Helicobacter pylori: comparison of DNA fingerprints provides evidence for intrafamilial infection


Abstract
Although a high prevalence of antibodies to Helicobacter pylori has been documented within families, culture and DNA typing of strains from infected children and their parents has not been evaluated. This study aimed to analyse H pylori infection within family groups. Endoscopy, gastric biopsy, and H pylori culture were performed on all eight parents of four children who presented with dyspepsia and who had a positive H pylori culture. All biopsy specimens were cultured on Columbia based blood agar under microaerophilic conditions for four days. The DNA from each strain was extracted and electrophoretic patterns were compared after digestion with restriction endonucleases Hae III or Hind III. Ribotyping using a biotinylated cDNA probe prepared from 16S and 23S rRNA of H pylori NCTC 11638 was also used. Seven of the parents were positive for H pylori on urease testing, histology, and on culture. DNA typing showed the same or a similar strain to be present in at least two family members in three of the four family groups. In family 1, the mother, father, and child all had an identical strain; in family 2, father and son had a similar related strain; father and mother had the same strain in family 3; and all strains were unique in family 4. These data provide evidence for either intrafamilial cross infection or a common source of infection within family groups.

Methods
The parents of four H pylori positive children who had originally presented at a paediatric clinic with abdominal pain were investigated. Three antral biopsy specimens were taken – one each for culture, histology, and CLO test (Delta West). Personal and family history relevant to the gastroduodenal tract was recorded.

CULTURE
Biopsy specimens were transported in sterile normal saline 0-9% and cultured on Columbia agar with 5% horse blood (Oxoid) in a microaerophilic environment (5% O2, 10% CO2, 85% N2) for four to seven days. Colonies of typical appearance and Gram stain which produced oxidase, catalase, and urease, were identified as H pylori.

DNA TYPING
H pylori isolates from four children and their parents, plus two isolates chosen randomly from unrelated individuals were incubated on brain heart infusion agar supplemented with 5% v/v horse blood and 1% v/v isovitalex (Oxoid) for 48 hours at 37°C, under microaerophilic conditions (5% O2, 5% CO2, 2% H2, 88% N2). Chromosomal DNA was isolated and purified from each isolate using the guanidine thiocyanate reagent method. The purified DNA was incubated with the restriction endonuclease (Hae III) for four hours at 37°C and the digests were electrophoresed at 30 v for 16 hours in a horizontal agarose gel. After electrophoresis the gels were stained with ethidium bromide and photographed. Strain DNAs which did not cut with Hae III were subjected to Hind III digestion. For ribotyping, the gels were then transferred to nylon membranes by means of vacuum blotting. A biotinylated cDNA probe was prepared from 16S and 23S rRNA of H pylori NCTC 11638 using reverse transcriptase. Biotinylation was achieved by the incorporation of biotin-16-dUTP. The membranes were then hybridized by standard procedures for 16 hours at 42°C, using the biotinylated cDNA probe. Restriction digest patterns and ribopatterns were compared. Details of DNA typing methods have been described elsewhere.

HISTOLOGY
Biopsy specimens were fixed in formalin and examined using haematoxylin and eosin, Giemsa, and Warthin-Starry staining techniques.
Results

Seven of the eight parents tested were currently infected with *H pylori*. The one parent who was *H pylori* negative has histological evidence of mild inflammation. A course of ampicillin and metronidazole had been taken by this subject within four weeks of endoscopy. All subjects in the study had antral gastritis. There was complete concordance between culture, CLO test, and histology for the detection of *H pylori*. A combination of the use of total DNA restriction digest patterns (Fig 1) and ribopatterns (Fig 2) showed that the same strain of *H pylori* or subtype of the same strain was present in more than one member of three out of four families tested. DNA types were designated by the first initial of each family surname, and DNA subtypes by the addition of ‘a’ or ‘b’ (Table).

DNA from 10 of the 13 isolates of *H pylori* in the study was digested with the restriction endonuclease *Hae III*. After hybridisation with the biotinylated probe, family K isolates F1, F2, and F3 yielded identical ribopatterns (ribotypes). The ribopatterns gave identical high intensity signals with variation in the minor bands only (DNA subtypes). Isolates F6, F7 (family B), F12 (family M), and F9 and F13 (both unrelated) had unique ribopatterns. Family H isolates of *H pylori* (F4 and F5) were very closely related or subtypes of the same strain since their *Hae III* digest patterns seemed identical except for the intensity of two bands, and ribopatterns differed on the secondary (lower intensity hybridisation signal) bands, but the main bands were common to both.

DNA extracts from isolates F8 (family B), and F10 and F11 (family M) were not digested by *Hae III* (Fig 1). Isolate F8 was therefore different from the other isolates from members of this family (F6 and F7). DNA of which was cut by *Hae III*. Also, the *Hind III* hybridisation pattern showed that F8 was different from F6 and F7. Family M isolates F10 and F11, however, had the same *Hind III* ribopattern, but the third member of this family set, F12, harboured a unique strain of *H pylori*.

Discussion

This study has shown a high prevalence of *H pylori* infection in the parents of children who are *H pylori* positive. No comparison has been made between this group and parents of *H pylori* negative children, but work from other sources provides serological evidence for an increased prevalence of infection in family contacts.14 More importantly it has been shown that an indistinguishable strain of *H pylori* as assessed by its DNA fingerprint, is present in the gastric mucosa of more than one member of a family group. Members of some families harbour strains of *H pylori* with the same DNA fingerprint (family K, three isolates; family M, two isolates); some harbour subtypes or strains with similar DNA fingerprints (family H, two isolates); and some harbour different strains (family B, three isolates; family M, one isolate).

The molecular techniques used are reproducible and highly discriminatory.14,15 *Hae III* followed by *Hind III* have been found to be the most discriminatory restriction endonucleases with which to cut *H pylori*.14,15 The combination
of the use of one or other of these enzymes and the ribotyping technique applied in this study provide a highly discriminatory means of strain comparison. Thus, we suggest that our results provide strong evidence for a common source of infection with *H pylori* in some members of three of the four families studied. This study provides evidence that supports the occurrence of intrafamilial cross infection with *H pylori*, although no comment can be made from these data about the direction of transmission. An alternative explanation is that familial infection is occurring from a point source.

In family B, all members had unique strains of *H pylori* as did one member of family M and the two unrelated individuals. This suggests that other reservoirs of *H pylori* infection also play a role in its epidemiology within a family setting. The finding of identical or similar strains in more than one individual differs from those of Simor et al who included two groups of adult siblings in Canadian families. In this case different strains were found in the family members. Others have found similar strains only in repeated samples from the same individual. The epidemiology of *H pylori* infection, as far as is known, supports a faecal-oral, or oral-oral route of spread. The finding of *H pylori* in dental plaque has also been reported and supports close contact and an oral-oral route as a mode of spread. Further studies are needed to clarify the presence of *H pylori* in saliva or plaque, and also in faeces.

This work presents a preliminary investigation into the possibility of cross infection within a family. Further studies need to be carried out to determine whether the finding of similar strains of *H pylori* in different members of the same family is a frequent occurrence. It would also be important to distinguish between children and adults, and to ascertain the route of transmission if this occurs. In investigations of this type, it is unlikely to be acceptable for children to be subjected to endoscopy as a part of studies in which a parent is the index case. Studies such as this one where parents become volunteers are probably at the limit of ethical acceptance.

If further investigation supports intrafamilial cross infection as a mode of spread for *H pylori*, these findings may have important implications for the investigation of index cases and for future treatment within the family.