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# Tonsillar colonization is unlikely to play important role in *Helicobacter pylori* infection in children

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**KEYWORDS**
*Helicobacter pylori*;
Tonsils;
Children;
Risk factors

**Summary**

*Objective:* To determine (i) seroprevalence of *Helicobacter pylori* (HP) infection in children undergoing tonsillectomy, (ii) possible HP colonization on tonsils of children and its importance in HP transmission, and (iii) if four examined socio-economic factors are the risk factors for HP transmission and HP colonization on tonsils in children.
*Methods:* Rapid urease test (RUT) of tonsils, and serologic blood tests for HP were performed in 77 children (aged 4—14 years) in Bosnia and Herzegovina (B-H), undergoing tonsillectomy. RUT positive tonsils were cultured for HP. RUT positive children were tested using $^{13}$Carbon—urea breath test ($^{13}$C—UBT). Information about socio-economic potential risk factors was obtained from the parents.
*Results:* Out of 139 pharyngeal and palatine tonsils, 17 palatine tonsils in 14 children were RUT positive and had negative HP culture. Eight children had positive both RUT and $^{13}$C—UBT. There was no significant difference between children with hypertrophy and those with recurrent tonsillitis comparing their serologic tests results. There was no significant difference between seronegative ($n = 61$) and seropositive ($n = 16$) children comparing their age, sex, parental education level, owning a family courtyard, attending a children's collective, and owning a pet cat.
*Conclusions:* The results in this prospective study do not support the notion that tonsils are an important reservoir for HP transmission in children in B-H. The examined socio-economic factors did not enhance HP seropositivity rate in children.


## 1. Introduction

*Helicobacter pylori* (HP) is a bacterium that colonises the gastric mucosa, causes gastritis, and pre-

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disposes peptic ulcer disease, gastric cancer and mucosa associated lymphoid tissue (MALT) lymphoma [1]. Transmission and natural reservoirs for HP are poorly defined. Tonsils, a component of MALT, participate in the immune function, but they may serve as a reservoir for bacteria [2]. The suggestion that tonsillectomy may protect the host against HP infestation of the stomach arose from the study of Minocha et al. [3]. They found a decreased prevalence of HP gastric colonization in subjects with a history of tonsillectomy. In the study of Unver et al., 11 of 19 Turkish patients had positive Campylobacter-like organism test of tonsil tissue [4]; while in the study of Skinner et al., the tonsils of all 50 Irish patients tested negative for the same test [5]. Cirak et al. found HP DNA in tonsillar samples [6]. Yilmaz et al. showed a growth of HP in tonsillar cultures [7].

Poor socio-economic conditions in early childhood are considered to be a major risk for the acquisition of HP infection. The war in Bosnia and Herzegovina (B-H) from 1992 to 1995 made living conditions worse for children. The collapsed economy and the destroyed infrastructure for sanitation may have enhanced the transmission of HP infection. The crowded household in childhood [8] and low educational standard [9] were considered as risk factors for HP infection. HP was found in field soil [10] and domestic animals [10,11].

This prospective study was undertaken to determine (i) seroprevalence of HP infection in children undergoing tonsillectomy, (ii) possible HP colonization on tonsils of children and its importance in HP transmission, and (iii) if four examined socio-economic factors are the risk factors for HP transmission and HP colonization on tonsils in children.

## 2. Materials and methods

From January 2003 through February 2004, 77 children from southern and western Herzegovina, part of B-H, who attended the Department of Otorhinolaryngology, Mostar University Hospital in Mostar, for tonsillectomy under general anesthesia, were recruited into this study. The inclusion criteria were: age 4—14 years, recurrent tonsillitis or tonsillar hypertrophy as indications for surgery. The exclusion criterion was the use of the following antibiotics in the preceding 4 weeks: amoxicillin, metronidazole, clarithromycin, tetracycline, and azithromycin. The design of this study has been approved by the Ethical Committee of the Mostar Medical School. The informed consent was obtained from the parents. In this study, a serological rapid blood test for HP, rapid urease test (RUT), $^{13}$Carbon—urea breath test ($^{13}$C—UBT), and a HP bacterial culture were performed. All RUT and serological tests were interpreted by the first author respecting the instructions of the manufacturers. Local anesthetics and disinfectants were not used during the surgery, or manipulation with tonsillar specimens. Tonsillectomy was performed by dissection and snare technique. Adenoid curettes were used for adenoidectomy.

In this study, each RUT positive tonsil was recognized positive for HP colonization if its positive RUT was associated with positive $^{13}$C—UBT, or with positive HP culture, or with positive both $^{13}$C—UBT and HP culture. Our intention was to compare the data of the children with HP colonization on tonsils, with the data of the children without HP colonization on tonsils.

The data were analyzed according to the indications for surgery. According to serological tests children were divided into HP seropositive group and HP seronegative group.

### 2.1. Serology test

Perioperatively, two drops of whole vein blood were assessed by immunochromatographic rapid blood test (Ulcognost PK test, Biognost, Zagreb, Croatia) for detecting HP antibodies.

### 2.2. RUT

Immediately after the surgery, a 3-mm-diameter sample was cut from the surface of each removed tonsil, using a different sterile instrument for each. Each sample was rinsed in 0.9% saline and placed in its test container under room temperature. RUT (Controloc Hp test, Cambridge Life Sciences Ltd., Ely, UK) detected the urease enzyme activity in tonsillar samples. A test was considered positive if a color change to pink/red occurred within 24 h.

### 2.3. HP culture

Immediately after the surgery, a 10-mm-diameter sample was cut from each removed tonsil using a different sterile instrument. Each sample was placed in 0.9% saline at 4 °C. Only RUT positive tonsils were processed for culture. If a certain 3-mm sample was RUT positive, only an appropriate 10-mm sample, taken from the same tonsil, was inoculated in glycerol containing medium and transported to microbiology. The sample was homogenized and cultured on Brucella-Agar with 5% sheep blood, vancomycin, polymixin and trimethoprim (Merck, KgaA Darmstadt, Germany). The plates were incubated at 37 °C under microaerophilic conditions (5% $O_2$, 10% $CO_2$, 85% $N_2$) (GENbox microaer,