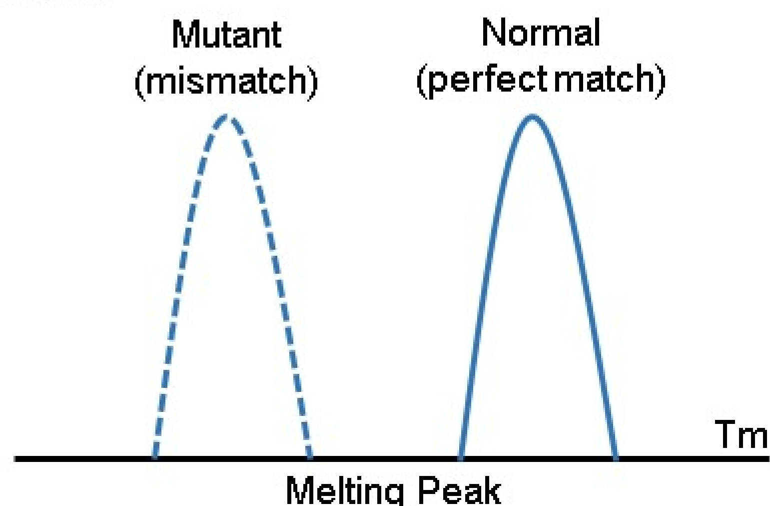


Figure 1b



Results

A total of 343 gastric tissue samples were cultured for H. pylori and concurrently tested by this PCR assay. 237 samples were H. pylori positive by both PCR and culture. 89 samples were H. pylori negative by both PCR and culture. 17 samples were H. pylori positive by PCR, but negative by culture. (Table 1a)

For susceptibility testing, genotype results were compared with phenotypic results. In phenotypic CLA sensitive group (n=174), melting peak could correctly assign 100% samples as sensitive genotype. In phenotypic CLA resistant/intermediate group (n=44), melting peak could correctly assign 36 samples as resistant strains, equal to 82% accuracy. (Table 1b)

Table1a

	Culture Results		
PCR Results	Cultural Positive	No Growth	Grand Total
HP DNA Det	237	17	254
HP DNA ndet		89	89
Grand Total	237	106	343

Table1b

	Culture Phenotypic Results			
Genotype Results	Sensitive	Resistant	Intermediate	Grand Total
Sensitive	174	7	1	182
Resistant		34	2	36
Grand Total	174	41	3	218

Conclusion

This PCR assay provides an accurate method to detect H. pylori infection and simultaneously allows for culture-independent clarithromycin susceptibility testing. The advantage of molecular method is rapidity and waive stringent aerial conditions to keep the organism viable. However, this PCR assay cannot be used for susceptibility testing of all antibiotics and does not detect resistance caused by mutations other than those in the probe region.

SingHealth MedSG200 Defining Tomorrow's Medicine

ORGANISER
SingHealth DukeNUS ACADEMIC MEDICAL CENTRE

SECRETARIAT
SingHealth Academy