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T2182C Mutation in 23S rRNA Is Associated with Clarithromycin Resistance in *Helicobacter pylori* Isolates Obtained in Bangladesh

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Twelve clarithromycin-resistant (MIC, ≥1 μg/ml) *Helicobacter pylori* isolates were analyzed for point mutations in the 23S rRNA gene. Sequence analysis of all of the resistant isolates revealed a T-to-C transition mutation at position 2182. Transformation experiments confirmed that a single T-to-C transition mutation at position 2182 is associated with clarithromycin resistance.

Eradication of *Helicobacter pylori* infection by treatment with two antimicrobials (clarithromycin and amoxicillin or metronidazole) and a proton pump inhibitor is recommended by various consensus groups (5, 13). The prevalence of antimicrobial susceptibility of *H. pylori* varies with geographical regions, and clarithromycin resistance is the major cause of treatment failure (3, 5, 20). Alteration in either one or both copies of the *H. pylori* 23S rRNA gene is associated with resistance to clarithromycin, and the mechanism is a point mutation (an adenine-to-guanine transition at either position 2142 or 2143 or an adenine-to-cytosine transversion at position 2142) of the 23S rRNA (2, 8, 15, 18, 19, 23). However, a T2182C mutation has also been proposed to be associated with clarithromycin resistance (10). In the present study we have examined clarithromycin-resistant (Cla^r) *H. pylori* (CRHP) isolates and characterized a T2182C mutation involved in clarithromycin resistance.

Twelve CRHP isolates and three clarithromycin-susceptible *H. pylori* (CSHP) strains isolated from individual pretreatment patients attending the Dhaka Medical College hospital during 1999 to 2001 for routine endoscopy were examined. The bacteria were grown in brain heart infusion (BHIB) agar with 7% sheep blood and incubated at 37°C in 5% O_2–10% CO_2–85% N_2 for 3 to 6 days. A pure culture from a single colony was stored at −70°C until further study.

The MICs of clarithromycin for the isolates were determined by the agar dilution method (MIC breakpoint, ≥1 μg/ml) and by the E-test strip (AB Biodisk, Solna, Sweden) as described elsewhere (11, 12, 16). All tests were repeated twice, and CSHP strain 26695 (MIC, 0.032 μg/ml) was used as a control. Chromosomal DNA of the isolates was extracted by the cetyltrimethylammonium bromide method. A 1,300-bp fragment of the 23S rRNA (bp 1627 to 2926; GenBank accession number U27270) was amplified with primers Cla1 (5′-GGCTCTTTGAGTCCTTTTAGGAC-3′) and Cla4 (5′ GCATTACTGCGCTCACACAT-3′) as described previously (21). The PCR product was purified with a Microcon centrifugal filter device (Millipore Corporation, Bedford, Mass.), and a cycle sequencing reaction was performed with the same primers. DNA sequencing was performed under standard conditions with an ABI PRISM 310 automated sequencer (Perkin-Elmer Applied Biosystems, Foster City, Calif.). DNA sequence editing and analysis were performed by DNASTAR package 5.06 (DNASTAR Inc.) software. In order to confirm the role of the T2182C mutation, the 1,300-bp PCR fragment from CRHP isolate Gj50 (MIC, 4 μg/ml) was transformed to CSHP strain DM26B as described earlier (19). Briefly, exponentially growing CSHP strain DM26B cells were inoculated and grown on cold BHIB agar plates for 5 h; 1 μg of donor DNA (1,300-bp PCR fragment from CRHP isolate Gj50 containing the site-specific mutation) was added to the bacterial lawn. Cells were grown for 3 days, and Cla^r transformants were selected on BHIB agar plates containing clarithromycin (1 μg/ml). Isolated single colonies were used for MIC determination and sequencing.

The MIC for 25% (3 of 12) of the Cla^r isolates was 1 μg/ml,

TABLE 1. MICs determined by E-test and agar dilution and mutation profiles of CRHP and CSHP isolates in Bangladesh

Isolate	Phenotype[b] and clarithromycin MIC (μg/ml)		Presence of mutation at position:			
	E-test	Agar dilution	1821 (A to G)	1826 (G to A)	1830 (T to C)	2182 (T to C)
DM26B	S (<0.016)	S (<0.125)	−	−	−	−
DM19B	S (<0.016)	S (<0.125)	+	−	+	−
DH36	S (<0.016)	S (<0.125)	+	−	−	−
DH182	R (1.0)	R (1.0)	+	−	−	+
DM1A	R (1.0)	R (1.0)	+	+	+	+
Gj49	R (1.0)	R (1.0)	−	−	−	+
DH181	R (2.0)	R (2.0)	−	−	−	+
Gj29	R (2.0)	R (2.0)	−	−	−	+
DM13B	R (2.0)	R (2.0)	−	−	−	+
DH218	R (2.0)	R (2.0)	+	+	+	+
DH51	R (4.0)	R (4.0)	+	+	+	+
DH64	R (4.0)	R (4.0)	+	+	+	+
DH142	R (4.0)	R (4.0)	+	+	+	+
Gj48	R (4.0)	R (4.0)	+	+	+	+
Gj50	R (4.0)	R (4.0)	−	−	−	+
$DM26B^{Ra}$	R (4.0)	R (4.0)	−	−	−	+

[a] Transformant strain.
[b] S, susceptible; R, resistant.

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FIG. 1. Nucleotide sequence of the 1,300-bp amplicon from the 23S rRNA gene from a resistant *H. pylori* isolate. The arrow indicates a single point mutation, that is, a T2182C transition. The arrows at the 5′ and 3′ termini indicate primer sequences.

for 33.3% (4 of 12) it was 2 μg/ml, and for 41.7% (5 of 12) it was 4 μg/ml (Table 1) (7, 16). The nucleotide sequences of all CRHP and CSHP isolates and transformants were compared with known sequences of the 23S rRNA (GenBank accession numbers U27270, AE000641, and AE001553). None of the CRHP or CSHP isolates had the expected mutation at positions 2142 and 2143 (4, 6, 24). Rather, all of our CRHP isolates and Cla^r transformant $DM26B^R$ had a single mutation, the T-to-C transition at position 2182 (Fig. 1 and Table 1). However, one to three or no base substitutions at positions 1821, 1826, and 1830 were also observed in some isolates compared to the *H. pylori* 23S rRNA sequence (GenBank accession number U27270), as shown in Table 1. The MIC for the transformants was equal to that for donor CRHP strain Gj50.

Early studies have demonstrated that clarithromycin resistance is attributed to point mutations mainly at positions 2142 and 2143 (cognate *Escherichia coli* positions, 2058 and 2059) within the peptidyltransferase-encoding region in domain V of the 23S rRNA, and these mutations confer resistance by altering the macrolide binding target (19, 23). The prevalence of mutations among the CRHP strains varies in different parts of the world (1, 9, 10, 17, 22, 23). In our study, all 12 of the Cla^r strains, for which the MICs ranged from 1 to 4 μg/ml, showed no mutation at position 2142 or 2143; however, a mutation similar to that in the present study has been identified in a study by Kim et al. in which 33.3% of the isolates with high levels of clarithromycin resistance (MIC range, 16 to ≥64 μg/ml) had the T2182C mutation (10). Recently, it was reported that a T-to-C mutation at position 2717 was also associated with low levels of clarithromycin resistance (MIC = 1 μg/ml) and accounted for 58.3% of the clarithromycin resistance found (6). Single base substitutions at three different positions of the 23S rRNA (within positions 1627 to 2926) along with or without the mutation at position 2182 were also observed in some isolates. As these substitutions were observed in both CSHP and CRHP isolates, they might not have any role in resistance to clarithromycin. However, these substitutions might indicate the identity of Asian isolates as they were absent from Western isolates. Although the underlying mechanism of this phenomenon is not known, the T2182C mutation, which falls within domain V of the 23S rRNA (14), seems to be associated with low levels of clarithromycin resistance in the *H. pylori* isolates studied as (i) all of the CRHP isolates present a single point mutation at position 2182 that is not present in the CSHP strains, (ii) the expected mutation at position 2142 or 2143 was absent in all of the CRHP isolates, (iii) the resistance phenotype can be transformed by natural transformation of resistance determinants to CSHP isolates, and (iv) the clarithromycin MIC was the same for the transformants and isolates with the resistance phenotype, and they had the same T2182C mutation. Further studies are essential to understand the molecular mechanism of macrolide-resistant *H. pylori*.

Nucleotide sequence accession numbers. The sequences obtained during this study have been deposited in the GenBank database under accession numbers AY598832 to AY598847 for strains DM26B, DM19B, DH36, DH182, DM1A, Gj49, DH181, Gj29, DM13B, DH218, DH51, DH64, DH142, Gj48, Gj50, and $DM26B^R$, respectively.

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