# Impact of household hygiene and water source on the prevalence and transmission of *Helicobacter pylori*: a South Indian perspective

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## ABSTRACT

**Introduction: In developing countries, the *Helicobacter pylori (H. pylori)* infection rate is high, especially in lower socioeconomic groups. The populace in developing countries lives in conditions that are highly conducive to the acquisition of microorganisms. Poor hygiene, crowded household conditions and deficient sanitation mark their day-to-day life. We aimed to find out the roles of household hygiene and water source in the prevalence and transmission of *H. pylori* infection among the South Indian population using polymerase chain reaction (PCR) assay.**

**Methods: The selected population consisted of 500 adults of varying ages ranging from 30 to 79 years, with upper gastrointestinal tract symptoms. Each participant in the study was given a questionnaire to complete. Samples to assess *H. pylori* infection included three gastric biopsies (two from the antrum and one from the corpus region). Infection was detected by PCR amplification of the 16S rRNA gene of *H. pylori*. The data was then examined statistically by univariate and multivariate analyses.**

**Results: The overall prevalence of *H. pylori* was detected to be 80 percent. Prevalence increased with an increase in age and it was found to be 90 percent in the 70-79 year age group (p-value is less than 0.01). The prevalence of infection among people who drank water from wells was 92 percent compared with 74.8 percent of those who drank tap water (p-value is less than 0.001). *H. pylori* infection prevalence was found to be higher in people with low clean water index (CWI) (88.2 percent) than in those with higher CWI (33.3 percent) (p-value is less than 0.001). While the prevalence of *H. pylori* in the subjects with lower socioeconomic status was 86.1 percent, in higher groups, it was 70 percent (p-value is less than 0.001). The prevalence of *H. pylori* was also found to be higher in subjects who lived in overcrowded houses. It was 83.7 percent with high crowding index, 76.6 percent with medium crowding index, and 71.3 percent with low crowding index (p-value is less than 0.05).**

**Conclusion: The results of the present study suggest that the risk of acquisition and transmission of *H. pylori* can be prevented to a large extent by following improved household hygienic practices, proper waste disposal measures as well as the regular use of boiling water for drinking purposes.**


**Keywords: *Helicobacter pylori*, household hygiene, hygiene, polymerase chain reaction assay, water source, 16S rRNA gene**

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## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) was isolated in 1983 for the first time and since then, it has been associated with gastritis and gastritis-related diseases, peptic ulcer, gastric adenocarcinoma, and primary gastric lymphoma.[1,2] The *H. pylori* infection has emerged as one of the most common chronic bacterial infections worldwide, and affects more than half of the world's population, with most of the infections occuring in the first decade of life.[3] The prevalence of *H. pylori* infection differs equally among and within populations, and is inversely related to standards of living and sanitary practice. The increased risk of *H. pylori* infection is especially high among those living in developing countries and in lower socioeconomic groups in the developed world,

possibly because they are exposed to conditions that are conducive to the acquisition of the microorganisms. These conditions include precarious hygiene standards, crowded households and deficient sanitation.[4] Interaction with potentially contaminated environmental sources, such as local drinking water, swimming in rivers, and the ingestion of faecal-contaminated vegetables, have also been reported as risk factors for the acquisition of *H. pylori* infection.[5-11] These factors play a vital role not only in escalating the prevalence of *H. pylori* infection, but also in the transmission of this bacterium.

It has been found that the rate of *H. pylori* infection differs among groups as well as within the population. Attempts have been made to understand the reasons for this. It might be due to the genetic susceptibility to *H. pylori* infection and its outcome, but this appears to be less important than environmental factors. This, in turn, might be influenced by cultural background, as well as social, dietary, or other environmental factors.[12] This explains why different population groups have different *H. pylori* prevalence rates and this also points to the importance of environmental factors and cultural background, in the mounting prevalence and transmission of *H. pylori* infection.

As with the rest of the world, *H. pylori* has remained at the centre stage of medical research in India. Yet in India, very critical aspects of this bug still have not been satisfactorily addressed. The explanation may be that most of the epidemiological studies conducted in our country may have suffered from methodological shortcomings. However, using of contaminated drinking water, improper disposal of human excreta, lack of personal and food hygiene, and improper disposal of solid and liquid waste, have all been reported to be the major causes of many diseases in developing countries like India.[13] This shows a direct relationship between water, sanitation, health, nutrition, and human well-being. According to previous studies, the environment in India is contaminated and gastrointestinal infections – both symptomatic and asymptomatic – are very common.

These points prepared the ground for the present study which attempts to find out the roles of household hygiene and water source in the prevalence and transmission of an emerging infection, i.e. *H. pylori* infection, with the help of polymerase chain reaction (PCR) assay.

Due to the difficulty of culturing from sites other than gastric mucosa,[14] and the need for the noninvasive diagnostic methods, interest has grown in the use of molecular techniques for detection of this species. The use of gene-specific probes has been described for the detection of *H. pylori* in biopsy specimens,[14,15] and progress has been made with the use of PCR. The PCR provides a specific and highly sensitive means of detecting microbial pathogens in clinical material. PCR assays have detected *H. pylori* DNA in fresh gastric biopsy specimens,[16-20] faeces,[21,22] saliva,[19,20] and dental plaque. These PCR assays are mostly based on the urease gene sequences, the 16S ribosomal RNA (rRNA) gene and adhesin gene, etc. The 16S rRNA gene of *H. pylori* is a highly specific target for amplification and has been used previously.[23,24] Weiss et al demonstrated the specificity of unique *H. pylori* gene primer to identify the organism in paraffin-embedded gastric biopsy specimens.[25] The aim of the current study was to find out the roles of household hygiene and water source in the prevalence and transmission of *H. pylori* infection in the populace of South India with the help of PCR assay.

## METHODS

The subjects consisted of 500 adults (300 males and 200 females), with ages ranging from 30 to 79 years, and who visited the Deccan College of Medical Sciences and Research Centre Hyderabad with upper gastrointestinal tract symptoms. Those who used antacids, antibiotics, or $H_2$-receptor antagonists were excluded from the study. Informed consent was obtained from all participants. Each participant in the study was asked to fill a questionnaire. Individuals were questioned regarding the presence and the regularity of symptoms referable to the upper gastrointestinal tract, including indigestion, heartburn, vomiting sensations and any type of epigastric or hypochondrial pain. Questions were related to social and economic data (subjects' education, occupation, and family income). Household hygiene questions concerning the water source used for drinking and bathing (i.e. tap or well), and excreta disposal facilities (indoors or outdoors) were also posed. Questions regarding sanitation practices included details about frequency of bathing, reuse of water, and boiling of water before drinking. We also obtained information about the living conditions of the subjects, such as the number of rooms, number of persons residing in the home, etc. Other questions included the growing up conditions of the subjects, i.e. in the city or villages.

Clean water index (CWI) and crowding index were prepared as described in an earlier study.[29] Categorisation was based on a combination of three factors: regularity of boiling water before drinking, frequency of restoring and reusing water, and frequency of bathing and showering. Three levels were identified, ranging from high to low. The density or crowding in the home was assessed using a crowding index defined as the total number of family members in the home divided by the total number of rooms in the home. The crowding index was scored as low (scores 0–1), middle (scores 2–3), or high (score > 3).[29] Categorisation of socioeconomic status was based on the occupation and educational level of subjects using a modification of the Hollingshead index.[46] Four

educational levels and five occupational categories were used to identify socioeconomic conditions. Two socioeconomic statuses were identified in our population: upper and lower.

Samples to assess *H. pylori* infection included three gastric biopsies (two from the antrum and one from the corpus). The three gastric biopsies were collected, one in urea solution for rapid urease test, one in 10% buffered formalin for histological analysis, and one in phosphate buffer saline for DNA isolation for PCR assay. Genomic DNA was isolated from all samples as per the standard protocol by the cetyltrimethyl ammonium bromide method.[26] Two 20-base oligonucleotide primers designated 16S rRNA-F (5'-TAAGAGATCAGCCTATATGTCC-3') and 16S rRNA-R (5'-TCCCACGCTTTAAGCGCAAT-3') as reported in our earlier study were selected.[27] The amplified product of these two primers with DNA prepared from the clinical isolates, and from the type strain of *H. pylori*, (ATCC 26695) was a 534 bp fragment.

PCR amplification was performed, which included initial denaturation at 95°C, for five minutes. A total of 40 cycles were performed, with one cycle consisting of 30 seconds at 94°C, 30 seconds at 52°C, and one minute at 72°C. The final cycle included a ten-minute extension step to ensure full extension of the PCR products. Amplification was performed in a thermocycler (M J Research Inc, Watertown, USA). DNA of the ATCC-type strain was used as a positive control in each set of PCR assays while negative control consisted of all the reagents of the master mix except the template DNA. The PCR-amplified products were analysed by agarose gel electrophoresis. Samples were scored as positive when a band of 534 bp was detected on the agarose gel. The Chi-square test was used to assess the associations between each independent factor of the study and the prevalence of *H. pylori* infection. Univariate analyses were calculated for *H. pylori* positivity associated with the study variables. Risk factors that are more significant in univariate analyses and are more prone to transmission of this bacterium were used in the multiple logistic regression models. These models help to assess the relative importance of *H. pylori* risk factors while controlling other risk factors. The data was analysed using the Statistical Package for Social Sciences version 12.0 (SPSS Inc, Chicago, IL, USA).

## RESULTS

A total of 500 subjects participated in the study. These were adults ranging in age from 30 to 79 years, and the overall prevalence of *H. pylori* was 80%. *H. pylori* prevalence increased to 90% at ages 70 and older. With the increase in age, the prevalence of *H. pylori* infection also increased. At the age of 30–39 years, the prevalence was 74.8%. At the age of 40–49 years, it increased to 85.8%; at the age of 50–59 years, the prevalence was 88.3%; at the age of 60–69 years, the prevalence was 90%; and by the age of 70–79 years, it was 90% ($p < 0.01$). There was no significant difference in the overall prevalence of *H. pylori* infection between males and females. It was 81% in males while it was 78.5% in females ($p > 0.05$).

*H. pylori* prevalence was found to be variable with respect to the source of drinking water. The prevalence of infection among well-water drinkers was found to be 92% as compared to 74.8% among those who drank tap water ($p < 0.001$). The infection was significantly higher among subjects with low CWI (88.2%), when compared to those with middle (80%) and high CWI, (33.3%) ($p < 0.001$). *H. pylori* infection was significantly more common among those who used outdoor toilet facilities, compared to those who used indoor facilities. The infection rate was 77.1% for those using indoor toilets, while it was 86.6% for those using outdoor toilets ($p < 0.05$). Those households, in which the participants' educational and occupational levels were low, had an 86.1% incidence of *H. pylori* infection, compared to 70% in those from the upper socioeconomic strata ($p < 0.001$). The prevalence of *H. pylori* was also found to be higher in those subjects whose houses were overcrowded. It was 83.6% for households with a high crowding index while it was 76.6% with a medium index, and 71.2% with a low crowding index ($p < 0.05$). We also found that those subjects who spent their formative years in villages were more prone to *H. pylori* infection in comparison to those who spent their childhood in cities. The prevalence rates were 90% and 75%, respectively ($p < 0.001$).

## DISCUSSION

The prevalence of *H. pylori* infection is diverse in different parts of the world. In our present study, we found that the prevalence of *H. pylori* increased with age, going up to 90% in the 70–79 year age group. The higher prevalence of infection in older population groups is explained as follows: the existence of lower socioeconomic conditions during the childhood of older people may have resulted in a higher incidence of infection when they were young, with the chances of contracting an infection increasing subsequently with age.[28] (Table I).

In our current study, we also found a successful association between the prevalence of *H. pylori* infection and use of water, e.g. CWI, as publicised in one of the earlier studies.[29] We also found that CWI was a very simple and accurate marker of household hygiene (Table II).[29] With the help of CWI, we found that those subjects who exhibited high CWI had a low prevalence rate (33.3%), those with middle CWI had a prevalence of 80%, whereas those who never boiled water before drinking,

took a bath less than once a week, and always stored and reused water showed a prevalence rate of 88.2%. The results we obtained are similar to the results from a study conducted in Kazakhstan using the same parameters for household hygiene.[29] The findings of the present study indicated that those subjects who use municipal water for drinking purposes are more susceptible to *H. pylori* infection than those who took boiled water regularly. The current results are supported by the fact that *H. pylori* DNA has been detected in water samples in India by PCR.[30]

We found that household hygiene also plays an important role in the transmission of this bacterium, and enhanced household hygiene can be helpful in reducing bacterium transmission. We also studied the prevalence of *H. pylori* in relation to the socioeconomic status of the subjects, which is also considered to be a reliable marker, to gauge the level of household hygiene.[31] According to the results obtained, we did not find any significant correlation between the prevalence of *H. pylori* infection and socioeconomic status of the subjects. The prevalence of *H. pylori* infection in subjects of high and low socioeconomic status was 70% and 86.1%, respectively (Table I). These results are supported by a study done in Peru, which suggests that water source is more important than socioeconomic status of the subjects in *H. pylori* acquistion and its transmission.[8]

Another important parameter of household hygiene is the crowding within the houses. This is also considered as one of the significant indicators of household hygiene, and one of the major risk factors in the transmission of *H. pylori* infection.[32-37] By reemploying this marker in our study, we found a high prevalence of *H. pylori* infection in those households with a high crowding index (83.7%). For those with a medium crowding index, the prevalence rate was 76.7%, and in the low crowding index, it was 71.3% (Table I). This indicates that intrafamilial transmission of this bacterium is possible. These results are supported by an earlier study conducted in the northern part of India. An infection rate of 83% was found in the spouses of *H. pylori* positive subjects, in comparison to 28.5% in spouses of *H. pylori* negative subjects.[38]

Another parameter related to household hygiene, was the role of indoor and outdoor toilets. In this study, we found that those subjects who were using indoor toilet facilities were less prone to develop *H. pylori* infection (77.1%) than those who were using outdoor toilet facilities (86.6%) (Table I). The results of our study are supported by the fact that the faeces disposed in the environment near the home may be easily accessible by animals or children. After the detection of *H. pylori*

**Table I. Prevalence of *H. pylori* infection according to the study variables.**

| Parameters | Total subjects | *H. pylori* positive | Prevalence (%) |
|---|---|---|---|
| Age (years) | | | |
| 30–39 | 290 | 217 | 74.8 |
| 40–49 | 120 | 103 | 85.8 |
| 50–59 | 60 | 53 | 88.3 |
| 60–69 | 20 | 18 | 90.0 |
| 70–79 | 10 | 09 | 90.0 |
| Gender | | | |
| Male | 300 | 243 | 81.0 |
| Female | 200 | 157 | 78.3 |
| Source of drinking water | | | |
| Tap water | 350 | 262 | 74.8 |
| Well/river | 150 | 138 | 92.0 |
| Socioeconomic status | | | |
| Upper | 190 | 133 | 70.0 |
| Lower | 310 | 267 | 86.1 |
| Sanitation practices | | | |
| Indoor | 350 | 270 | 77.1 |
| Outdoor | 150 | 130 | 86.6 |
| Place of growing up | | | |
| City | 350 | 265 | 75.7 |
| Village | 150 | 135 | 90.0 |
| CWI | | | |
| High | 60 | 20 | 33.3 |
| Middle | 100 | 80 | 80.0 |
| Lower | 340 | 300 | 88.2 |
| Crowding index | | | |
| High | 300 | 251 | 83.7 |
| Middle | 120 | 92 | 76.7 |
| Low | 80 | 57 | 71.3 |

**Table II. Parameters used in calculating clean water index.**

| Clean water index | Storing and reusing water | Frequency of bathing | Boiling water before drinking |
|---|---|---|---|
| High | Never storing or reusing water | At least 2–3 times per week | Consistently boiling water before drinking |
| Middle | Sometimes storing and reusing water | Less than 2–3 but more than once per week | Sometimes boiling water before drinking |
| Low | Always storing and reusing water | Once per week or less | Never boiling water before drinking |

in faeces, we were able to conclude that easy access to faecal deposits may lead to the transmission of *H. pylori* infection. The use of indoor toilets may therefore prevent *H. pylori* transmission.[39,40]

When comparing people who consume unboiled tap water with those who use well water for drinking, we found that those subjects who drink unboiled tap water showed a prevalence of 74.8%, against those who were consuming well water (92%) (Table I). The current study supports our previous study in which we have shown that the prevalence of *H. pylori* infection is higher in those who were drinking tap water without prior boiling. This observation is also supported by the fact that *H. pylori* is possibly present in surface or ground water[11,41-43] (Fig. 1). In another example, a study from Ethiopia also reported a high prevalence of *H. pylori* among those who drank well water compared to those who consumed river or piped water.[44] Shallow wells are well known to be susceptible to contamination, particularly if there is inadequate sealing of the well close to the surface. Even in the United States, 42% of private wells are reported to be contaminated by bacteria as shown by an increase in coliform bacteria.[45]

Another parameter, which is also considered as one of the important parameters to uncover the status of personal hygiene and household hygiene, is the place where the subjects spent their childhood, because epidemiologic data obtained in adults suggests that the actual colonisation of *H. pylori* is determined by childhood factors. In our study, we obtained information about where the subjects

**Table III. Prevalence of *H. pylori* infection in rural and urban population in relation to the clean water index.**

| CWI | High | Middle | Low |
|---|---|---|---|
| Urban | 60/20 (33.3%) | 90/72 (80.0%) | 200/173 (86.0%) |
| Rural | 20/11 (55.0%) | 10/8 (80.0%) | 120/116 (96.6%) |
| Total | 80/31 | 100/80 | 320/289 |

**Fig. 1** Photograph shows the sewage water pipeline is one of the potential hazards causing *H. pylori* transmission in India. The pipeline is leaking near the gutter and the buffalos are grazing in it. This is a common picture in most of the cities in India presenting the view of water contamination and transmission of water-borne diseases.

**Table IV. Logistic regression of variables responsible for transmission of *H. pylori* infection and its association with *H. pylori* positivity.**

| Variables | Total subjects | Total positive | Percentages | Odds-ratio | 95% CI |
|---|---|---|---|---|---|
| Tap water | 350 | 262 | 74.8% | Referent | Referent |
| Well/river | 150 | 138 | 92.0% | 3.413 | 1.66–6.977 |
| Socioeconomic status | | | | | |
| Upper | 190 | 133 | 70.0% | Referent | Referent |
| Lower | 310 | 267 | 86.1% | 2.923 | 1.704–5.016 |
| Place of growing up | | | | | |
| City | 350 | 265 | 75.7% | Referent | Referent |
| Village | 150 | 135 | 90.0% | 2.836 | 1.461–5.508 |
| CWI | | | | | |
| High | 60 | 20 | 33.3% | Referent | Referent |
| Middle | 100 | 80 | 80.0% | 8.847 | 3.982–19.657 |
| Low | 340 | 300 | 88.2% | 13.921 | 6.994–27.709 |
| Crowding index | | | | | |
| Low | 80 | 57 | 71.3% | Referent | Referent |
| Middle | 120 | 92 | 76.7% | 1.318 | 0.614–2.830 |
| High | 300 | 251 | 83.7% | 2.590 | 1.309–5.126 |

grew up, in a rural or urban setting, in order to gauge the prevalence of *H. pylori* infection. The results obtained were 90% and 75.7% infection rates, respectively. These results are quite similar to the results obtained from the previous studies done in developing countries, showing high prevalence of *H. pylori* infection in the rural population in comparison to the urban population.[47] But one question arises as to why rural inhabitants showed a high prevalence of *H. pylori* infection in contrast to urban dwellers. A previous study done in India showed a higher prevalence of *H. pylori* infection in an urban population compared to a rural population[48] (Table I).

To answer these questions, we correlated the rural and urban populace with CWI, which is considered as one of the most reliable markers of household hygiene.[29] According to the results obtained, we found that the prevalence of *H. pylori* infection was low with high CWI in an urban population (33.3%), while with middle CWI, it was 80% and with low CWI, it was 86%. When we compared these results with the rural populace, we found that the prevalence of *H. pylori* infection was comparatively very high (55%) with high CWI; comparable (80%) with middle CWI, and highest (96.6%) with low CWI (Table III). The statistical analysis has shown no significant impact, though percentage-wise, it was high (Table IV). This indicates that the high percentage of *H. pylori* infection in the rural population, in comparison to the urban population, may have arisen because of precarious hygiene, poor water supply, and lack of sufficient clean water, all of which are considered important determinants in the acquisition of the bacterium.

In conclusion, the outcome of the present study shows that prior household hygiene is a very important factor in increasing the prevalence and transmission of *H. pylori*. It also suggests that the source of drinking water is very important in affecting the prevalence and transmission of *H. pylori*. The present study also shows the importance of drinking boiled water regularly. With the regular use of boiled water for drinking purposes, along with good household hygiene, and proper disposal of waste and faecal material, we can reduce the prevalence and rate of transmission of *H. pylori* to a large extent.

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