

Research article

## **Helicobacter pylori (H pylori) infection in Greece: the changing prevalence during a ten-year period and its antigenic profile**

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### **Abstract**

**Background:** To evaluate changes in H pylori infection prevalence in Greece during a ten-year period, and to examine its antigenic profile.

**Methods:** Three groups of patients were studied. Group O-87: Banked serum samples of 200 consecutive adult outpatients, from the Hepato-Gastroenterology clinic of a teaching hospital at Athens, collected in 1987. Group O-97: Serum samples of 201 similarly selected outpatients from the same Unit, collected in 1997. Group BD-97: Serum samples of 120 consecutive blood donors from the same hospital, collected in 1997. H pylori IgG antibody seroprevalence was studied by a quantitative ELISA. Antigenic profile was studied by western-blot IgG assay, in 62 IgG positive patients of O-97 and BD-97. Results were analyzed by conventional statistics and multivariate regression analysis.

**Results:** The H pylori seroprevalence increased with age in the three tested groups. In O-97, seroprevalence did not differ from that, in BD-97. On the contrary, there was a significant decrease in seropositivity between O-87 and O-97 (59.5% vs 49.2%,  $p = 0.039$ ). Multiple regression analysis showed that age over 35 years (OR:3.45, 95% CI:1.59–7.49,  $p = 0.002$ ) and year of patients' selection – that is 1987 or 1997 – (OR:1.73, 95% CI:1.14–2.65 for 1987,  $p = 0.010$ ), were independent risk factors of H pylori infection. The seroprevalence of CagA+ and VacA+ strains was 77.4% and 58.5%, respectively, and type I(CagA+/VacA+) strains were significantly more common than type II(CagA-/VacA-) strains (59.7% vs 22.6%,  $p < 0.001$ ).

**Conclusions:** During a ten-year period, we found a significant decrease of H pylori infection in Greece and our data support the birth cohort phenomenon as an explanation for the age-dependent increase of H pylori infection. The prevalence of CagA and/or VacA positive strains is relatively high, in a country with low incidence of gastric cancer.

## Background

It is known that *H pylori* infection occurs mainly in early childhood and the link between the infection and risk factors such as socioeconomic status and living conditions in childhood is well documented [1–3]. Thus, there is a marked difference in the prevalence of *H pylori* infection between developing and developed countries during early childhood [4]. However, everywhere in the world, serological data have shown that the prevalence of the infection increases with age [5–7]. In particular in Greece, according to a study carried out in the early '90s [8] seroprevalence increased about 10% per 10 years, from 40% in people aged 21–40 years to 77% in those older than 60 years.

Long-term follow-up studies in developed countries, with low rates of *H pylori* infection, suggest that the age-dependent increase of seropositivity is mainly due to the decreasing rate of childhood infections [9–13], since many studies have proved that during adulthood the rates of seroconversion and seroreversion are almost equal [9,11,14,15]. This phenomenon, is often referred as a "birth cohort phenomenon". In contrast to this theory, there is also evidence suggesting that a continuous risk of acquisition rather than a cohort effect, best explains the age-dependent increase of seropositivity [16]. However, in populations with higher rates of infection than that observed in occidental and Scandinavian countries, there are no data available to evaluate if the cohort phenomenon or the continuous risk of acquisition could better explain the age dependent increase of *H pylori* infection.

Nevertheless, it has been shown that some *H pylori* genes (*vacA*, *cagA*) confer different biological properties which could enhance the *in vivo* pathogenicity of the bacteria [17,18]. Patients infected with *cagA*-positive strains of *H pylori* demonstrate enhanced expression of various cytokines [19] and these patients present a higher grade of gastric inflammation and accelerated epithelial damage [20]. Thus, recently, there has been an interest in detecting the *H pylori* immunophenotype and in particular, the *CagA* and/or *VacA* status.

The aim of this study was two-fold: first, to examine the seroprevalence of *H pylori* infection in two samples of Greek adult population that were collected in 1987 and in 1997, that is ten years apart, in order to find out if the "birth cohort phenomenon" or the continuous risk of acquisition could better explain the age-dependent increase of *H pylori* seropositivity, in a country of south-east Europe with relatively high rate of *H pylori* infection; and second, to evaluate the antigenic profile of the *H pylori* infection (*Vag*, *Cag* etc) in a Greek adult population.

## Material and Methods

### Subjects

The study population included three groups of patients:

#### Group O-87

Banked serum samples of 200 consecutive adult outpatients, irrespective of their socioeconomic status and the cause of admission, (107 men and 93 women, aged 15–82 years, mean age 44.3 ± 17 years), from Hepatology Section of the Gastroenterology clinic of 1<sup>st</sup> Department of Propedeutic Medicine (Athens University School of Medicine, "Laikon" General Hospital). The used serum samples were originally obtained in 1987, for the study of viral hepatitis.

#### Group O-97

Serum samples of 201 similarly selected outpatients (123 men and 78 women, aged 16–85 years, mean age 45.9 ± 15.2 years), from the same Section, that were collected in 1997.

#### Group BD-97

Serum samples of 120 consecutive blood donors from the same hospital (102 men and 18 women, aged 18–62 years, mean age 40.1 ± 10.8 years), collected in 1997.

### Study design and definition of variables

Group O-97 was compared with group BD-97. Subsequently, group O-97 was compared with group O-87. The analyzed variables were age and gender. Both groups (O-87 and O-97) comprised of consecutive outpatients from the same central hospital in Athens. Thus, the demographic data and the average socioeconomic profile of patients could be considered roughly similar in both groups, taking of course into consideration the total development of the socioeconomic and educational level in our country during the last decade.

The antigenic profile of the infection was studied in 62 randomly selected *H pylori* IgG antibody positive patients of O-97 and BD-97 groups (the first 40 and 22 consecutive *H pylori* IgG antibody positive samples of O-97 and BD-97 groups, respectively).

### Assays

All serum samples were stored at -70°C and had not been thawed before the current analysis, except for the samples of O-87 group which had been thawed once before.

#### Assay for antibodies to *H pylori*

*H pylori* seroprevalence was studied determining IgG antibodies by a quantitative enzyme-linked immunosorbent assay (ELISA, Diasorin Diagnostics Srl-Manufactured by Hycor Biomedical GmbH). The antigen used was extracted by sonication from the *H pylori* strain NTCC 43054.

The main protein fractions which predominantly included in H pylori antigen coated to ELISA microtiter plates, were: p120-130, p83-87, p67, p63/66, p50-59, p28-31, p19 and p14. These immunogenic proteins were enriched and purified by a designated developed biochemical procedure. All assays were performed in duplicate and the intra-assay and inter-assay variations were <5%, as was estimated with the positive and negative control sera. Validation of the ELISA used, was performed, at the same period, in 40 consecutive patients of the Endoscopy Unit of the same hospital. Using histology (modified Giemsa) as "gold-standard", the estimated sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of that ELISA were 87%, 94%, 95% and 84%, respectively.

#### Antigenic profile of H pylori infection

The antigenic profile of the infection was studied using Immunoblot Helicobacter pylori IgG (Microgen GmbH, Germany). This test permits the safe identification and localization of specific antibodies on the solid phase because the antigens are electrophoretically separated. The test results were evaluated according to the manufacturer's instructions and the main detected bands were: p120(CagA), p87(VacA), p62(UreB), p58(HspA&B), p54(FlaA) and p29(UreA). Using histology and/or microbiology as "gold standard", the sensitivity and specificity of this immunoblotting test was 95% and 83%, respectively (Mikrogen molekularbiologische Entwicklungs-GmbH, Immunoblot Helicobacter pylori IgG, Instructions for use version: GIIBHPE004.DOC).

#### Statistical analysis

Chi-square test was used to test for an association between H pylori infection and year of patients' selection, by age group and overall. Multivariate logistic regression was used to determine risk factors associated with H pylori infection. A model containing all variables was considered. Variables that did not contribute to the model, based on their Wald statistic, were eliminated and the new model was compared to the old through the likelihood ratio statistic. Variables whose exclusion gave a non-significant likelihood ratio statistic ( $p > 0.05$ ) were omitted from the model.

#### Results

##### H pylori seropositivity in O-97 and BD-97 groups (table 1, fig. 1)

In 1997, seroprevalence of H pylori infection in all patients and in the patients' age groups did not differ from that of blood donors. Thus in group BD-97, seropositivity was 11% up to 24 years, increased of about 136% per decade up to 44 years and continued to increase at an average of 13% per decade up to 60 years. In group O-97 seropositivity was 14.2% up to 24 years, increased of about 96% per decade up to 44 years and continued to increase at an average of 16.5% per decade up to 64 years.

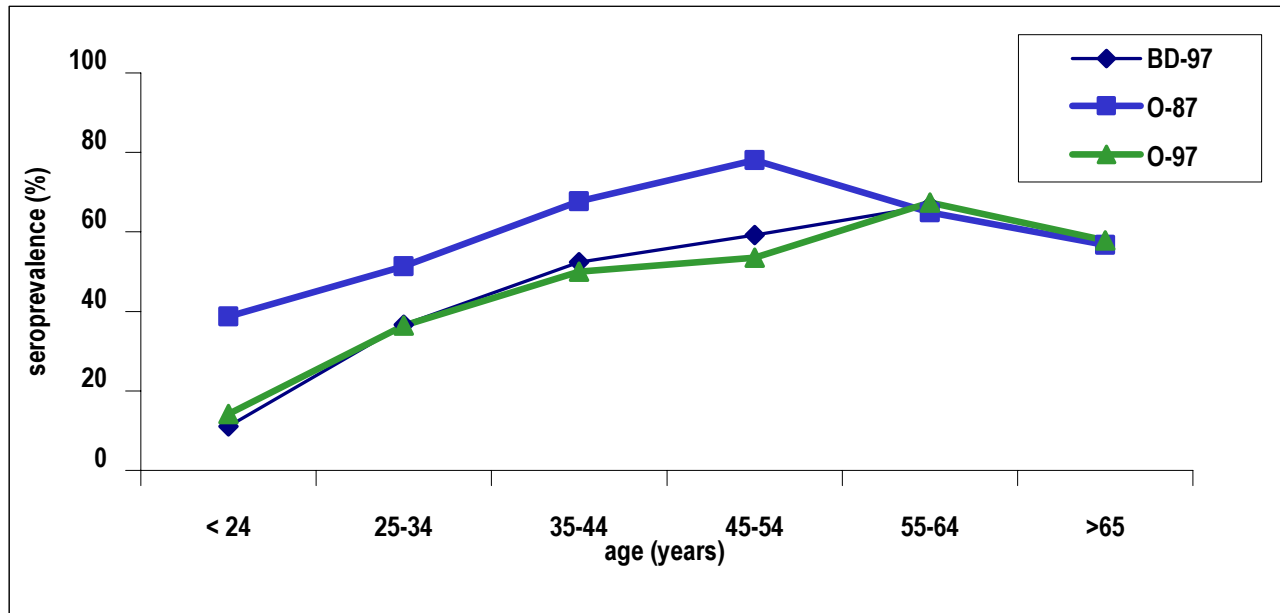
##### Prevalence of antibodies in O-87 and O-97 groups of patients (table 1, figure 1)

The prevalence increased with age in both tested groups of patients (O-87 and O-97). More specifically, in 1987, the increase was continuous up to 45-54 years, at an average rate of 26% per decade. After the age of 55 years, a decrease of seropositivity at an average of 10% per decade was observed.

**Table 1: Seroprevalence of H pylori antibodies (IgG) in O-87, O-97 and BD-97 groups.**

Age (years)	Studied number			H pylori IgG(+) (%)		
	O-87	O-97	BD-97	O-87	O-97	BD-97
15-24	31	21	9	12(38.7)	3(14.2)	1(11.1)
25-34	39	33	30	20(51.3)	12(36.4)	11(36.6)
35-44	31	42	42	21(67.7)	21(50.0)	22(52.4)
45-54	32	43	27	25(78.1)†	23(53.5)†	16(59.2)
55-64	37	43	12	24(64.8)	29(67.4)	8(66.6)
> 65	30	19	-	17(56.7)	11(57.9)	-
<b>Total</b>	<b>200</b>	<b>201</b>	<b>120</b>	<b>119(59.5)‡</b>	<b>99(49.2)‡</b>	<b>58(48.3)</b>

†  $p = 0.032$ , ‡  $p = 0.039$



**Figure 1**  
Seroprevalence of H pylori antibodies (IgG) in O-87, O-97 and BD-97 groups.

**Table 2: Independent risk factors of H pylori seropositivity in each group of tested patients (O-87, O-97) (multivariate logistic regression analysis).**

Variables Age(years)	O-87 Odds ratio	95% CI	p	O-97 Odds ratio	95% CI	p
15 – 24	1			1		
25 – 34	1.68	(0.63, 4.47)	<b>0.29</b>	3.34	(0.81, 13.73)	<b>0.09</b>
35 – 44	3.28	(1.13, 9.49)	<b>0.02</b>	5.54	(1.41, 21.88)	<b>0.01</b>
45 – 54	6.05	(1.96, 18.65)	<b>0.002</b>	7.12	(1.80, 28.07)	<b>0.005</b>
55 – 64	3.28	(1.19, 9.02)	<b>0.02</b>	13.51	(3.35, 54.51)	<b>&lt; 0.001</b>

In 1997, the prevalence was increasing up to the 55–64 years group, at an average rate of 50% per decade. The average increasing rate was highest for up to 44 years (96% per decade) and continued at an average of 16.5% per decade up to the 55–64 years group. In the oldest age group (>65 years), a decrease of seropositivity of about 14% per decade was noticed.

Logistic regression analysis in the group O-87 with independent variables such as age and gender, showed that age over 35 years was the only independent risk factors of H pylori infection (table 2). Gender was not associated with

positivity, but there was a small but insignificant increase of seropositivity in males (OR: 1.24, 95% CI: 0.68–2.25, p = 0.47).

During a ten-year period (1987–1997) the crude H pylori seroprevalence fell significantly by 10% (from 59.5% in group O-87, to 49.2% in group O-97, p = 0.039), and it was constantly lower in each age group (up to 55–64 years) by 15 – 24%.

Logistic regression analysis in all patients (group O-87 and group O-97, 401 patients) with independent varia-

bles such as age, gender and year of patients' selection (that is 1987 or 1997), showed that age over 35 years and the year 1987 were independent risk factors for H pylori infection (table 3).

**Table 3: Independent risk factors of H pylori seropositivity in O-87 and O-97 groups of patients (N:401) (multivariate logistic regression analysis).**

Variables Age(years)	Odds ratio	95% CI	p
15 – 24	1		
25 – 34	2.02	(0.94, 4.37)	0.073
35 – 44	3.45	(1.59, 7.49)	0.002
45 – 54	5.07	(2.31, 11.11)	< 0.001
55 – 64	5.60	(2.57, 12.19)	< 0.001
> 65	3.49	(1.51, 8.10)	0.004
<b>Period</b>			
1997	1		
1987	1.73	(1.14, 2.65)	0.010

The peak of H pylori seroprevalence differs by one decade in the two tested samples (fig 1). This may suggest that the risk of infection is associated with the year of patients' birth. Indeed, in both groups of patients the highest risk for H pylori seropositivity was found in patients who were born during the decade 1933–1942 in comparison with those born after 1953) (table 4).

**The antigenic profile of H pylori infection (table 5)**

The seroprevalence of CagA and VacA antigens was 77.4% and 59.7%, respectively. These rates were constant across gender, age and group of patients (O-97 and BD-97). Type I (CagA+/VacA+) strains were significantly more common than type II (CagA-/VacA-) strains (59.7% vs. 22.6%, p <

0.001). The prevalence of the other antigens ranged from 33.9% (FlaA) to 82.2% (UreB).

**Discussion**

Our findings show that the prevalence of H pylori IgG antibodies increases with age in the three tested groups of patients (O-87, O-97 and BD-97). Provided that the ELISA used had 87% sensitivity and 94% specificity, the results reflect satisfactorily H pylori infection rate. H pylori seroprevalence did not differ between the group O-97 and the healthy controls (group BD-97)(49.2% vs 48.3%, respectively, p = 0.87). On the contrary, comparing the group O-87 with the group O-97, we found a significant decrease of the infection rate in 1997 (59.5% vs 49.2%, respectively, p = 0.039).

The dynamic of infection in 1987 seems to be similar with that seen in the developing countries. Ten years later, in 1997, the picture changed toward what was found in the developed countries. Thus, the crude seroprevalence was reduced about 10% units; multiple regression analysis showed that a person of the O-87 group had a significant higher risk of seropositivity than a comparable person of the O-97 group (OR:1.73, 95% CI: 1.14–2.65, p = 0.010).

As we did not found paired serum samples of the same patient in groups O-87 and O-97 we could not know the rate of seroconversion in our population. But, in both developing and developed countries, there are many studies suggesting that, during adulthood, the rate of seroconversion and seroreversion is almost the same[9,14,15]. Thus, though our data showed that increasing age does increase the risk of H pylori infection, the fact that patients from group O-87 had higher rates of infection than comparable patients from group O-97, supports the "birth cohort phenomenon" as the main cause for the increasing occurrence of the infection seen with age.

**Table 4: Decade of birth as an independent risk factor of H pylori seropositivity (multivariate logistic regression analysis).**

Year of patients' birth	1987			1997		
	Odds ratio	95% CI	p	Odds ratio	95% CI	p
> 1953	1			1		
1943–1952	2.49	1.01 – 6.14	0.047	2.08	1.00 – 4.34	0.050
1933–1942	4.54	1.71 – 12.03	0.002	3.57	1.67 – 7.62	0.001
1923–1932	2.47	1.07 – 5.73	0.035	2.87	0.96 – 8.56	0.058
< 1923	1.69	0.70 – 4.06	0.24	2.08	0.10 – 28.38	0.704

**Table 5: Western-blot analysis in 62 H pylori (IgG antibodies) positive persons.**

	Studied number		Rate (%)		
	O-97 (N = 40)	BD-97 (N = 22)	O-97	BD-97	Total (N = 62)
p120 (CagA)	32	16	80	72.7	77.4
p87 (VacA)	25	12	62.5	54.5	59.7
p63 (UreB)	33	18	82.5	81.8	82.2
p58 (HspA&B)	32	12	77.5	54.5	71
p54 (FlaA)	17	4	42.5	18.2	33.9
p29 (UreA)	24	16	60	72.7	64.5

The highest risk of infection in both groups of patients, was found in those born around 1940, that is the period of war deprivation. This finding, in association with the reduction in the infection rate during the studied 10-year period is compatible with the hypothesis that better socio-economic conditions and improved hygiene have reduced the risk of H pylori infection [4].

In both groups of patients (O-87 and O-97) we found a constant decrease of seropositivity in the oldest age group. This may be due to many factors like spontaneous seroreversion [14], increased antibiotics and NSAIDs consumption [21] and advanced gastric atrophy in association with the natural senility of the immunological system [22].

In this study the prevalence of CagA positive and VacA positive strains of H pylori were 77.4% and 59.7%, respectively; type I strains were significantly more common than type II strains (59.7% vs. 22.6%,  $p < 0.001$ ). According to the Eurogast study group, Greece has low incidence of gastric cancer in comparison with other European countries [23]. It has been suggested that CagA positive infections are more likely to predispose to gastric cancer than the CagA negative ones [24]. Nevertheless, we found a high rate of CagA positive and type I strains of H pylori in our sample. In accordance with previous studies [20,25,26], these data suggest that CagA and/or VacA status are not the only factors that influence gastric cancer rates.

To conclude, a significant decrease of H pylori infections' rate was noticed in Greece during a ten-year period. Even in a country of south-east Europe, with relatively high rate of H pylori infection, our data support the birth cohort phenomenon as an explanation for the age-dependent increase of H pylori infection. The prevalence of CagA and/or VacA positive strains is relatively high for the reported incidence of gastric cancer in Greece.

### Competing interests

None declared.

### Authors' contributions

PA carried out the immunoassays, participated in its design and sequence alignment and drafted the manuscript. IV participated in its design and coordination. MT participated in the sequence alignment. DK performed the statistical analysis. NK participated in its design and coordination. AA conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

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