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T2182C Mutation Is Not Associated with Clarithromycin Resistance in *Helicobacter pylori*

In a recent article, Khan et al. described a new mutation in the 23S rRNA gene, T2182C, conferring clarithromycin resistance to *Helicobacter pylori* (1). An A-to-G mutation at either position 2142 or position 2143 and an A-to-C mutation at position 2142 in the 23S rRNA gene have been shown to confer resistance to clarithromycin in *H. pylori* (5). In 2000, we isolated a clarithromycin-resistant (Cla^r) strain from a French patient (MIC $>$ 256 μg/ml). Sequencing the 23S rRNA gene revealed a single T2182C mutation. To confirm the role of the T2182C mutation, we used the PCR fragment containing this mutation to transform a susceptible receptor strain. We obtained Cla^r transformants. These results were accepted for oral presentation at the 11th International Workshop on Campylobacter, Helicobacter and Related Organisms held in 2001 (3), but a final checking of the MIC determined by E-test revealed a mixed set of colonies. We observed small colonies in the susceptible area of the plate and big colonies in the resistant area. Subcultivation of isolated colonies provided two different clones. A Cla^s clone (MIC, 0.016 μg/ml) harbors the T2182C mutation and a wild-type sequence at positions 2142 and 2143. A Cla^r clone (MIC, 256 μg/ml) harbors both the T2182C mutation and an A2143G mutation. Randomly amplified polymorphic DNA patterns from the Cla^s and Cla^r isolates demonstrated that they are genetically identical. Coming back to the frozen gastric biopsy sample, we isolated 53 single colonies of *H. pylori*. Fifty-two colonies were from the Cla^s clone, and only one was from the Cla^r clone.

It is possible that Khan et al. were misled, like we were, by a mixed population of a Cla^s clone and a Cla^r clone. It is surprising that they mention in their article the use of "pure culture from a single colony [for] further study" (1).

T2182C is not a new mutation, and it has been already described in previous reports (2, 4, 6). In 1998, Wang et al. (6) first reported a Cla^r strain with A2143G and T2182C mutations. In vitro site-directed mutagenesis experiments suggested that this additional mutation is not associated with clarithromycin resistance (6). In 1999, Matsuoka et al. (4) reported the T2182C mutation in both sensitive and resistant colonies. In 2002, Kim et al. (2) described the same T2182C mutation for four Cla^r isolates, but they did not indicate that they worked on single-colony isolates.

Attention must be paid to experiments used as evidence that a mutation is responsible for resistance in *H. pylori*. Experiments have to be realized on several isolated colonies, since a mixture of strains can lead to a false determination of sequence. Transformation with PCR fragments could lead to resistant clones growing on antibiotic plates, since the error rate of *Taq* polymerase spontaneously generates random mutations containing the well-known A2142G or A2143G mutations (5).

The existence of a Cla^s isolate (20-222S) harboring a T2182C mutation in the 23S rRNA genes led to the conclusion that the T2182C mutation is not associated with clarithromycin resistance in *H. pylori*. The susceptible clone 20-222S is available on request.

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Christophe Burucoa*
Cédric Landron
Martine Garnier
Jean-Louis Fauchère
EA 3807 Faculté de Médecine et de Pharmacie de Poitiers, France
Laboratoire de Microbiologie A CHU Poitiers Poitiers 86021, France

*Phone: (33) 549 454991
Fax: (33) 549 443888
E-mail: c.burucoa@chu-poitiers.fr

Authors' Reply

We thank Burucoa et al. for their comments and concerns regarding the association of the T2182C mutation and clarithromycin resistance in *H. pylori*. The frequency of isolates resistant to a given drug varies geographically, but most clarithromycin resistance in *H. pylori* from the United States, Europe, and East Asia has been shown to be associated with point mutations, generally A to G or C at position 2142 or 2143 of the 23S rRNA gene (1, 5, 13, 14). Resistance due to mutations at other positions of the 23S rRNA gene, e.g., A2115G, G2141A, T2182C, G2224A, C2245T, T2289C, and T2717C (2, 4, 5, 6, 7), and resistance without mutations in the 23S rRNA have also been reported (2, 12, 15). In *H. pylori*, resistance to clarithromycin may be low or high level, and the molecular mechanism of low- and high-level resistance is poorly understood. However, a mutation at position 2143 is usually associated with different levels of resistance, with MICs ranging from ≤0.016 to ≥256 μg/ml, while MICs of strains with a mutation at position 2142 frequently were ≥64 μg/ml (3, 15). Mutations at other positions or no mutation in the 23S rRNA has also been associated with different levels of Cla^r.

We would like to respond to points raised by Burucoa et al. The association of the T2182C mutation and Cla^r was first reported by Kim et al. (7), and in our study we confirmed the earlier finding by transformation and sequencing of 23S rRNA from single-colony isolates. It is apparent from the letter that Burucoa et al. performed all of their initial analysis (isolation, MIC determination, DNA extraction, PCR, sequencing, and

transformation) with pooled isolates and subsequently realized that they were working with mixed isolates. It seems that they used only the E-test for MIC determination, and in our experience the determination of the MIC for low-level clarithromycin-resistant *H. pylori* needs careful reading of E-test results and confirmation by the agar dilution method. It is not clear at which concentration of clarithromycin they selected the transformants, and it seems that they did not confirm the 23S rRNA gene of the transformants by DNA sequencing. In most standard laboratories, the MIC determination is usually done several times to confirm the reproducibility of the result, and it is not clear whether Burucoa et al. got small and big colonies each time or only the last time. When they cultured the frozen biopsy sample, they identified 52 sensitive colonies and only one resistant colony. It might be important to analyze the 23S rRNA gene sequences of the sensitive and resistant isolates for confirmation of their finding. However, it was also not clear whether they indicated that they found the T2182C mutation in both copies of the 23S rRNA gene of their sensitive isolates.

Many laboratories, including ours, store *H. pylori* isolates as a pool and as propagation from a single colony, which is standard procedure for storage of *H. pylori* in order to avoid confusions of mixed infection. Burucoa et al. worked with only one high-level Cla^r isolate (they initially worked with mixed isolates and subsequently realized that fact but have not shown any data on the isolates from the frozen biopsy sample), and it might be difficult to come to a definite conclusion based on the results from one isolate. In contrast, we worked with 12 individual, pretreatment Cla^r isolates, and the MICs of clarithromycin for the isolates were determined by both the agar dilution and the E-test methods at least twice in every step where the results were reproducible. Finally, we confirmed the association of the T2182C mutation by transformation and DNA sequencing. It should be noted that our strains exhibit only low levels of Cla^r (MIC, 1 to 4 μg/ml) and that the T2182C mutation was absent in the sensitive strains tested ($n = 3$) (MIC, $\leq$0.016 to 0.5 μg/ml). Burucoa et al. rightly mentioned the error rate of *Taq* polymerase, but it is unusual to get the error each time at the same position.

We agree that some authors have shown that the T2182C mutation has no role in clarithromycin resistance (8, 16), at least in the isolates tested. This fact is not surprising because it is known that the genetic character of *H. pylori* in different areas is different (10, 11, 17), as are the genetic characters of resistant *H. pylori* strains (9). The following reasons are several possible explanations for the T2182C mutation and clarithromycin resistance among *H. pylori* isolates in Bangladesh. (i) The use of macrolides in this society became widespread only recently (since the late 1990s), and incomplete therapy is common. Under these circumstances, T2182C may represent a transient state, a relative hotspot for mutation, and might be subject to replacement later on by alleles that confer higher-level resistance (e.g., changes at position 2142 or 2143). (ii) The predominance of duodenal ulcer disease in South Asia, in contrast to gastric ulcer disease in other societies, might offer a selective advantage to the T2182C allele or be less costly than other resistance alleles in the usual Bangladeshi gastric environments. (iii) Differences in predominant genotypes of South Asian *H. pylori* strains from those from Europe, East Asia, and the Americas might favor T2182C or be more effective in conferring resistance due to its interaction with other ribosomal protein components or rRNA motifs.

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Rasel Khan*
Motiur Rahman*
Laboratory Sciences Division, ICDDR, B
Centre for Health and Population Research
GPO Box 128
Dhaka 1000, Bangladesh

*Phone: 880 2 9886464
Fax: 880 2 8812529
E-mail for Rasel Khan: rasel@icddrb.org
E-mail for Motiur Rahman: motiur@icddrb.org