The Journal of Laryngology & Otology (2008), ***122***, *905–911.*

doi:10.1017/S0022215107000989

Main Article

A critical evaluation of the evidence on a causal relationship between *Helicobacter pylori* and otitis media with effusion

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Abstract

Objective: There is growing interest in the presence of *Helicobacter pylori* in the upper aerodigestive tract, and in the middle ear in patients with otitis media with effusion. Some studies have reported detecting *H pylori* in the middle ear, although reports to the contrary exist. In this study, we critically evaluate the evidence for the theory that *H pylori* in the middle ear plays a role in otitis media with effusion.

Material: We undertook a systematic review of all available studies investigating the presence of *H pylori* in the middle ear of patients with otitis media with effusion. The current literature was critically analysed using the key words and phrases '*Helicobacter pylori*' 'otitis media with effusion', 'serous otitis media', 'glue ear' and 'middle ear'. Six original research papers were identified, studying a total of 203 patients and 27 controls; two of these papers were randomised, controlled studies and four were prospective, cohort studies.

Results: At present, there is poor evidence for the existence of *H pylori*-associated otitis media with effusion.

Conclusions: Further research in the field is needed in order to delineate the presence of *H pylori* and its role in the pathogenesis of otitis media with effusion.

Key words: Otitis Media With Effusion; Gastro-esophageal Reflux; *Helicobacter Pylori*; Middle Ear

Introduction

Otitis media with effusion (OME) is one of the most common infectious diseases in the paediatric age group. Several predisposing factors have been suggested, including eustachian tube dysfunction, allergy, passive smoking, viral upper respiratory tract infections, and adenoidal hypertrophy or adenoiditis.[1,2] Recent studies have investigated the possibility of a causal relationship between OME and the presence of *Helicobacter pylori* in the middle ear.[3–8]

Helicobacter pylori is a microaerophilic, Gram-negative, spiral organism which inhabits the mucus layer overlying the gastric epithelium.[9] Worldwide, it is one of the most frequent infections. The prevalence rate of the disease is 20–50 per cent in developed countries and 80–90 per cent in underdeveloped countries. It has been shown to be associated with chronic gastritis, gastric and duodenal ulcer, and gastric cancer.[10] These bacteria are most probably transmitted by the gastro–oral route, and have been identifiable in saliva and dental plaque.[11,12] *Helicobacter pylori* has also been discovered in tonsil and adenoidal tissue, nasal polyps, nasal mucosa, and maxillary sinus mucosa.[7,8]

Direct transmission of *H pylori* from the stomach to the middle ear by reflux may occur.[13] High pepsin and pepsinogen levels have recently been demonstrated in persistent middle-ear effusions in children, suggesting a role for gastroesophageal reflux in middle-ear disease.[14] A recent series of papers linking *H pylori* to OME contains contradictory data, and the detection methods employed, and, indeed, the study groups, are inconsistent.[3–8]

In this study, we aimed to clarify the role of *H pylori* in the pathogenesis of OME, based on the current evidence base.

Material and methods

Literature search

We undertook a literature search on 21st September 2006, using the Medline database, using search terms

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Accepted for publication: 2 August 2007. First published online 26 November 2007.

including: '*Helicobacter pylori*', 'otitis media with effusion', 'glue ear' and 'middle ear'. Six original research papers were identified.[3–8] Two of these were randomised, controlled studies[3,8] and four were prospective, cohort studies.[4–7]

Randomised, controlled studies

In the first of the two randomised, controlled studies, Agirdir and co-workers investigated 45 patients between the ages of three and 13 years with a diagnosis of chronic otitis media with effusion (OME) and adenoidal hypertrophy.[3] The study group comprised 30 patients (18 males (60 per cent) and 12 females (40 per cent)). Adenoidectomy with myringotomy, with bilateral ventilation tube insertion, was performed in this group. Middle-ear effusion samples and fragments of adenoid tissue were collected and the presence of *H pylori* was investigated by rapid urease testing (termed the campylobacter-like organism test) (Table I). The control group, comprising 15 age-matched patients (nine males (66.7 per cent) and six females (33.3 per cent)) also underwent adenoidectomy with myringotomy. These patients had a dry tap on myringotomy. Middle-ear washings and adenoid tissue samples were collected from the control group. Using the campylobacter-like organism test, these control samples were also tested for the presence of *H pylori*. The campylobacter-like organism test was positive for the middle-ear effusion samples of 20 (66.6 per cent) patients in the study group. None of the control group patient middle-ear washings had a positive campylobacter-like organism test result. There was no significant difference between the campylobacter-like organism test positivity for the adenoid tissue samples of the two groups. Agirdir *et al.* concluded that their findings demonstrated the presence of *H pylori* in middle-ear effusion fluid, and surmised that this may be responsible for the pathogenesis of OME.

The second randomised, controlled trial, by Yılmaz and co-workers, studied aspirated middle-ear fluid and biopsies from the middle-ear promontory of 22 children with OME (nine girls and 13 boys, age range two to 13 years).[8] All Children diagnosed with OME, adenoid hypertrophy and chronic tonsillitis underwent bilateral ventilation tube insertion and adenoidectomy and tonsillectomy. The control group comprised 20 children undergoing adenotosillectomy for chronic adenotonsillitis, without any signs of middle-ear disease. In this control group, a small biopsy was taken from the promontory after myringotomy. In both groups, 5-mm deep tissue specimens were obtained from the tonsils and adenoids, together with gastric juice samples, for *H pylori* analysis (Table I). Polymerase chain reaction

TABLE I

SUMMARY OF STUDIES ADDRESSING RELATIONSHIP BETWEEN *HELICOBACTER PYLORI* AND OTITIS MEDIA WITH EFFUSION

Study	Tissue	Controls (*n*)	Method	Sample size (*n*)	Positive detection rate (%)	Conclusion
Agirdir *et al.*[3]	Middle-ear effusion Adenoids		CLO	45	66 33	Presence of *Hp* in middle-ear effusion
Yılmaz *et al.*[8]	Middle-ear effusion Middle-ear biopsy	20	PCR	22	15 35	Bacteria may be involved in OME pathogenesis
Bitar *et al.*[4]	Middle-ear fluid Adenoids		Rapid-urease reaction Culture PCR	28	77	No evidence that *Hp* colonises nasopharynx of children with middle-ear disease
Morinaka *et al.*[6]	Middle-ear effusion (smears) Gastric mucosa	7	Immunohistochemistry Giemsa CLO	15 12	80 93 20	*Hp* may exist in middle-ear effusion of some OME patients
Yılmaz *et al.*[7]	Middle-ear fluid Tonsils Adenoids		PCR	38	47	Presence of *Hp* in middle ears of children with chronic OME
Karlidag *et al.*[5]	Middle-ear effusion		PCR	55	16 3	Interesting implications for possible role of *Hp* in OME

CLO = campylobacter-like organism test; *Hp* = *Helicobacter pylori*; PCR = polymerase chain reaction; OME = otitis media with effusion

https://doi.org/10.1017/S0022215107000989 Published online by Cambridge University Press

analysis and microbiological culture indicated a statistically significant ($p < 0.05$) increase in the presence of *H pylori* in gastric juice and in tonsil, adenoid and middle-ear promontory samples in the study group, compared with the control group.

Prospective, cohort studies

Bitar and colleagues studied 18 consecutive paediatric patients (mean age 4.4 years; age range three to eight years; equal gender distribution) undergoing myringotomy and adenoidectomy for chronic OME or recurrent otitis media.[4] Aspirated middle-ear fluid samples were cultured on three types of agar (Brucella + laked horse blood, Brucella + sheep blood, and 'chocolate'). A double polymerase chain reaction test was used to detect urease-C and adhesion subunit genes. Adenoid specimens underwent rapid urease enzyme and polymerase chain reaction testing (Table I). Middle-ear cultures were negative in all three media, for all 28 middle-ear fluid samples. Twenty-one of the 28 samples contained deoxyribonucleic acid (DNA), but polymerase chain reaction analysis revealed that none of this DNA was from *H pylori*. Ten of the 13 adenoid specimens obtained were positive for *H pylori* on rapid urease testing, but none were positive on polymerase chain reaction.

Morinaka and co-workers performed another prospective, nonrandomised study with nonpaired, nonmatched controls.[6] They collected 15 effusion fluid samples from 15 patients with OME (male:female = 4:13; age range three to 85 years; mean age 54 years). Twelve gastric tissue biopsy samples from seven *H pylori* infected patients with gastric or duodenal ulcers were used as positive controls. Microscopy smears were prepared from the samples and immunostained with anti *H pylori* antibody, as well as being subjected to Gram and Giemsa staining. In addition, the middle-ear effusion and gastric tissue biopsy samples underwent campylobacter-like organism testing (Table I). The immunostained smears revealed spiral or curved, immunoreactive organisms in 12 (80 per cent) of the 15 middle-ear effusion specimens. The Giemsa-stained smears showed blue, spiral or curved organisms in 14 patients' samples. Three patients with positive immunohistochemistry also had a positive campylobacter-like organism test for OME samples. All 12 control gastric biopsy specimens from the seven positive control patients tested positive for *H pylori* using immuno- and Giemsa staining. Gram-staining showed Gram-negative bacteria.

Yılmaz and colleagues divided 38 consecutive children (average age 7.85 years) with adenoid hypertrophy and/or OME into two groups.[7] The first group comprised 18 subjects with OME and adenoid hypertrophy, and the second group comprised 20 subjects having only adenoid hypertrophy. Each patient underwent the appropriate surgical procedure: myringotomy, placement of tympanostomy tubes and/or adenoidectomy. At myringotomy, middle-ear effusion samples were collected using a suction and collection device, and a core biopsy specimen was taken from each piece of adenoidal tissue following adenoidectomy. Deoxyribonucleic acid extracted from these samples was used to amplify the 23S ribosomal ribonucleic acid gene of *H pylori* by real-time polymerase chain reaction. In the first group, 34 effusion samples were obtained from the ears of 18 patients (two had unilateral OME). Real-time polymerase chain reaction analysis for *H pylori* was positive for 12 of the 18 children (67 per cent) and for 16 of the 34 ears (47 per cent). No positive reaction was seen in these patients' adenoid tissue samples. In the second group, a positive reaction was seen in the adenoid tissue of only one patient.

Karlidag and co-workers studied 38 patients with OME.[5] In all cases, myringotomies were carried out (age range two to 12 years; male:female = 23:15). Aspirated middle-ear effusion samples were analysed by polymerase chain reaction. A total of 55 aspiration samples was collected. Seventeen patients had bilateral effusions, whereas 21 had unilateral effusions. Nine (16.3 per cent) of the 55 middle-ear effusion samples were shown to be *H pylori* positive by polymerase chain reaction.

Discussion

The behaviour of pathogenic bacteria within the middle ear is poorly understood. In recent years, advances in animal model research have led to major improvements in our knowledge of host–pathogen interactions in otitis media with effusion (OME).[13,15] However, OME, characterised by the persistence of a middle-ear effusion for three months or more, remains an unsolved problem. Recently, the relationship between *H pylori* and OME has been evaluated.

Bacteriological studies of OME using highly sensitive molecular biology techniques, such as polymerase chain reaction, have demonstrated that traditional culturing methods are inadequate to detect many viable bacteria present in OME. The presence of pathogens attached to the middle-ear mucosa as a bacterial biofilm, rather than as free-floating organisms in a middle-ear effusion, has previously been suggested to explain these observations.[16]

The majority of the authors of papers identified in our systematic review concluded that they found interesting indications for a possible role of *H pylori* in OME. All of these authors proposed that further studies are needed to investigate the role of *H pylori* in the aetiology of OME.

Other authors who detected the presence of *H pylori* in the upper aerodigestive tract and middle ear have speculated that the presence of *H pylori* in the middle ear, adenoid and tonsillar tissue is most probably related to reflux of gastric contents into the pharynx.[17] Acid reflux leads to mucosal inflammation and oedema in the nasopharynx, disturbing eustachian tube clearance and thus enabling nasopharyngeal bacteria to enter the middle ear. *Helicobacter pylori* may be one such

bacterial species. Adenoid tissue may also act as a nidus of infection, disseminating *H pylori* to the middle ear via the eustachian tube in patients with OME. This theory is supported by evidence of significantly increased *H pylori* colonisation in the adenoids of patients with OME, compared with those with adenoid hypertrophy without OME.[8]

Is there a suitable microenvironment to sustain H pylori growth?

In order to reproduce itself, *H pylori* needs a microaerobic environment (i.e. 10 per cent CO_2). Such a microaerobic environment is present in the middle ears of OME patients. The pH value of the luminal side of the mucus layer is 1.0–2.0, but on the mucosal side, which *H pylori* inhabits, it is approximately 5–7.[9] The pH value of middle-ear effusions in chronic OME patients was found to be between 7.0 and 9.0. Such an alkaline environment will allow *H pylori* to survive; however, its growth will be impeded. *Helicobacter pylori* requires an acidic environment in order to survive in the presence of urea.[18,19] None of the reports of its presence in extragastric sites are completely proven, since *H pylori* is highly localised to its ecological niche in the gastric mucus and needs dedicated conditions for growth.[20] The presence of *H pylori* in other tissues might be explained by its existence as a transient pathogen. The reported finding of *H pylori* in the upper aerodigestive tract may give a false impression of its ability to survive in a 'hostile', non-acidic environment, as demonstrated by Berloco *et al.*,[21] who showed daily variation in the amount of *H pylori* genomic material detected in the saliva of *H pylori* infected patients. More studies are needed to clarify this issue before a clear and definitive conclusion is reached regarding the significance and behaviour of *H pylori* detected in the upper aerodigestive tract.

How is the infection transmitted?

The exact route of *H pylori* transmission is still not clear. Most infections seem to be caused by human-to-human contacts within the family[22,23] through a 'gastro–oral' route.[24] Faecal and oral shedding of *H pylori* from healthy but infected children has been demonstrated.[25] A faecal–oral route,[26,27] possibly involving contaminated water,[28] has been discussed. Data on intrafamilial transmission of *H pylori* among children continue to be produced. Due to improved living standards, the prevalence of *H pylori* infection in industrialised countries has fallen to 5–50 per cent, depending on age.[29,30] As it is not present in blood, *H pylori* may travel into the middle ear via gastro-esophageal reflux.

Tasker *et al.* investigated the presence of gastric juice in the middle ear in 65 children with chronic OME; they found that 91 per cent of their effusion samples contained pepsin and pepsinogen at levels that made gastric juice the most likely source.[14] However, the antigens used in this study were not specific and showed massive cross-reactivity in middle-ear samples.

A study by Rowland *et al.* also examined prospectively the age-specific incidence of *H pylori* in children aged younger than 24 months.[31] They found that children in a developed country such as Ireland become infected at a very young age, usually before five years of age. According to these authors, because of the limitations of the ^{13}C urea breath test in very young children, the incidence of infection could not be evaluated in children younger than two years.

Which test to apply?

Many diagnostic methods have been developed to detect *H pylori* infection, both invasive (rapid urease test, histological analysis, microbiological culture and polymerase chain reaction) and non-invasive (serological analysis, urea breath test and, more recently, analysis for *H pylori* antigen in faeces). At present, no single test is absolutely reliable in detecting *H pylori* infection. If feasible, a combination of two tests is recommended.

The rapid urease test is a simple, inexpensive, quick and reliable method for detecting urease activity in a specimen, and it is considered by clinicians to be the initial test of choice for the diagnosis of *H pylori*.[32] The campylobacter-like organism test, an agar gel test, is the most widely used and best-studied rapid urease test.[33] The rapid urease test is based on the ability of *H pylori* to produce urease, which is not usually present in the stomach. Urease produced by the organism converts urea in the medium to ammonia, resulting in a rise of pH to values above 8 in the weakly buffered medium, causing a colour change in a pH indicator. Rapid urease tests are inexpensive, quick, reliable and can easily be performed in a local laboratory. This is in contrast to culture and histology, which usually take several days to obtain a result. The campylobacter-like organism test had a specificity and positive predictive value of 100 per cent, although its sensitivity is in the range of 90–99 per cent.

The rapid urease test has a high sensitivity in adults but a low sensitivity in children, possibly because the test depends on the concentration of *H pylori* for adequate urease activity.[34] A positive rapid urease test in non-gastric tissue does not necessarily indicate the presence of *H pylori*, as the test is designed to detect a pH rise in an acidic sample, as in *H pylori* samples in the stomach. Use of the rapid urease test in a non-gastric, non-acidic tissue such as the adenoids and middle ear may lead to a high rate of false positive results, due a non-urease-dependent pH rise and to a high number of other bacteria, some of which may produce urease. Therefore, the campylobacter-like organism test will become positive in testing samples with a high buffer value at pH values above 8 (such as middle-ear effusion samples, in the case of OME) and will remain negative in neutral solutions with low buffer values (such as washings from a dry tap). Similar false positive results have been reported in achlorhydric patients, who are believed to have stomachs colonised by commensal organisms capable of producing urease. False

https://doi.org/10.1017/S0022215107000989 Published online by Cambridge University Press

positive results were investigated by Bitar *et al.*;[4] they found a high level of evidence for the presence of *H pylori* in adenoid specimens, using the campylobacter-like organism test. However, polymerase chain reaction testing of the same adenoid specimens failed to detect *H pylori*.

Although several diagnostic tests are available for the detection of *H pylori* infection, all of them have both advantages and disadvantages, and none can be considered as a single 'gold standard'. A combination of biopsy-based methods (histological examination and culture) usually gives the most reliable diagnosis.

Assessing the six studies analysed, Agirdir *et al.* detected *H pylori*, using the campylobacter-like organism test, in 66.6 per cent of OME cases.[3] Yılmaz *et al.*, using a combination of polymerase chain reaction and culture, found *H pylori* in 45 per cent of their OME patients.[8] Morinaka *et al.* found that 80 per cent of OME fluid samples analysed were positive for *H pylori* by immunostaining; however, only 20 per cent had viable bacteria which reacted positively in the campylobacter-like organism test.[6] Yılmaz *et al.* detected *H pylori*, using polymerase chain reaction, in 47 per cent of OME samples.[7] Using the same method, Karlidag *et al.* detected *H pylori* in 16.3 per cent of OME samples.[5] Bitar *et al.* failed to detect *H pylori* by either culture or polymerase chain reaction, and concluded that *H pylori* plays no part in OME in children and does not colonise adenoid tissue.[4]

In the studies assessed, bacterial culture of *H pylori* had the highest rate of false negative results, which decreased its sensitivity (80 per cent), in comparison with other tests (86–100 per cent). However, its specificity was high (100 per cent), as it constituted definite proof of the presence of viable bacteria.[35] Despite this, difficulties in the isolation and culture of *H pylori*, and the demanding technical requirements involved, do not allow culture to be used as a single gold standard.

- **There is growing interest in the presence of *Helicobacter pylori* in the upper aerodigestive tract and in the middle ears of patients with otitis media with effusion (OME)**
- **Direct transmission of *H pylori* from the stomach to the middle ear by reflux may occur**
- **This study critically evaluated the evidence for a possible role of *H pylori* in the middle ear with OME**
- **At present, there is poor evidence for the existence of *H pylori*-associated OME, and further research in the field is needed**

Bacterial culture indicates the presence of living bacteria; however, polymerase chain reaction indicates the presence of bacterial DNA rather than the bacteria itself. Each method complements the other. When both are applied together, the sensitivity and specificity of results increase.

The anti *H pylori* antibody test has an accuraty between 80–90 per cent.[36] Giemsa staining has been reported to have a sensitivity of 91 per cent and a specifity of 100 per cent.[37]

In the study by Morinaka *et al.*, even though the majority of OME samples were positive for the presence of *H pylori*, only three of the 15 samples showed viable organisms.[6] This shows that, although there may be reflux of gastric juice into the nasopharynx or middle ear, this in itself fails to prove that *H pylori* can survive in the middle ear and influence its physiology.

Can we distinguish between H pylori detection and infection?

The reason why some individuals remain infected with *H pylori* for life without displaying any symptoms, while others develop severe disease, has only been partially clarified. Presumably, it depends on multifactorial interactions between host immunological and physiological factors, bacterial virulence determinants, and environmental influences modulating the host response. The ability of *H pylori* to overcome defence mechanisms on mucosal surfaces, as well as to modulate the immune response by interfering with the gastric epithelial and immune cells seem to be the crucial factors for the infection[38], however, a lot of questions are still unsolved. In contrast to the widely accepted "oral-oral" route of *Helicobacter pylori* transmission, it is possible to detect molecular traces of *H. pylori* in the environment.[39] Since it is not possible to culture *H. pylori* from these samples, molecular traces do not indicate a colonization. In the studies assessed, efforts to isolate *H pylori* from OME effusion samples by culture also often failed. In some cases this may indicate a correct detection of *Helicobacter* traces without colonisation or infection.

Is the control group reliable?

Rees *et al.* showed that, in 13 per cent of patients with proven middle-ear effusion prior to anaesthesia, there was some displacement of the effusion during the early stages of nitrous oxide anaesthesia.[40] In the study by Bitar *et al.*, general anaesthesia was induced via thiopental and continued using a combination of nitrogen protoxide, oxygen and sevoflorane, in each patient. Therefore, the selection of controls in the studies assessed seems inappropriate and implies a methodological bias.

Conclusion

From the studies assessed, we found no conclusive evidence for the existence of *H pylori*-associated otitis media with effusion (OME) in children. Reported data may suggest that acid reflux is the underlying aetiology explaining the presence of *H pylori* as a contaminant in the middle ear. All reviewed papers used indirect methods for the detection of *H pylori*, such as the campylobacter-like

https://doi.org/10.1017/S0022215107000989 Published online by Cambridge University Press

organism test, polymerase chain reaction without sequence confirmation, or immunohistochemistry. All currently published findings require additional confirmation. Further research in this field is strongly needed in order to delineate the presence of *H pylori* and to establish its role in the pathogenesis of OME.

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Prof Dr H Sudhoff takes responsibility for the integrity of the content of the paper.
Competing interests: None declared

https://doi.org/10.1017/S0022215107000989 Published online by Cambridge University Press