Prevalence of *Helicobacter pylori* resistant to clarithromycin, metronidazole and amoxicillin isolated from pediatric patients in China

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Background: **Antimicrobial resistance in *Helicobacter pylori* (*H. pylori*) is an increasing serious problem, and the development of methods for detecting the resistance and the constant surveillance of both prevalence and evolution of *H. pylori* resistance in children would greatly improve the selection of antibiotics used to treat gastroduodenal infection with this organism. This study was designed to investigate the prevalence of *H. pylori* resistance to clarithromycin, metronidazole and amoxicillin isolated from pediatric patients in China.**

Methods: **A total of 44 *H. pylori* isolates from pediatric patients with gastritis and peptic ulcer undergoing endoscopy were cultured during the period of October 2002 to November 2003 at Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China. The susceptibilities of the 44 *H. pylori* isolated strains to clarithromycin, metronidazole and amoxicillin were tested by the agar dilution test to determine the minimum inhibitory concentration (MIC).**

Results: **The range of MIC was as follows: clarithromycin 0.125-64 μg/ml; metronidazole <0.125-128 μg/ml; and amoxicillin <0.125-64 μg/ml. The rate of *H. pylori* resistant to clarithromycin, metronidazole and amoxicillin in the children was 18.2% (8 strains), 31.8% (14 strains) and 9.1% (4 strains), respectively. Three strains (6.8%) were simultaneously resistant to the 3 antibiotics.**

Conclusions: **The prevalence of *H. pylori* resistance to metronidazole is high in children, and the rate of resistance to clarithromycin is higher than that in adults. Amoxicillin-resistant strains are found, and multi-drug-resistant strains of *H. pylori* to clarithromycin, metronidazole and amoxicillin are also found.**

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Key words: ***Helicobacter pylori*;**
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Introduction

The emergence of antibiotic resistance is now recognized as a major cause of failure in the eradication of *Helicobacter pylori* (*H. pylori*).[1,2] In such patients, *in vitro* susceptibility testing may be helpful in excluding antibiotic resistance and thereby aids in treatment selection. However, data concerning *H. pylori* resistance in children are limited. The purpose of this study was to investigate the local primary resistance prevalence by examing *in vitro* susceptibility of clinical *H. pylori* against antimicrobial clarithromycin, metronidazole and amoxicillin isolated from pediatric patients during the period of 2002-2003 in Hangzhou, China.

Methods

Bacterial strains

From October 2002 to November 2003, a total of 44 *H. pylori* strains were isolated from pediatric patients (25 boys, 19 girls; aged 3 to 14 years; mean age, 8.7 years) who had undergone endoscopy at Children's Hospital, Zhejiang University School of Medicine. Biopsy specimens were taken from the gastric antrum or body for testing *H. pylori*. The diagnoses included gastritis

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(*n*=37) and duodenal ulcer (*n*=7).

Isolation and identification of *H. pylori*

Fresh gastric biopsy specimens obtained during gastroscopy from symptomatic patients with a positive rapid urease assay were transported to the laboratory in Brucella broth supplemented with 30% glycerol and minced and homogenized, then inoculated immediately on selective medium plates (Columbia agar) enriched with 7%-10% fresh sheep blood and incubated in a microaerophilic environment (CO_2 10%, O_2 5%, N_2 85%) at 37℃ for about 3 to 5 days. Suspected isolates were identified as *H. pylori* by conventional methods using Gram staining and biochemical tests for positive urease, oxidase and catalase activities. All isolates were stored at -70℃ in Brucella broth for later determination of antibiotic susceptibility.

Antibiotic susceptibility test

The susceptibilities of *H. pylori* isolates to clarithromycin, metronidazole and amoxicillin were determined by an agar dilution technique with Mueller-Hinton agar plates supplemented with 5% fresh sheep blood as described by NCCLS recommendations.[3] The bacterial suspension was adjusted to a final concentration of 3×10^8 CFU/ml. The plates containing serial two-fold dilutions of each antibiotic at concentrations ranging from 0.125 to 128 μg/ml were inoculated with 10 μl of bacterial suspension (approximately 10^6 CFU) and incubated at 37℃ in a microaerophilic environment for 72 hours before reading the minimal inhibitory concentrations (MIC). The antibiotic breakpoints of susceptibility (S) and resistance (R) were determined according to published reports. The breakpoints for clarithromycin used according to the National Committee of Clinical Laboratory Standards (NCCLS) were determined as <1 and 1 μg/ml, respectively. For metronidazole and amoxicillin, these were accepted to be < 8 and 8 mg/L, respectively.

Results

MICs range of antibiotic resistance

Table shows the MIC ranges of *H. pylori* isolates resistant to the 3 antimicrobial agents (clarithromycin, metronidazole and amoxicillin) tested against the total number of strains. The range of MIC varied respectively: clarithromycin 0.125-64 μg/ml; metronidazole <0.125-128 μg/ml; and amoxicillin <0.125-64 μg/ml.

MIC_{50}s (MIC at which 50% of the isolates tested are inhibited) of clarithromycin, metronidazole and amoxicillin were <0.125 μg/ml, 0.5 μg/ml and <0.125 μg/ml respectively, and MIC_{90}s (MIC at which 90% of the isolates tested are inhibited) were 16 μg/ml, 128 μg/ml and 0.5 μg/ml respectively.

Table. Minimal inhibitory concentrations of the three antibiotics for 44 isolates of *H. pylrori*

MIC (μg/ml)	No. of *H. pylori* isolates			No. of calculative isolates		
	CLM	AMO	MET	CLM	AMO	MET
<0.125*	33	31	21	33	31	21
0.125	1	0	0	34	31	21
0.25	0	4	0	34	35	21
0.5	2	5	1	36	40	22
1	0	0	3	36	40	25
2	0	0	3	36	40	28
4	0	0	2	36	40	30
8	4	0	0	40	40	30
16	3	3	0	43	43	30
32	0	0	1	43	43	31
64	1	1	3	44	44	34
128	0	0	10	44	44	44

Prevalence of antibiotic resistance

Of all 44 isolates, the rate of *H. pylori* resistant to clarithromycin, metronidazole and amoxicillin in children was 18.2% (8 strains), 31.8% (14 strains) and 9.1% (4 strains) respectively. Three strains (6.8%) were simultaneously resistant to 3 antibiotics.

Discussion

Triple treatment with a proton-pump inhibitor (PPI) or bismuth salt plus two antibiotics is now commonplace in all patients diagnosed and has been demonstrated to have high eradication rates.[4,5] Clarithromycin, metronidazole and amoxicillin are the most frequently used antibiotics for the treatment of *H. pylori* infection. However, antibiotic resistance frequently causes failure of eradication of *H. pylori*. With regard to antibiotic resistance, however, there have been few reports on antibiotic resistance in children, and close attention should be paid by clinicians. It is time to consider routine susceptibility test to guide treatment of individual patient and surveillance of antibiotic resistance.

Previous studies showed that the prevalence of primary resistance to clarithromycin appeared to be lower than 10%,[6] but the rate of clarithromycin resistance has been increasing in recent years and a high prevalence of clarithromycin resistance has also been found in pediatric population. A study conducted during 1993-1996 in Poland[7] revealed

that 17% (22/130) of *H. pylori* strains isolated from children were resistant to clarithromycin, and during 1998-2000 23.5%, showing an increasing resistance over the last 3 years. In a Portuguese study,[8] the rate of clarithromycin resistance was higher in *H. pylori* isolates from children than in those from adults. It is thought to be associated with that macrolides are now frequently used to treat respiratory infections in children and an increasing overall use of newer macrolides in clinical practice.[9] Our study shows a high level of *H. pylori* resistance to clarithromycin (18.2%), which is higher than that in adults (5.6%),[10] but an increasing resistance to clarithromycin in adults in China has also been described elsewhere.[11]

Metronidazole resistance is extremely common in *H. pylori*. A difference in the resistance to metronidazole ranging from 10% to 50% has been found in adult populations from western countries. Higher resistance rates in developing countries varied from 77% to 95%,[12] which were attributed to the extensive use of metronidazole, for instance, in the treatment of parasitic, genital or dental infections. The prevalence of metronidazole-resistant strains in Shanghai, China has been reported to be improved from 42% to 70% during the period of 1995-1999,[13] whereas in some areas reached 90%.[10] The prevalence of metronidazole resistance in our study was 31.8% (14 strains), which was lower than that in adults because of the rare use of metronidazole in children. But all the resistant strains to metronidazole showed a high resistance level with high MIC ranging from 32 to 128 mg/L, the highest being MIC_{90}.

Resistance to amoxicillin has been considered to be low or absent, for example, 0 in pediatric patients in Japan.[14] In recent years, however, reports on the isolates of resistant strains have been increasing worldwide, with a high frequency in particular geographical regions. Dore et al[15] reported the unusually high prevalence of *H. pylori* resistance to amoxicillin (31% and 45%) with MIC greater than 256 mg/L of all strains. Resistance to amoxicillin was observed in 4 strains (9.1%) of the isolates, reflecting the importance of its use in children in China.

However, three strains (6.8%) were found simultaneously resistant to clarithromycin, metronidazole and amoxicillin. In Mexico, a study showed an increasing multi-drug-resistant *H. pylori* strains isolated from children and adults.[16] Lang et al[17] reported that multiple resistance was found in 4.3% (94 strains) of the isolates in Costa Rica. Although it is rare, the appearance of multi-drug-resistant *H. pylori* strains, especially in children, is of utmost importance, because the eradication of *H. pylori* infection in similar cases could be a treatment challenge. There is a need for constant surveillance of both prevalence and evolution of *H. pylori* resistance in children.

Regarding the mechanism of clarithromycin resistance, mutations within domain V of the 23S rRNA gene cause a decreased affinity of clarithromycin for the 23S ribosomal component, resulting in the impaired activity of clarithromycin against *H. pylori*.[18-21] In contrast to clarithromycin, the mechanisms of resistance to metronidazole in *H. pylori* are less clear. It has been hypothesized that mutations in the rdxA gene, which encode an oxygen-insensitive NADPH nitroreductase, are responsible for the resistance.[22,23] Some investigators, however, have questioned the mutational inactivation of the rdxA gene and suggested that there are many different pathways for the resistance. Changes in PBP1 have been regarded to be involved in the amoxicillin resistance of *H. pylori* isolates.[24] A better understanding of antibiotic resistance mechanisms in *H. pylori* is essential to selecting therapeutic choices and alternative strategies for combating these infections.

In conclusion, we documented a considerable prevalence of *H. pylori* resistance to commonly used antibiotics such as clarithromycin and metronidazole. Amoxicillin-resistant strain was found and multi-drug-resistant strains which were resistant to the three antimicrobial agents also emerged. We confirmed that implementation of a resistance surveillance program may be advisable in the near future to define resistance and/or multi-drug-resistant patterns of pediatric *H. pylori* isolates before the selection of a treatment regimen.

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