Analysis of allelic variants of \( rdxA \) associated with metronidazole resistance in \textit{Helicobacter pylori}: detection of common genotypes in \( rdxA \) by multiplex allele-specific polymerase chain reaction

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\textbf{ABSTRACT.} Resistance to metronidazole (Mtz) in \textit{Helicobacter pylori} is a major problem worldwide, especially in developing countries. Alterations in Mtz nitroreductase enzymes, such as oxygen-insensitive NADPH nitroreductase (RdxA) and NADPH flavin oxidoreductase (FrxA), are the major contributing factors for this resistance. In this study, \( rdxA \) and \( frxA \) were amplified, sequenced, and analyzed in 34 Mtz-resistant \( H. pylori \) isolates (MIC \( \geq 8 \) \( \mu \)g/mL) using multiple allele-specific polymerase chain reaction (MAS-PCR); this method was developed to target the most common genotypes of \( rdxA \) in \( H. pylori \). In this study, the \( rdxA \) and \( frxA \) genes in Mtz-resistant \( H. pylori \) strains
displayed a large number of point mutations. The \textit{rdxA} and \textit{frxA} genes of Mtz-resistant clinical isolates showed a higher percentage of missense mutations (97.1 and 78.6\%, respectively) compared to 26695 reference strains; additionally, missense mutations were more common than frameshift (20.6 and 32.1\%) and nonsense mutations (8.8 and 10.7\%, respectively) in these genes. The most common missense mutations in \textit{rdxA} were D 59 N (94.1\%), T 31 E (88.2\%), and R 131 K (85.3\%). The most common missense mutations in \textit{frxA} were F 72 S (57.1\%), G 73 S (57.1\%), and C 193 S (53.6\%). The developed MAS primers, specific to position 175 and 392 of \textit{rdxA}, successfully amplified the common alleles and distinguished the variants. MAS-PCR could be a useful tool for epidemiological studies of \textit{H. pylori}, associated with Mtz resistance. \textit{rdxA} variants must be screened in order to ensure the effectiveness of Mtz-based \textit{H. pylori} therapies in developing countries.

\textbf{Key words:} \textit{Helicobacter pylori}; Metronidazole; \textit{rdxA} gene; \textit{frxA} gene; MAS-PCR