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# *Helicobacter pylori* Reinfection Is Virtually Absent after Successful Eradication

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**This study examined whether reinfection or recrudescence accounts for the reappearance of *Helicobacter pylori* infection after apparent successful eradication. In a prospective study, 173 patients cured from *H. pylori* infection underwent follow-up endoscopies, with biopsies for culture and histopathology, every 3 months during the first year after treatment. Subsequently, elective half-yearly endoscopies were performed in 124 patients; the remaining 49 underwent follow-up endoscopy only in 1995. At reappearing infection, DNA profiles of pretreatment and recurrent strains were compared. After 3.5 years (range, 1.0–9.2), *H. pylori* infection recurred in 9 patients (5.2%). Reappearing infections were classified as endoscopically transmitted reinfection ($n = 2$), unclassified because of loss of pretreatment isolate ($n = 1$), or recrudescence (identical DNA patterns before and after treatment; $n = 6$). The reappearance rate of infection, discarding endoscopic transmission, was 1.2% (7/601 *H. pylori*–negative patient-years). There was virtually no reinfection with *H. pylori* after eradication in this adult Western population. These data do not rule out acquisition of *H. pylori.***

The pathogenic role of *Helicobacter pylori* in chronic active gastritis and the association with duodenal ulcer disease in 95%–99% of patients are well established [1–3]. Eradication of *H. pylori* infection cures peptic ulcer disease and, conversely, relapses of peptic ulcer disease are associated with reappearance of *H. pylori* infection [4].

Data on reappearance of *H. pylori* infection following successful eradication are conflicting and vary from 0 to 47% [5–8]. Comparison of the results from reinfection studies is hampered by the difference in assessment of cure of the infection, such as assessment within 4 weeks after completion of therapy (leading to an overestimation of cure and reinfection rates) [6, 8], sampling error following eradication therapy [4, 6–10], the use of less sensitive tests [10, 11], and absence of pretreatment *H. pylori* isolates [4–13]. Iatrogenic transmission of *H. pylori* due to inappropriately disinfected endoscopic equipment may also occur. In a previous study, DNA profiles of *H. pylori* strains were used to distinguish between reappearance of *H. pylori* infection owing to iatrogenic transmission or to late recrudescence [14]. If the DNA profile is missing, interpretation of reappearing infection is not possible [4–15].

We conducted a long-term follow-up study to assess the rate of reappearance of *H. pylori* infection in a cohort followed since 1984. If *H. pylori* infection reappeared, DNA profiles of pretreatment and recurrent strains were compared by restriction fragment length polymorphism (RFLP) or random-amplified polymorphic DNA (RAPD) techniques to distinguish between reinfection (different strain) and recrudescence (identical strain).

## Materials and Methods

*Patient selection.* Dyspeptic *H. pylori*–positive patients referred for diagnostic upper gastrointestinal endoscopy were included in this follow-up study. All patients were enrolled between 1984 and 1994 and participated in various *H. pylori* eradication trials. Patients who had previous gastric surgery; who were alcohol abusers; who had cardiac, pulmonary, hepatic, or renal disease; or who were taking anticoagulants were excluded.

*Administered therapies.* Eradication therapies have varied over the years and consisted of bismuth monotherapy (colloidal bismuth subcitrate [CBS]), bismuth dual therapy (CBS-metronidazole or CBS-amoxicillin), bismuth-triple therapy (CBS-amoxicillin-metronidazole or CBS-tetracycline-metronidazole), or proton pump inhibitor (PPI) dual therapy (omeprazole-amoxicillin or omeprazole-clarithromycin). Patients received sequential eradication attempts until cure of *H. pylori* infection was achieved.

*Follow-up scheme.* After successful eradication therapy, patients ($n = 173$) were followed by endoscopy every 3 months during the first year. Thereafter, elective follow-up endoscopy was performed half-yearly in 124 patients. The remaining 49 patients were contacted again in 1995 to undergo follow-up endoscopy. Records were kept of the sequence in which the patients were examined per endoscopy session and of the endoscopic equipment used.

*Disinfection of endoscopic equipment.* The endoscopic disinfection procedure used was as follows: Before 1988, after each endoscopy, the shaft of the endoscope, the instrument channels, and the valves were mechanically cleaned with a brush, water, and detergent; soaked in 70% ethanol for 3 min; then rinsed with sterile

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water and dried. One single endoscope was repeatedly used in a single endoscopy room until the end of the program.

After 1988, endoscopic cleaning was intensified when it became clear that endoscopic transmission of *H. pylori* was still possible [14]. Before each endoscopy, the endoscopes were thoroughly cleaned with water and detergent and subsequently disinfected for 30 min with aqueous 2% alkaline glutaraldehyde in a disinfecting machine (ETD; Olympus Europe). The endoscopes were then rinsed with sterile water and dried with hot air before reuse. From this time on, several endoscopes were used in a single endoscopy room.

Biopsy forceps and other instruments were cleaned with detergent (with rocking), disinfected with 70% ethanol, rinsed with sterile water, and autoclaved after each examination.

*H. pylori assessment.* *H. pylori* presence was assessed before and 4–6 weeks after completion of each *H. pylori* eradication attempt and at all subsequent endoscopies during the follow-up period. Antrum biopsies were taken for culture ($n = 2$) and histopathology ($n = 2$). Corpus biopsies were added to the follow-up procedures after PPIs became part of therapy.

For histopathologic examination, formalin-fixed gastric biopsies were stained with hematoxylin-eosin, and two histopathologists independently examined the slides. No additional special staining was done.

For culture, gastric biopsy specimens were smeared on horse blood agar plates (Colombia agar base, Oxoid CM 331; Unipath, Basingstoke, UK) and horse blood agar plates containing Skirrow supplement (Oxoid, Unipath). Gram-negative; oxidase-, catalase-, and urease-positive; and spiral or curved rods were identified as *H. pylori.* The *H. pylori* colonies were collected by sweeping with swabs. Each swab was shaken in 2 mL of 8% glycerol peptone, and the suspensions were stored at −70°C. These bacterial suspensions are depicted as the primary cultures [14, 15].

*H. pylori chromosomal DNA isolation.* After being thawed, bacterial suspensions were grown on horse blood agar plates at 37°C in a microaerophilic environment for 3 days. From these plates, 10 single *H. pylori* colonies were picked, cultured on horse blood agar, and harvested again. From the cultures of individual colonies, chromosomal DNA was isolated as described [14, 16].

*Genome typing by RFLP.* *H. pylori* chromosomal DNA was digested by *Hin*dIII-endonuclease (Boehringer Mannheim, Mannheim, Germany). The DNA fragments were separated in a horizontal gel with ethidium bromide as described [14, 15].

*Genome typing by RAPD-PCR (polymerase chain reaction).* PCR-based RAPD profiles were obtained using the method of Akopyanz et al. [17], with 20 ng of chromosomal DNA (Perkin-Elmer Nederland, Gouda, Netherlands) and 5 pmol of one of the following primers: 1254 (CCGCAGCCAA), 1281 (AACGCGCAAC), 1283 (GCGATCCCCA), or 1247 (AAGAGCCCGT). PCR was carried out in 25 $\mu$L of a mixture of 10 m*M* TRIS-HCl (pH 8.8), 50 m*M* KCl, 3.0 m*M* $MgCl_2$, and 0.1 mg/mL bovine serum albumin. The reaction conditions were as follows: 3 cycles of 5 min at 94°C, 5 min at 36°C, and 5 min at 72°C, followed by 29 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C, followed by 10 min at 72°C. The PCR fragments were analyzed by horizontal agarose (1%) gel electrophoresis as described [16].

*Whole cell ELISA.* Anti–*H. pylori* IgG antibodies in patient sera were detected by using the ELISA developed by Pena et al. [18]. Serum samples used to determine the presence of *H. pylori* IgG antibodies were obtained at the time of each follow-up endoscopy.

*H. pylori infection, reinfection, and recrudescence: working definitions.* *H. pylori* infection was present if culture, histopathologic assessment, or both were positive. Cure of *H. pylori* infection was defined as absence of *H. pylori* in both culture and histopathologic examination at least 4 weeks after cessation of eradication therapy.

Reappearance of the *H. pylori* infection was defined as the presence of *H. pylori* by culture or histopathologic assessment after apparently successful eradication. A reinfection was present if pretreatment and recurrent strains had different DNA profiles. Recrudescence was present if pretreatment and recurrent strains had identical DNA profiles.

## Results

The follow-up cohort consists of 310 patients followed for a mean of 5.05 years (range, 1.5–10.9). *H. pylori* eradication was successful in 173 patients (56%), who were subsequently followed for a mean of 3.5 years (range, 1.0–9.2). In 164 of these 173 patients, *H. pylori* infection remained absent for the follow-up period of 606 patient-years. In 9 (5.2%) of 173 patients, reappearing infection was diagnosed after a mean *H. pylori*–negative period of 14.6 months (range, 3–32). During this *H. pylori*–negative period, repeated endoscopies (average, 5/patient) confirmed cure of the infection.

Two of these 9 patients (patients A and B) were infected iatrogenically via contaminated endoscopic equipment: Patient A had two reappearing infections; one 3 months after successful bismuth therapy and the other 22 months later. Patient B had a reappearing infection 32 months after successful eradication. DNA profile by RFLP of the *H. pylori* isolates of these 2 patients proved iatrogenic transmission from the patient who underwent the immediately previous endoscopy [14]. After 1988, when endoscopic cleaning procedures were improved, no additional cases of endoscopic/iatrogenic *H. pylori* transmission were encountered.

After excluding these 2 iatrogenically infected patients, reappearance of infection occurred in 7 (4.1%) of 171 patients, corresponding to a reappearing infection rate of 1.2%/patient-year. The pretreatment isolates of 1 of the remaining 7 patients were lost. In the remaining 6 patients (C–H), DNA profiles of *H. pylori* isolates were determined by RAPD.

In patient C, 14 months after successful eradication of *H. pylori,* a reappearing infection was diagnosed, with recurrence of histologic gastritis. Initially, a reinfection was assumed, since DNA profiles (figure 1) of the pretreatment (lanes a, e) and recurrent (lanes c, g) isolates from the antrum were different. However, further analysis revealed that a pretreatment strain isolated from the fundus (lanes b, f) was identical to the recurrent antrum strain (lanes c, g), indicating recrudescence of *H. pylori* already present in that patient before treatment. This was confirmed by the antrum strain isolated 1 year later (lanes d, h), which appeared to be identical to the pretreat-

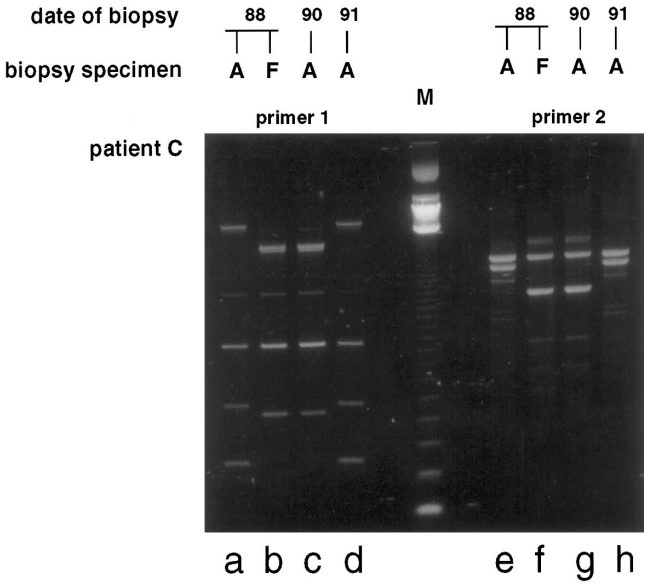


**Figure 1.** *H. pylori* chromosomal DNA patterns by polymerase chain reaction–based random-amplified polymorphic DNA assay of isolates from patient C, who had recurrent infection with different types of *H. pylori.* Lanes a and e, pretreatment antrum strain in 1988; lanes b and f, pretreatment fundus strain in 1988; lanes c and g, antrum strain at recurrent infection in 1990; lanes d and h, recurrent antrum strain in 1991. Pretreatment and recurrent antrum strain are genetically different (lanes a and e, c and g). Pretreatment fundus strain (lanes b and f) and recurrent antrum strain (lanes c and g) are identical, whereas pretreatment (1988) antrum strain (lanes a and e) and 1991 antrum strain (lanes d and h) are also identical, indicating intraindividual heterogeneity and late recrudescence.

ment antrum strain (lanes a, e). This patient is illustrative of the difficulties in interpreting the cause of reappearance if the microorganism originates from a heterogeneous *H. pylori* population present in an individual. To contrast this finding, DNA profiles of *H. pylori* isolates from patients without successful cure of the infection are demonstrated in figure 2. It illustrates identical pre- and posttreatment strains in individual patients and the heterogeneity of strains between persons.

In the remaining 5 patients (D–H), the DNA profiles from pretreatment and recurrent *H. pylori* isolates were identical. Again, this finding strongly favors recrudescence of the pretreatment *H. pylori* strain, although reinfection by an identical *H. pylori* type from a common source cannot be excluded. Histologic examination at the time of reappearing infection showed reactivation of gastritis. Histologic examination of culture-negative controls revealed a decrease in gastritis activity and absence of *H. pylori.* Whole cell ELISA revealed a sustained high level of anti–*H. pylori* IgG antibodies in the sera of 5 of 6 recrudescence patients during the complete follow-up period (figure 3); usually a gradual fall of antibody titers should be noticed after apparent successful eradication, as is illustrated for another 10 patients with successful eradication and no reinfection (figure 4).

## Discussion

In this long-term follow-up study, *H. pylori* infection reappeared in only 5.2% of patients. The DNA profiles of pretreatment and reappearing *H. pylori* strains indicated recurrent infections to be due to either iatrogenic transmission ($n = 2$) or recrudescence ($n = 6$ or 7). It should be realized that recurrent infection by identical *H. pylori* from a common source can never be ruled out; however, if it does occur, this cohort study indicates that it is rare. Evidently this does not mean that adults cannot be spontaneously infected.

After proven iatrogenic reinfection before 1988 [14], endoscopic disinfecting procedures in our institution were improved. Ever since, no additional iatrogenic transmission of *H. pylori* has been observed. After exclusion of the 2 iatrogenically infected patients, the recurrent infection rate was 1.2%/patient-year.

The frequency of reappearing infections varies in the literature because of several pitfalls. Assessment of *H. pylori* infec-

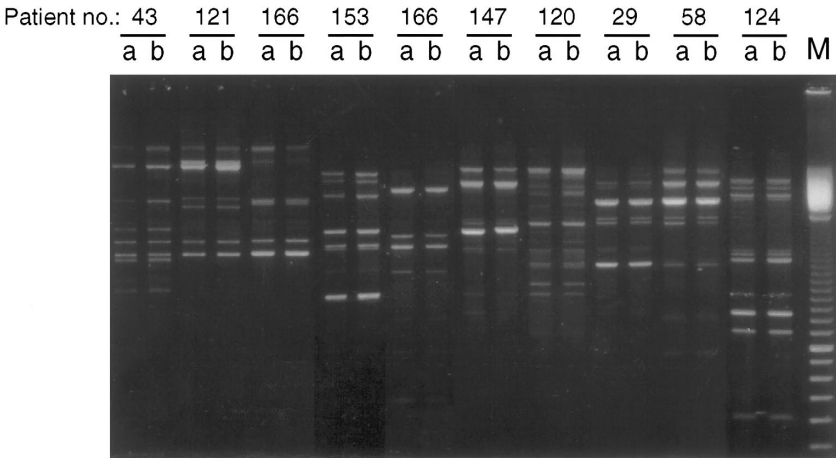


**Figure 2.** *H. pylori* chromosomal DNA profiles by polymerase chain reaction–based random-amplified polymorphic DNA assay of isolates from 10 patients in whom eradication therapy failed. Lanes a and b represent pre- and posttreatment strains isolated from 1 person.

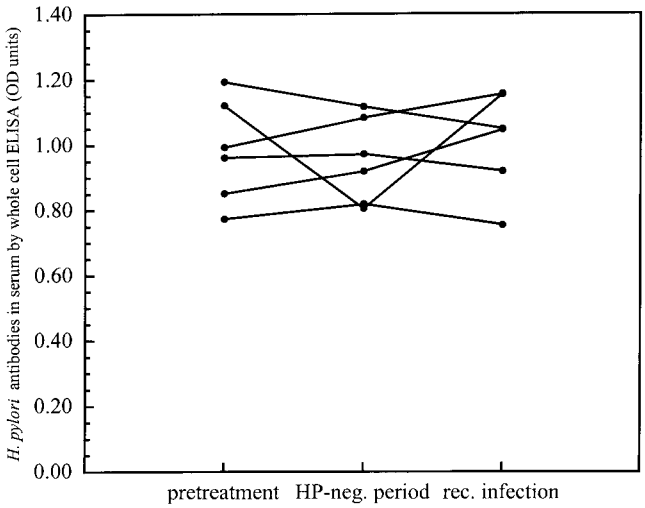


**Figure 3.** Serial serum IgG antibody titers against *H. pylori* in patients with reappearance of *H. pylori* infection after apparent successful eradication. Titers obtained before eradication therapy (pretreatment), after successful cure (HP-neg. period), and at reappearing (rec.) infection are depicted. Lowest levels of antibody titers during *H. pylori*–negative period are presented. Mean antibody titers at 3 sampling times are not significantly different. Significant rise in antibody level was noted in only 1 patient after recurrent infection.

tion too early after eradication therapy may result in overestimation of cure rates and a high reappearance rate [4, 9]. The 4-weeks rule used for posttherapy assessment is generally accepted, because most of the recrudescent infections occur during this period [7]. In the present study, the 4-weeks posttreatment *H. pylori* assessment represents a specificity of 96%. In contrast, the specificity in the study of Coghlan et al. [8] was much lower, since cure was assessed immediately after cessation of bismuth therapy, and *H. pylori* infection reappeared in 33% of patients. Despite assessment of cure 4 weeks after cessation of therapy, Xia et al. [10], from the same group, reported a reappearing infection rate of 18.9% (range, 9.2%–47.1%). Those authors concluded that this was obviously not reappearance of infection but recrudescence after use of less effective therapies, which may only suppress rather than eradicate the microorganism. Moreover, they suggested more sensitive techniques to assess *H. pylori* infection [10].

We have demonstrated that recrudescence remains possible 12 and even 33 months after successful eradication with less-effective bismuth therapies. Our 6 patients with recrudescence were treated with bismuth monotherapy, bismuth dual therapy, or bismuth triple therapy. The relation between high rate of reappearance of infection and less-effective *H. pylori* eradication regimens is also supported by the near-zero reappearance rates that have been noted since more-effective eradication regimens have been used [19–21].

Sampling error (e.g., low number of biopsies, only one detection method, sampling from a single site), especially after eradication regimens containing bismuth compounds or omeprazole, may also lead to falsely high eradication rates and falsely high reappearance rates [9, 10, 22]. The use of two diagnostic methods is more sensitive than the use of only one [23]. Many studies rely on only [$^{13/14}$C]UBT (urea breath test) for *H. pylori* assessment after eradication therapy. Since the sensitivity of UBT is lower than that achieved with the combination of culture and histopathology, rates of reappearing infection may be underestimated [23]. On the other hand, false-positive [$^{13}$C]UBTs can occur, as Cutler et al. [11] found in 8 of 12 patients and confirmed by subsequent endoscopy.

Once reappearance of infection is diagnosed, the DNA profiles of pretreatment and recurrent *H. pylori* strains must be determined to distinguish between reinfection and recrudescence [13–15, 24]. The results of this technique should be interpreted carefully, since an individual patient can carry a heterogeneous *H. pylori* population, as did our patient C. Therefore, multiple colonies from specimens taken simultaneously should be analyzed because they may contain several subtypes of *H. pylori* [15, 25].

Discrimination between recrudescence and reinfection by an identical *H. pylori* strain from the same common source is virtually impossible. Transmission between family members living close together is suggested and is supported by the circumstantial evidence of familial clustering of *H. pylori* [26] but remains difficult to prove [27], especially since heterogeneity of *H. pylori* strains also occurs within families [25]. Recently, Schutze et al. [13] found that 2 patients with reappearing infection and their respective spouses were infected with identical strains. The authors concluded that person-to-person transmission had occurred. It should be realized that recrudescence or reinfection from the same environmental source with an *H. pylori* strain identical to the pretreatment strain could not be excluded.

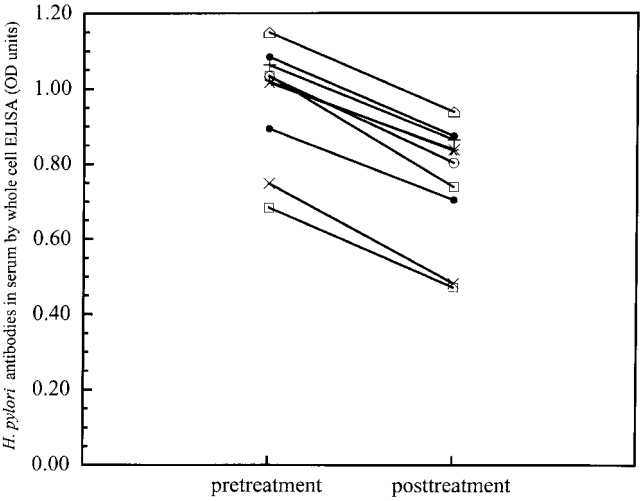


**Figure 4.** Serial serum IgG antibody titers against *H. pylori* in patients who were successfully cured of infection and who had no reappearing infection. Titers obtained before eradication therapy (pretreatment) and after successful cure (posttreatment) are presented.

In our study, persistently high anti–*H. pylori* antibody titers in 5 of 6 study patients suggests ongoing infection in undetectable niches. However, the simultaneously obtained histologic specimens showed a decrease in gastritis, concurrent with apparent disappearance of the microorganism, after successful eradication and subsequent increased inflammatory activity at the time of overt reappearance of infection. The discrepancy between histologic and serologic findings may be explained by two factors: There is a significant drop in antibody titers usually after ~6 months [28, 29] (2 patients had recurrent infection within 3–5 months), and *H. pylori* is not detectable in the antrum after therapy because of suppression and migration of *H. pylori* to the corpus or fundic area. In 1 patient, the lowest serologic titer was 28% lower than the pretreatment titer. However, serologic titers obtained 3 months before and 1 month after measurement of the lowest titer were only 9% and 17% lower, respectively, than the pretreatment value. Culture and histology results remained negative during this period.

In conclusion, after apparent eradication, reappearance of *H. pylori* infection was rare and due to either iatrogenic transmission or recrudescence, the latter even after 3 years, in a Western adult population. However, spontaneous aquisition of *H. pylori* infection can occur in adults. To confirm the statement ''once cured is cured forever,'' studies should be carried out in areas with high infection acquisition rates, on the condition of adequate endoscopic cleaning procedures and DNA profiling of strains in reappearing infections.

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