Serological Assessment of the Early Response to Eradication Therapy Using an Immunodominant Outer Membrane Protein of Helicobacter pylori

AKIRA NISHIZONO,1* TAKAYUKI GOTOH, 2 TOSHIRO FUJIOKA, 1 KAZUNARI MURAKAMI,2 TOSHIHIRO KUBOTA,2 MASARU NASU,2 MAKOTO WATANABE,3 AND KUMATO MIFUNE4

Department of Infectious Diseases Control, 1 The Second Department of Internal Medicine, 2 and Departments of Biochemistry 3 and Microbiology, 4 Oita Medical University, Oita, Japan

Received 12 March 1998/Returned for modification 22 April 1998/Accepted 23 July 1998

Eradication of Helicobacter pylori infection cures gastritis and prevents recurrence of peptic ulcers. Endoscopy is usually used to evaluate the effectiveness of eradication therapy. We designed a new noninvasive assay system for the early evaluation of eradication of H. pylori infection in which a crude H. pylori outer membrane protein preparation (HPOmp) is used as an antigen, and we determined the sensitivity and specificity of the serological assay system. Immunoblot analysis showed that anti-HPOmp antibodies reacted to a protein with a molecular mass of approximately 29 kDa. In those patients who responded to therapy, the anti-HPOmp immunoglobulin G (IgG) titers measured by enzyme-linked immunosorbent assay (ELISA) at 1 month after the end of therapy were significantly lower than those before treatment (34.8% reduction; P < 0.001), and the posttreatment reduction in the antibody titer was significantly greater than that of the titer measured with a commercially available anti-H. pylori IgG ELISA (34.8% versus 16.1%; P < 0.001). When a 25% reduction of anti-HPOmp IgG titer at 1 month after the end of treatment was taken as the cutoff value for H. pylori eradication, the sensitivity and specificity of our new assay were 75% (51 of 68 treatment responders) and 96% (22 of 23 nonresponders), respectively. Our results indicate that the novel serological test with HPOmp might be a clinically useful tool for assessment of eradication of H. pylori.

Helicobacter pylori is an important pathogen which causes gastritis, peptic ulcer, and intestinal metaplasia, and long-term infection with this organism is a risk factor for gastric carcinoma (11). Therefore, eradication of H. pylori is important, especially in patients with peptic ulcers (5). Apart from serological detection and the urea breath test (UBT), invasive tests involving endoscopy are the main methods for evaluation of the efficacy of eradication therapy. Although the currently available serological tests are convenient and the UBT offers a highly sensitive and specific means of detection of H. pylori, the former tests cannot detect reductions in antibody titer in the early post eradication period (8), while the latter test is expensive and not readily available to the majority of general practitioners, especially in Japan. Thus, a noninvasive and sensitive method that detects eradication of the organism is desirable.

In the present study, we describe the design and evaluation of a new serological assessment test for the eradication of H. pylori in which a crude H. pylori outer membrane protein preparation (HPOmp) is used as an antigen.

MATERIALS AND METHODS

Patients and sera. One hundred two patients (61 males and 41 females; mean age, 52.4 years; range, 13 to 76 years) were diagnosed with H. pylori infection in the Second Department of Internal Medicine between 1989 to 1996. The diagnosis was based on the following tests: bacterial culture, histopathological examination, and rapid urease test. The sample consisted of 38 patients with chronic gastritis, 27 with gastric ulcer, 36 with duodenal ulcer, and 1 patient with normal findings on endoscopic examination. All patients received a proton pump inhibitor or histamine blocker (H2 blocker) combined with amoxicillin (1,500 mg/day) or clarithromycin (400 to 800 mg/day) and metronidazole (500 mg/day) for 7 days. H. pylori was not detected by bacterial examination at 1 month after the end of eradication therapy in 68 patients (responders). H. pylori was not eradicated in the remaining 34 patients (nonresponders).

Blood samples were obtained just before treatment and at 1, 3, 6, and 12 months after the end of therapy. Among nonresponders, we were able to obtain serum samples from only 23 patients at 1 month after the end of therapy. Control sera used in this study were obtained from 19 individuals (10 males and 9 females; mean age, 38.9 years) who were negative for H. pylori infection by bacterial examination and from 23 newborn babies (14 males and 9 females). Each patient gave informed consent after receiving a full explanation of the purpose and design of the study.

Preparation of HPOmp. The H. pylori type strain ATCC 43504 was used for preparation of the antigen in the present study. H. pylori was grown on blood agar plates with 10% defibrinated sheep blood (GBICO BRL, Grand Island, N.Y.) in an atmosphere of 10% CO2 and 5% O2 with CampyPak-Plus (BBL Microbiology Systems, Cockeysville, Md.). H. pylori was scraped and collected from plates and pulverized by a French press (12,000 lb/in2, three times), and the particulate fraction was pelleted by ultracentrifugation at 200,000 × g for 3 h. The resulting whole particulate fraction was subjected to linear sucrose density gradient (SDG) separation from 25% to 65% (wt/wt). After centrifugation at 120,000 × g for 20 h, the gradient was divided from the bottom into six fractions. In order to identify the fraction containing the outer membrane, we determined the insolubility of each fraction with 1% N-lauroylsarcosine, the electrophoretic patterns of proteins detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the reactivities of electrophoresed proteins with anti-urease monoclonal antibody (kindly provided by Kumiko Nagata, Hyogo Medical University) detected by immunoblotting and by the presence of urease activity. After the outer membrane was identified by its insolubility with N-lauroylsarcosine, the fraction that contained the outer membrane proteins was pelleted by ultracentrifugation at 200,000 × g for 4 h. The resulting pellet was re-suspended in an aliquot of membrane buffer consisting of 0.25 M sucrose, 50 mM trithanolamine, and 1 mM dithiothreitol and used as the crude HPOmp antigen.

ELISA and immunoblotting. The serum sample was subjected to two types each of enzyme-linked immunosorbent assay (ELISA) and Western blotting (WB) analyses. The first ELISA was a conventional ELISA performed by using a commercial GAP immunoglobulin G (IgG) test (Biomerica, Newport Beach, Calif.) (Pilkakrape Helicobacter II; Fuji Rebio Inc., Tokyo, Japan). Determination of ELISA units (EU) was performed with 1:200-diluted test sera and an accompanying positive control serum according to the instructions provided by the manufacturer. In the second type of ELISA, HPOmp at 10-μg/ml concen-
tration was used as a coated antigen (HPOmp ELISA) and the assays were performed with serial dilutions of the test serum. For standardization of antibody titer in the serum, we used a positive control serum obtained from an H. pylori-infected patient who was confirmed to have a high antibody titer (by GAP IgG test) (12). The EU value of the HPOmp ELISA was considered to be 300 EU at 1:5,000 dilution. This was based on the results of two series of experiments. In the first experiment, a linear relationship was detected between the reciprocal two-fold dilutions of the positive serum from 1:250 to 1:32,000 and the optical density at 414 nm (OD414). In the second experiment, four other antibody-positive sera showed a similar linear relationship. EU of the test serum was determined from the standard curve of the positive control serum. The second antibody of HPOmp ELISA was used with peroxidase-labeled anti-human immunoglobulin g-F(ab')2 fragments (American Qualex, La Miranda, Calif.). Absorbance was measured at OD 414 in the presence of 2,2'-azinobis(3-ethylbenzthiazoline-sulfonic acid) (Wako Pure Chemical Industries, Osaka, Japan) as a substrate.

WB was performed as described previously (10). Briefly, the antigen used in ELISA was subjected to SDS–12.5% PAGE and electroblotted onto a polyvinylidene difluoride membrane. The blotted membrane was reacted with 1:5,000-diluted sera and subsequently reacted with 1:5,000-diluted peroxidase-conjugated goat anti-human immunoglobulin g-chain F(ab')2 fragments (American Qualex, La Miranda, Calif.). Absorbance was measured at OD414 in the presence of 2,2'-azinobis(3-ethylbenzthiazoline-sulfonic acid) (Wako Pure Chemical Industries, Osaka, Japan) as a substrate. The membrane was subjected to an ECL kit-WB detection system (Amersham Japan, Tokyo, Japan) and exposed to X-ray film.

Statistical analysis. Data are expressed as the means ± standard deviations. Differences between groups were analyzed for statistical significance by the Student’s t test. All P values were two-sided, and values of < 0.05 were considered statistically significant.

RESULTS

Presence in H. pylori-infected patients of antibodies that reacted with a 29-kDa protein of HPOmp. Membrane proteins were fractionated by SDG separation. The main proteins that floated onto the membrane fraction between 25 and 45% of the SDG (Fig. 1A, lanes 1 to 3) appeared to be large, urease subunit B (UreB), and small, urease subunit A (UreA) subunits as well as heat shock protein 60, based on their molecular sizes. However, these membrane fractions were considered to be part of the inner membrane consisting of plasma membrane structure (data not shown), since the fractions were completely solubilized with N-lauroylsarcosine. On the other hand, a single, opalescent brownish band generated at approximately 55% (wt/wt) of SDG (Fig. 1A, lane 5) was different from the upper fractions in the SDS-PAGE pattern and was resistant to N-lauroylsarcosine. SDS-PAGE profiles of this fraction showed relatively clear bands with medium to large molecular sizes and fuzzy bands with small molecular sizes (Fig. 1A, lane 5). The urease activity assay (data not shown) and WB with anti-UreA monoclonal antibody showed that urease did not contaminate this fraction, suggesting that the fraction consisted mainly of HPOmp (Fig. 1C, lane 5).

In the initial step, we examined, using WB, the immunoreactivity of the fraction (HPOmp) against IgG of a patient infected with H. pylori (Fig. 1B) and serum samples obtained from H. pylori-infected patients (Fig. 2). For each subject, immunoblot analysis showed as the major band a band with a molecular mass of approximately 29 kDa, which was reactive to the serum sample (Fig. 2A). Because the HPOmp fraction could not have been contaminated with UreA, the major band showing a 29-kDa mobility was considered to react with an H. pylori protein different from urease.

The results of WB analysis of serum samples obtained from 12 patients before eradication therapy and 6 months after the end of the therapy showed that the intensity of the 29-kDa major band was diminished after treatment compared with that before treatment (Fig. 2A and B). Furthermore, there was a total absence of reactivity in several serum samples obtained at 6 months after treatment. Therefore, we used HPOmp in further studies in order to assess the antibody response in patients.
with *H. pylori* infection and to monitor the effectiveness of eradication therapy.

**Accuracy of anti-HPOmp ELISA.** We next assessed the specificity of the newly developed ELISA system with HPOmp as an antigen. Figure 3 shows the distribution of anti-HPOmp EU in *H. pylori*-infected patients and the control group. Although a few samples, even from the control group, exhibited somewhat high EU values on HPOmp ELISA, the EU for serum

---

**FIG. 2.** Immunoreactivities detect by WB analysis at pretreatment and at 6 months after treatment for responders. Fraction 5 shown in Fig. 1A was used for WB as HPOmp. Immunoreactivities against HPOmp protein of serum samples obtained from 12 patients before treatment (A) and at 6 months after therapy (B) are shown. The molecular mass markers (M) are shown in the left column.

**FIG. 3.** Distribution of EU for anti-HPOmp antibody detected in the sera of *H. pylori*-infected and control individuals. *, *P* < 0.005, compared with the infected group.
IgG antibody against HPomp in H. pylori-infected patients was clearly higher than that in the control group. It is not clear whether such moderately high titers in the control group arose due to a specific antibody; however, it is possible that antibodies against proteins of the outer membrane of other bacteria may exhibit nonspecific reactions.

When the cutoff was set at 30 EU (Fig. 3), HPomp ELISA had a sensitivity of 99% (101 of 102 H. pylori-infected patients) and specificity of 88% (37 of 42 controls). Therefore, the diagnostic accuracy of HPomp ELISA was almost equal to that of the commercially available ELISA in evaluating the status of H. pylori infection (3, 4). Interestingly, the titer on HPomp ELISA for pretreatment samples obtained from responders was higher than that for samples obtained from nonresponders after eradication therapy. However, these levels could not be used for the prediction of the effectiveness of therapy prior to this treatment (data not shown).

Changes in H. pylori-specific IgG antibody titers after eradication therapy. As shown in Fig. 4, the average titer of anti-HPomp IgG in responders (n = 68) at 1 month after the end of therapy was significantly decreased, by 34.8%, compared with pretreatment levels (P < 0.001). In addition, the titer was significantly lower than the average anti-H. pylori IgG titer determined by conventional GAP IgG ELISA (16.1% reduction; P < 0.001). At 3, 6, and 12 months after eradication therapy, the anti-HPomp IgG titers were further decreased to 44.9%, 37.5%, and 27.0% of the pretreatment titers, respectively. These levels were also significantly lower than the corresponding GAP IgG titers. On the other hand, in nonresponders (n = 23), the average titer of anti-HPomp IgG at 1 month after treatment was 101.5% of the average pretreatment titer, and during the 12 posttreatment months the titers did not diminish relative to pretreatment levels. Retrospective follow-up studies of responders showed the reappearance of H. pylori infection in four patients diagnosed at 6 months after therapy (Fig. 5, responders). In these patients, the reduction in antibody titer was minimal at 1 month after treatment and the titers increased somewhat in two patients. Antibody titers in these patients at 3 months after therapy were significantly higher than those at 1 month after therapy (data not shown). Such cases should be classified as H. pylori reinfection or recrudescence.

Usefulness of HPomp ELISA for the assessment of eradication at 1 month after therapy. When >25% reduction in anti-HPomp IgG titer at 1 month after treatment was taken as the cutoff for eradication of H. pylori for the test system employed in this study, the sensitivity and specificity of the test were 75% (51 of 68 responders) and 96% (22 of 23 nonresponders) (Fig. 5), respectively. As stated above, patients with recrudescence of H. pylori after 6 months showed relatively high titers on HPomp ELISA at 1 month after treatment, suggesting that amounts of residual organisms nondetectable by bacterial cultures caused a low level of stimulation of the immune system.

FIG. 4. Serial changes in titers of serum IgG antibody against HPomp protein for responders and nonresponders. Titers obtained before eradication therapy and at 1, 3, 6, and 12 months after the end of therapy are expressed as percentages of the individual pretreatment titers measured by HPomp ELISA and GAP IgG ELISA. Data are mean percentages of pretreatment titers ± standard deviations. Closed circles, titer measured by HPomp ELISA; open circles, titer measured by GAP IgG ELISA; solid lines, titer in responders; dotted lines, titer in nonresponders. † and *, P < 0.001 compared with the pretreatment titer and with the GAP IgG ELISA titer, respectively.

FIG. 5. Specificity and sensitivity of HPomp ELISA for assessment of response to therapy at 1 month after the end of therapy. Dotted lines indicate the relative percentages corresponding to 25 and 30% reductions compared to the pretreatment titer. Closed circles, percentages of pretreatment titers for HPomp ELISA for four individuals who were categorized as responders but showed evidence of reappearance of H. pylori infection at 6 months after therapy.
DISCUSSION

Eradication of *H. pylori* markedly improves the natural history of peptic ulcer in patients with duodenal or gastric ulcers (5). Confirmation of eradication of the causative organism usually requires noninvasive and invasive tests involving repeated endoscopic examinations. UBT and *H. pylori* antibody assay are widely used as noninvasive tests for detection of *H. pylori*. Since the results of UBT reflect the presence of *H. pylori* in the whole stomach, sampling error in collection of biopsy specimens very seldom occurs, and therefore, UBT is a clinically useful diagnostic test with a high sensitivity and specificity. However, the 13C-UBT requires a mass spectrometer, which is expensive and is not readily available to general practitioners (6). On the other hand, there are several problems with 14C-UBT such as protection against radiation pollution caused by the use of radioactive material and its unsuitability for infants and pregnant women (1). The serological tests are less expensive and easier to perform than UBT and may be preferable for the diagnosis of *H. pylori* infection. Therefore, serological diagnosis has been used in seroepidemiological studies and screening of *H. pylori* infection in large samples (3).

*H. pylori* causes a chronic infection of the gastric mucosa, and humoral and cellular immune responses persist for a long period unless the organisms are eradicated (9). The currently available *H. pylori*-specific antibody assay has not been used for the early monitoring of eradication, since the antibody titer gradually decreases after disappearance of the organism. Thus, it is important to establish an improved *H. pylori*-specific antibody assay for use as a noninvasive test for the early and accurate assessment of the response to treatment. In this study, we demonstrated that the 29-kDa outer membrane protein of *H. pylori* (HPOmp) is a major antigen capable of inducing a strong antibody response and that a new ELISA system incorporating the HPOmp is capable of detecting the eradication of *H. pylori* with high sensitivity and specificity compared to the serological tests so far established.

Several investigators reported that the titer of *H. pylori*-specific antibody decreases progressively in responders after treatment (2, 7, 8). The average time necessary for 50% reduction in antibody titer varies from one study to another. For example, Kosunen et al. (8) and Cullen et al. (2) reported that the average duration until antibody titers fell by 50% in response to treatment was 3 or 4 months. On the other hand, Hirschl and coworkers (7) reported that a 50% reduction in IgG titer was observed more than 6 weeks after treatment. The differences in the intervals between cessation of therapy and reduction in the antibody titer might be due to the different antigens used in the ELISAs: an acid-glycine *H. pylori*-extract was employed by the former groups of investigators and a 120-kDa protein of *H. pylori* was used by the latter investigators. The present study using HPOmp detected 34.8% and 55.1% reductions in antibody titers relative to the pretreatment level at 1 and 3 months after therapy, respectively.

The kinetics of posttreatment changes in antibody titer have shown some reduction during the early posttreatment period irrespective of the success of bacterial eradication (8). In our study, a few nonresponders also showed a decrease in the antibody titer (Fig. 5). Therefore, it is difficult to determine the success or failure of treatment in the early posttreatment period (8).

The major finding of the present study was the reduction in the intensity of the bands in the low-molecular-mass region detected 6 months after therapy by WB analysis with the outer membrane protein as the antigen. This feature indicates that HPOmp is a useful diagnostic indicator reflecting the efficacy of eradication therapy. Our new ELISA with HPOmp as an antigen was capable of detecting a significant reduction in the titer of *H. pylori*-specific antibody at 1 month after treatment with high sensitivity and specificity (75% and 96%, respectively; Fig. 5). The reason for the rapid reduction in the titer of HPOmp ELISA in responders compared with conventional ELISA is unknown at present. It is possible, however, that the mode of colonization of the body by *H. pylori* is limited and only superficial in the gastric mucosa and that the clearance of organisms caused by eradication therapy might induce a rapid decline in specific immune responses against the organism. It is also possible that HPOmp might be one of the immunodominant antigens of this organism reflecting infection with *H. pylori* since the HPOmp is probably located on the bacterial surface, while the antigens used in conventional ELISA consist of the whole antigenic material of the organism including cytoplasmic components.

We also evaluated the sensitivity and specificity of HPOmp ELISA in the early assessment of the efficacy of eradication therapy. Our results showed that the sensitivity and specificity of the novel tests are similar to those of UBT. Previous reports showed that the sensitivity and specificity of the UBT for eradication of infection at 6 weeks after treatment were 97 to 99% and 71 to 76%, respectively (13, 14). The sensitivity of the UBT is superior to that of serological tests. On the other hand, if the cutoff value of reduction in antibody titer as the criterion for bacterial eradication is set at >50%, the specificity of assessment of eradication by HPOmp ELISA is 100%, suggesting that our new HPOmp ELISA can accurately evaluate the success of bacterial eradication at an early stage and that the test is more specific than the UBT.

Finally, our results showed that a protein with a molecular mass of approximately 29 kDa induces a strong antibody response. We tentatively named the highly immunogenic protein HPOmp29. Our amino-terminal sequencing of the protein showed that HPOmp29 does not exhibit any identity to previously reported proteins (data not shown). However, according to comparison with the complete genome nucleotide sequence of *H. pylori* 26695 that was reported recently (15), HPOmp29 is categorized as a member of a family of outer membrane proteins of *H. pylori*. Further studies are necessary to characterize the structure and function of this newly isolated outer membrane protein.

ACKNOWLEDGMENT

We thank F. G. Issa from the Department of Medicine, University of Sydney, Sydney, Australia, for the careful reading and editing of the manuscript.

REFERENCES

1. Bell, G. D., J. Weil, G. Harrison, A. Morden, P. H. Jones, P. W. Gart, J. E. Trowell, A. K. Young, T. K. Daneshmend, and R. F. A. Logan. 1987. 13C urea breath analysis, a non-invasive test for Campylobacter pylori in the stomach. Lancet i:1367–1368.

2. Cullen, D. J. E., K. J. Cullen, B. J. Collins, K. J. Christiansen, and J. Epis. 1992. Serological assessment of Helicobacter pylori eradication. Lancet 340:1161–1162.

3. Evans, D. J., D. G. Evans, D. Y. Graham, and P. D. Klein. 1989. A sensitive and specific serologic test for detection of Campylobacter pylori infection. Gastroenterology 96:1084–1088.

4. Evans, D. J., D. G. Evans, K. E. Smith, and D. Y. Graham. 1989. Serum antibody responses to the N-acetylcarnamylaminolactose-binding hemagglutinin of Campylobacter pylori. Infect. Immun. 57:664–667.

5. Graham, D. Y., G. M. Lew, P. D. Klein, D. G. Evans, D. J. Evans, Z. A. Saeed, and H. M. Malaty. 1992. Effect of treatment of Helicobacter pylori infection on the long term recurrence of gastric and duodenal ulcers: a randomized controlled study. Ann. Intern. Med. 116:705–708.

6. Graham, D. Y., P. D. Klein, D. J. Evans, L. C. Alpert, A. R. Opekun, and T. W. Botton. 1987. Campylobacter pylori detected noninvasively by the
13C-urea breath test. Lancet 1:1174–1177.
7. Hirschl, A. M., G. Brandstatter, B. Dragosics, E. Hentschel, M. Kundi, M. L. Rotter, K. Schutze, and M. Taufer. 1993. Kinetics of specific IgG antibodies for monitoring the effect of anti- Helicobacter pylori chemotherapy. J. Infect. Dis. 168:763–766.
8. Kosunen, T. U., K. Seppala, S. Sarne, and P. Sipponen. 1992. Diagnostic value of decreasing IgG, IgA, and IgM antibody titers after eradication of Helicobacter pylori. Lancet 339:893–895.
9. Morris, A. J., M. R. Alle, G. I. Nicholson, G. I. Perez-Perez, and M. J. Blaser. 1991. Long-term follow up of voluntary ingestion of Helicobacter pylori. Ann. Intern. Med. 114:662–663.
10. Nishizono, A., M. Hiraga, K. Mifune, H. Terao, T. Fujisaka, M. Nasu, T. Goto, J. Misumi, M. Moriyama, Y. Arakawa, N. Hayashi, M. Esumi, and T. Shikata. 1993. Correlation of serum antibody titers against hepatitis C virus core protein with clinical features by Western blot (immunoblot) analysis using a recombinant vaccinia virus expression system. J. Clin. Microbiol. 31:1173–1178.
11. Nomura, A., G. N. Stemmermann, P.-H. Chyou, G. I. Perez-Perez, and M. J. Blaser. 1991. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N. Engl. J. Med. 321:1132–1136.
12. Perez-Perez, G. I., B. M. Dworkin, J. E. Chodos, and M. J. Blaser. 1988. Campylobacter pylori antibodies in humans. Ann. Intern. Med. 109:11–17.
13. Satoh, K., K. Kimura, Y. Yoshida, T. Kasano, K. Kihara, and Y. Taniguchi. 1991. A topographical relationship between Helicobacter pylori and gastritis: quantitative assessment of Helicobacter pylori in the gastric mucosa. Am. J. Gastroenterol. 86:285–290.
14. Slomianski, A., T. Schubert, and A. F. Cutler. 1995. [13C]Urea breath test to confirm eradication of Helicobacter pylori. Am. J. Gastroenterol. 90:224–226.
15. Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzgerald, N. Lee, M. D. Adams, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, and J. C. Venter. 1997. The complete genome sequence of the gastric pathogen Helicobacter pylori. Science 388:539–547.