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ORIGINAL ARTICLE

Resistance of *Helicobacter pylori* to tetracycline, amoxicillin, clarithromycin and metronidazole in Israeli children and adults

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The aim of this study was to examine *Helicobacter pylori*-resistance rate to different antibiotics: tetracycline, amoxicillin, clarithromycin and metronidazole, and to compare eradication rates in adults and children in Israel. The study was based on the hypothesis of high-resistance rates to clarithromycin and metronidazole especially in adults and overall low-resistance rates to tetracycline and amoxicillin. One seventy six biopsies from patients with dyspeptic symptoms were cultured of which 100 were from adults (19–79 years) and 76 from children (7–17 years). All positive cultures were examined by Epsilometer test for MIC determination against tetracycline, amoxicillin, clarithromycin and metronidazole. 48.3% (85 out of 176) were *H. pylori* positive, of which 44% were from adults and 54% from children. Antibiotic resistance was seen in 31 out of 44 (70.5%) for metronidazole, 1 out of 44 (2.3%) for amoxicillin, 10 out of 44 (22.3%) for clarithromycin and 1 out of 44 (2.3%) for tetracycline among adults. Antibiotic resistance was seen in 10 out of 41 (24.4%) for metronidazole, 5 out of 41 (12.2%) for amoxicillin, 10 out of 41 (24.4%) for clarithromycin and 1 out of 41 (2.4%) for tetracycline among children. High rates of *H. pylori* resistance to metronidazole and clarithromycin was found especially among adults. Therefore, to increase the success rate of anti-*H. pylori* treatment, other classes of antibiotics need to be considered.

INTRODUCTION

Helicobacter pylori (H. pylori) infection is one of the most common bacterial infections of the digestive system. *H. pylori* infection is an important factor in the development of serious upper gastrointestinal diseases: gastritis, duodenitis, gastric ulcers, duodenal ulcers (reported to develop in 1–10% of infected patients), gastric cancer (in 0.1–3%) and gastric mucosa-associated lymphoid-tissue lymphoma (in <0.01%).[1,2] Unfortunately, very little is known about the details of the modes of transmission of *H. pylori* and its routes of dissemination. The primary modes of transmission are thought to be fecal–oral and oral–oral, but some indirect evidence reports transmission via drinking water and other environmental sources.[3,4] The current most effective treatment includes proton-pump inhibitors in combination with two antibiotics. However, owing to bacterial resistance to antibiotic therapy, ~20% of patients fail to respond to the eradicative first-line treatment and remain *H. pylori* positive.[5] Prevalence of resistance to treatment is variable in different areas of the world and probably is related to indication, particularly for drugs that are used in the treatment of respiratory tract infections. For instance, in countries where the use of clarithromycin is rare, the resistance rate is low, while in countries where its use is wide, the resistance rate reaches 10–15%.[6,7] Similarly, metronidazole-resistance rate is much higher in developing countries (50–80%), for example in Mexico it is 76.3%, which is in contrast to a much lower-resistance rate in Japan (9–12%).[8] Additionally, resistance rates between adults and children are different.[7]

The aim of this study was to examine *H. pylori*-resistance rate in response to different antibiotics: tetracycline, amoxicillin, clarithromycin and metronidazole, and to compare response rate in adults and children in Israel. The study was based on the hypothesis of high-resistance rates to clarithromycin and metronidazole especially in adults and overall low-resistance rates to tetracycline and amoxicillin, reflected in treatment success or failure.

MATERIALS AND METHODS

Bacterial culture

The study population consisted of 176 patients with dyspeptic symptoms. Among them 71 were males and 105 females of which 100 were adults (19–79 years) and 76 children (7–17 years). All patients did not receive antibiotics in the months preceding the study. Gastric biopsies from the antrum and the corpus were taken from each patient. All samples were collected in the period June 2011–April 2012. The biopsies were cultured within 60 min on *H. pylori*-selective medium (Hy-lab, Rehovot, Israel) enriched with 5% horse blood/laked-lysed supernatant and contained vancomycin, trimethoprim and

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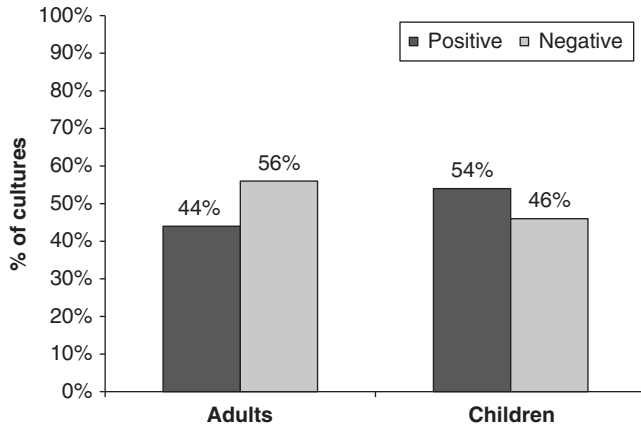
Figure 1 *Helicobacter pylori* prevalence among children and adults.

Table 1 Pattern of primary-bacterial resistance toward different antibiotics in all of the subjects ($n = 85$)

Antibiotic	Resistance, n (%)
Metronidazole	41 (48.8)
Clarithromycin	20 (23.5)
Amoxicillin	6 (7)
Tetracycline	2 (2.3)
Metronidazole & clarithromycin	12 (14.1)
Metronidazole & tetracycline	1 (1.2)
Metronidazole & amoxicillin	1 (1.2)

Table 2 Pattern of primary-bacterial resistance toward different antibiotics divided into age groups

Antibiotic	Adults, n = 44 Resistance (%)	Children, n = 41 Resistance (%)
Metronidazole	31 (70.5)	10 (24.3)
Clarithromycin	10 (22.3)	10 (24.3)
Amoxicillin	1 (2.3)	5 (12.2)
Tetracycline	1 (2.3)	1 (2.4)
Metronidazole & clarithromycin	9 (20.4)	3 (7.3)
Metronidazole & tetracycline	1 (2.3)	1 (2.4)
Metronidazole & amoxicillin	0 (0)	0 (0)

polymixin B to inhibit the growth of other microorganisms. Cultures were incubated for up to 8 days at 37 °C in microaerobic atmosphere (5% O_2 and 10% CO_2). *H. pylori* identification was carried out using Gram stain, positive oxidase, catalase and urease tests.

Determination of *in vitro* susceptibility
Suspensions from primary plates were prepared in 0.85% NaCl solution to a 2.0 McFarland standard (containing 1×10^7 to 1×10^8 CFU per ml) to perform the E-test on Mueller-Hinton agar with 5% sheep blood (Hy-lab) against tetracycline, amoxicillin, clarithromycin and metronidazole, and were incubated for 72 h at 37 °C in microaerobic atmosphere. An isolate was considered resistant to amoxicillin if MIC was > 1 mg l^{-1}, to clarithromycin if MIC was $\geqslant 1$ mg l^{-1}, to metronidazole if MIC was > 4 mg l^{-1} and to tetracycline if MIC was > 2 mg l^{-1}.[9,10] For MIC quality control the *H. pylori* 43504 strain was used.

Statistical analysis
For comparison between the subgroups, the analysis of variance single factor anal, χ^2-test, Fisher's exact test and *t*-test were used appropriately. A *P*-value of < 0.05 was considered significant.

RESULTS
Among 176 samples, 85 (48.3%) were *H. pylori* positive of which 44 (44%) were from adults (average age 48 years) and 41 (54%) from children (average age 12 years) (Figure 1). All patients' positive cultures were accepted both from the antrum biopsy and from corpus biopsy. Antibiotic resistance was seen in 31 out of 44 (70.5%) for metronidazole (mean MIC 8 μg), 1 out of 44 (2.3%) for amoxicillin (MIC 3 μg), 10 out of 44 (22.3%) for clarithromycin (mean MIC 4 μg) and 1 out of 44 (2.3%) for tetracycline (MIC 4 μg) in adults. Combined resistance was observed in one sample for tetracycline and metronidazole and in nine samples for metronidazole and clarithromycin among adults. Antibiotic resistance was observed in 10 out of 41 (24.4%) samples for metronidazole (mean MIC 3 μg), 5 out of 41 (12.2%) for amoxicillin (mean MIC 4 μg), 10 out of 41 (24.4%) for clarithromycin (mean MIC 3 μg) and 1 out of 41 (2.4%) for tetracycline (MIC 6 μg) among children. Combined resistance was observed in one isolate for amoxicillin and metronidazole and in three samples in metronidazole and clarithromycin in children (Tables 1 and 2) (*P*-value of 0.037 was significant). Patients who were diagnosed as positive for *H. pylori*, received triple (two antibiotics and a proton-pump inhibitor) therapy according to the results of the sensitivity tests. In patients who were resistant to two

of the three antibiotics from the first-line treatment, levofloxacin was used as a part of combined therapy.

DISCUSSION
H. pylori infection is very common, and it is one of the most frequent bacterial infections of the digestive system in the world.[1,2] Usually, the disease has chronic features unless it is treated by the combination of two proper antibiotics with proton-pump inhibitor or H_2 receptor antagonist. *H. pylori* management in the clinical practice remains a challenge for the physicians. Choice of appropriate antibiotics is crucial in the success of treatment and recovery from *H. pylori*-related diseases. The available tests for the diagnosis of *H. pylori* infection are generally divided into invasive methods (based on gastric specimens for histology, culture or other methods) and noninvasive methods (based on peripheral samples, such as blood, breath samples, stools, urine or saliva for detection of antibodies, bacterial antigens or urease activity). Usually, the treatment prescription is empirical. Bacterial culture and antibiotics sensitivity are not a part of routine checkup, but it is recommended in case of treatment failures.[11,12] The first-line treatment includes proton-pump inhibitor, and two of the three antibiotics: amoxicillin, clarithromycin or metronidazole. In cases of first-line treatment failure, either a bismuth-containing quadruple therapy or levofloxacin-containing triple therapy is recommended as second line treatment.[13,14] Knowledge of the sensitivity of *H. pylori* in specific geographic areas and among specific age group can significantly improve the treatment success rates. In the present study, we observed significantly high rate of *H. pylori* resistance to metronidazole and clarithromycin. Significantly higher rate of metronidazole resistance was seen among adults: however, resistance to clarithromycin was comparable in both adults and children. On the other hand, isolates from children showed high (but nonsignificant) resistance rate to amoxicillin when compared with adults. The rate of resistance to tetracycline in both groups was significantly low.

The resistance rates for each antibiotic may result from the before widespread use of antibiotics used in the treatment of other infections. A similar study that was conducted in Israel in 1999 showed high-resistance rate to metronidazole (67%), comparable with the rates established in the present study. Contrary to our findings, clarithromycin-resistance rate was relatively low (10%) and amoxicillin-resistance rate was not observed. In summary, amoxicillin- and clarithromycin-resistance rates increased since 1999 while metronidazole-resistance rate remained high and unchanged in Israel.[15] The low-resistance rate of *H. pylori* to tetracycline in Israel is similar to that observed in many other countries throughout the world.[16] Unfortunately, use of tetracyclines is limited in pediatric patients owing to their side effects, and tetracycline-based regimens usually require the concomitant use of preparations containing bismuth salts, which are no longer available in a number of European countries owing to possible side effects.[17] In conclusion, to raise the success rate of anti-*H. pylori* treatment, *H. pylori* cultures should be tested for antibiotic sensitivity. Second line of treatment antibiotics are currently used to treat patients with resistant *H. pylori*. Additionally we suggest replicating the study using a larger sample size to acquire information concerning *H. pylori*-resistance rates in Israel.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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