

# *Helicobacter pylori* in Children Who Are Prone to Upper Respiratory Tract Infections

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**Objective:** To examine the presence of *Helicobacter pylori* infection in children with recurrent upper respiratory infections, including acute otitis media.

**Design:** A prospective clinical trial.

**Setting:** Tertiary referral center.

**Patients:** A total of 20 children who were prone to upper respiratory infections and acute otitis and who were admitted for elective adenoidectomy and/or tympanostomy were included in the study.

**Main Outcome Measures:** Samples of adenoid tissue and middle ear fluid were cultured for *H pylori*.

Serologic analysis and fecal antigen detection testing were also carried out to determine the presence of *H pylori*.

**Results:** The results of serologic and antigen detection tests were positive for *H pylori* in 4 children (20%), but cultures of adenoid tissue and middle ear fluid samples were negative for *H pylori* in all children.

**Conclusion:** An association between *H pylori* infection and recurrent upper respiratory infections and otitis media could not be established.

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**H**ELICOBACTER PYLORI COLONIZES gastric mucosa and can be found in gastric juice. Reflux of gastric juice has been suggested to be associated with glue ear<sup>1</sup> and chronic sinusitis in children.<sup>2</sup> It has also been suggested that tonsil and adenoid tissues are potential reservoirs of *H pylori* infection.<sup>3-5</sup> These observations raise the question as to whether *H pylori* infection might have a role in frequent respiratory tract infections (FRTIs) and recurrent acute otitis media (rAOM) in respiratory infection-prone children.

The aim of the present study was to examine the presence of *H pylori* by culture of samples of adenoid tissue and middle ear fluid from children with FRTIs and rAOM. Furthermore, *H pylori* infection was studied by fecal antigen detection testing and by determination of serum antibody titers.

gee families of African origin who had been admitted to the Helsinki University Central Hospital, Helsinki, Finland, for elective adenoidectomy and/or tympanostomy between October 2002 and June 2003. All the children were admitted because of FRTIs and rAOM. A child was considered to have FRTIs and rAOM if he or she had experienced at least 9 upper respiratory tract infections and at least 4 episodes of AOM during the year before admittance. All the children underwent adenoidectomy under general anesthesia, and the adenoid tissue sample was sent for *H pylori* culture. Myringotomy was performed in the children with suspected effusion in the middle ear cavity. Middle ear effusions (8 children, 12 ears) were collected for *H pylori* culture. Serum samples were obtained from all 20 children during the anesthesia. The parents were asked to bring a fecal sample from their children for *H pylori* antigen detection, and 10 such samples were available. The study was accepted by the local ethical committee, and an informed written consent was obtained from the parents or guardians.

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## METHODS

The study included 20 children (14 boys; age range, 1-7 years [median age, 2 years]) from refu-

## CULTURE

Samples were cultured on *Brucella* agar plates (Becton Dickinson, Sparks, Md) supplemented with horse blood (7%). Selective *Bru-*

cella agar plates containing 1% IsoVitalax (Haartman Institute, University of Helsinki), vancomycin hydrochloride (6 mg/L), amphotericin B (2 mg/L), and nalidixic acid (20 mg/L) were also used. All the plates were incubated at +37°C in a micro-aerobic atmosphere for a maximum of 12 days.

### SEROLOGIC TESTS

Serum samples were stored at -20°C until analyzed for IgG and IgA antibodies to *H pylori* with an in-house enzyme immunoassay. The antigen used was an acid glycine extract from *H pylori* strain NCTC 11637.<sup>6</sup> The lower limits for the raised titers were 700 for IgG and 70 for IgA antibodies. In a series of adult outpatients, the assay showed a specificity of 93% and a sensitivity of 100% when compared with gastric histologic analysis.<sup>7</sup> The results of this test were seropositive in 94% of the children whose *H pylori* infection was verified by gastric biopsy-based methods (culture, histologic analysis, or biopsy urease test).<sup>8</sup>

### FECAL ANTIGEN TESTS

Fecal samples were stored at -20°C before analysis and were run in parallel in both antigen tests. The polyclonal antibody-based *H pylori* microtiter assay (Premier Platinum HpSA; Meridian Inc, Cincinnati, Ohio) was performed according to the manufacturer's instructions. Briefly, a small portion of the specimen was diluted with a sample diluent. The specimens were added to the microtiter wells with a peroxidase-conjugated polyclonal antibody and incubated for 1 hour at room temperature. After the specimens were washed, substrate was added, and the mixture was incubated for 10 minutes at room temperature. A stop dilution was added, and the results were read at 450 nm with a spectrophotometer (Titertek Multiskan; Eflab Oy, Helsinki). Cutoffs for optical density values were as suggested by the manufacturer. In cases with gray zone values, the same stool samples stored at -20°C were retested as recommended by the manufacturer.

For the monoclonal antibody-based test (Amplified IDEIA HpStAR; Dako Corp, Glostrup, Denmark), a stool suspension with sample diluent was centrifuged for 5 minutes. The supernatant and peroxidase-conjugated monoclonal antibodies were pipetted into the wells and incubated for 1 hour on a shaker. After 4 washes, followed by a 10-minute incubation with the substrate, the reaction was stopped and the results were read with a spectrophotometer. Optical density values were assessed according to the manufacturer's instructions.

### RESULTS

The fecal samples from 4 (20%) of the 20 children were positive for *H pylori* on serologic analysis and/or antigen detection testing: 3 of the 4 children were younger than 5 years and 1 was 7 years old. The results were positive in 3 of 10 fecal samples. Two of the 3 children whose samples were fecal antigen positive (aged 1 year 9 months and 7 years) had positive results on the fecal antigen tests and 1 child (aged 3 years) had positive results only on the monoclonal antibody test. The samples from 2 of these 3 children were positive for *H pylori* on serologic testing, and the sample from 1 child was seronegative. The fecal sample from 1 child (aged 3 years) whose results were seropositive was not available. The cultures of 20 adenoid samples and 12 middle ear fluid samples were all negative for *H pylori*.

In the present study, we evaluated the possible role of *H pylori* infection in respiratory infection-prone children. In this series, the samples from only 20% of the children with FRTIs and rAOM were *H pylori* positive on serologic testing and/or fecal antigen detection, and all *H pylori* cultures of adenoid tissue and middle ear fluid samples were negative, thus showing no association between *H pylori* infection and FRTI and rAOM. Our findings do not agree with those of some earlier studies that suggested that adenoid tissue acted as a reservoir of *H pylori*.<sup>3,5</sup> In those studies, more than 20% of adenoid biopsy specimens from children with either recurrent or chronic tonsillitis were positive for *H pylori* on polymerase chain reaction analysis.<sup>5</sup> Although the sensitivity of *H pylori* culture of gastric biopsy specimens is usually lower than that of other diagnostic tests, such as polymerase chain reaction analysis, high sensitivity figures can be obtained,<sup>9</sup> and as the specificity is 100%, it is a very convincing method with which to demonstrate the existence of live bacteria. Although the detection of *H pylori* by polymerase chain reaction analysis of samples of adenoid tissue may suggest that this tissue acts as a reservoir of *H pylori*, it may also represent transient rather than persistent colonization. Several studies have shown that *H pylori* may exist transiently in the mouth.<sup>10-12</sup> The transient existence of *H pylori* in the oral cavity seems to be associated with *H pylori* colonization in the stomach, but it is not known whether this reflects exposure to *H pylori* via an oral route or the presence of gastroesophageal reflux and the origin of this bacterium from the gastric lesions.<sup>10</sup>

Because the prevalence of *H pylori* is low and decreasing among Finnish children,<sup>13</sup> we studied children only from refugee families of African origin. Among African children living in Finland, *H pylori* infection is common, and 63% of the children with gastrointestinal symptoms are seropositive for *H pylori*.<sup>14</sup> In the present study, we found that samples from only 20% of the children with FRTIs and rAOM were *H pylori* positive on either serologic testing or fecal antigen detection. Since our study was conducted, several microbial treatments (often amoxicillin) have been made available that may suppress the growth of *H pylori*, but they have not been of sufficient strength to completely eradicate the bacteria. The levels of serum antibodies in children infected with *H pylori*, however, do not decrease until there is proper eradication of the bacteria.<sup>8</sup> This low rate of positivity for *H pylori* further suggests that *H pylori* infection has no role in FRTIs in children.

Although some authors who have used modern microbiological techniques have suggested that *H pylori* may have a role in upper respiratory tract infection,<sup>15,16</sup> to our knowledge no one has been able to culture the bacteria and we were not able to confirm *H pylori* colonization in samples of adenoid tissue or middle ear fluid from children with FRTIs and rAOM.

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