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BRIEF ARTICLE

Antibiotics resistance rate of *Helicobacter pylori* in Bhutan

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Abstract

AIM: To survey the antibiotic resistance pattern of *Helicobacter pylori* (*H. pylori*) strains isolated from Bhutanese population.

METHODS: We isolated 111 *H. pylori* strains from the gastric mucosa of *H. pylori*-infected patients in Bhutan in 2010. The Epsilometer test was used to determine the minimum inhibitory concentrations (MICs) of amoxicillin (AMX), clarithromycin (CLR), metronidazole (MNZ), levofloxacin (LVX), ciprofloxacin (CIP), and tetracycline (TET).

RESULTS: Nineteen of the isolated *H. pylori* strains were susceptible to all antibiotics tested. The isolated strains showed the highest rate of antibiotic resistance to MNZ (92/111, 82.9%). Among the 92 MNZ-resistant strains, 74 strains (80.4%) showed high-level resistance (MIC $\geqslant$ 256 μg/mL). Three strains were resistance to LVX (2.7%). These strains were also resistance to CIP. None of the strains showed resistance to CLR, AMX and TET.

CONCLUSION: CLR-based triple therapy is a more effective treatment approach over MNZ-based triple therapy for *H. pylori* infection in Bhutan.

Key words: *Helicobacter pylori*; Drug resistance; Bhutan

Core tip: In Bhutan, 82.9% of *Helicobacter pylori* isolates showed metronidazole resistance. Of these, 80.4% showed high-level resistance (minimum inhibitory concentration $\geqslant$ 256 μg/mL). Only 2.7% strains showed levofloxacin, ciprofloxacin resistance. Intriguingly, none of them were resistance to clarithromycin, amoxicillin, and tetracycline.

Vilaichone R, Yamaoka Y, Shiota S, Ratanachu-ek T, Tshering

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral, Gram-negative bacterium that infects more than half of the world's population and is thought to be involved in the pathogenesis of chronic gastritis, peptic ulcer diseases, gastric cancer (GC), and mucosa-associated lymphoid tissue (MALT) lymphoma[1,2]. Eradication of *H. pylori* infection not only improves the healing of peptic ulcers, but it also prevents its recurrence and reduces the risk of developing GC[3-7]. Furthermore, other *H. pylori*-associated disorders such as MALT lymphoma, atrophic gastritis, and intestinal metaplasia have been shown to regress after treatment with antibiotics[8-10].

Triple therapy regimens that include 1 proton pump inhibitor (PPI) and 2 antimicrobial agents such as amoxicillin (AMX), clarithromycin (CLR), metronidazole (MNZ), levofloxacin (LVX), ciprofloxacin (CIP), and tetracycline (TET) have been widely used to eradicate this bacterium[7,11,12]. Although the success of the treatment depends on several factors such as patient compliance and whether the patient is a smoker, antibiotic resistance is the most common factor causing treatment failure[13-15]. Prevalence of antibiotic resistance is now increasing worldwide and varies by the geographic area; it is generally higher in developing countries than in developed regions[16-18]. In addition, the antibiotic resistance rate often parallels the antibiotic consumption rate in the population[16,19-21].

Bhutan is a small landlocked country in South Asia, located at the eastern end of the Himalayas, and shares its borders in the south, east, and west with the Republic of India and to the north with the People's Republic of China. In Bhutan, the incidence of GC is reported to be quite high (24.2 deaths/100000 population) compared to the neighboring areas[22]. Effective therapies to eradicate *H. pylori* can contribute to the decrease of GC incidence in Bhutan. However, information about the prevalence of drug-resistant *H. pylori* strains in Bhutan, which is essential for designing effective eradication therapies, is lacking.

In this study, we aimed to determine the antibiotic susceptibility of *H. pylori* strains isolated from Bhutanese population toward AMX, CLR, MNZ, LVX, CIP, and TET.

MATERIALS AND METHODS

Subjects and sample collection

H. pylori strains were obtained from the gastric mucosa of *H. pylori*-infected Bhutanese volunteers who underwent endoscopy at 3 cities within the country (Thimpu, Punaka, and Wangdue) from December 6 to 9, 2010. Biopsy samples from the antrum were endoscopically obtained from each patient and used for culturing *H. pylori* by using standard methods. Cases of peptic ulcers and GC were identified by endoscopy, and GC was further confirmed by histopathology. Gastritis was defined as *H. pylori* infection-mediated gastritis in the absence of peptic ulcer or gastric malignancy. Written informed consent was obtained from all participants, and the protocol was approved by the Ethics Committee of Jigme Dorji Wangchuk National Referral Hospital, Bhutan.

Drug sensitivity testing

Epsilometer test (*E* test) was used to determine the minimum inhibitory concentrations (MICs) of AMX, CLR, MNZ, LVX, CIP, and TET. Mueller Hinton Ⅱ Agar supplemented with 10% horse blood was used as the culture medium and the culture suspension was used to inoculate the agar plates. The *E* test strip of the corresponding antibiotic was placed on the plate and incubated for 3-5 d at 37 ℃, under microaerophilic conditions. The MIC was defined by the point of intersection of the inhibition ellipse with the *E* test strip. Strains were considered "resistance" when the MIC values were $\geqslant$ 1 μg/mL for AMX, $\geqslant$ 1 μg/mL for CLR, $\geqslant$ 1 μg/mL for LVX, $\geqslant$ 8 μg/mL for MNZ, and $\geqslant$ 4 μg/mL for TET[23]. In accordance with previous studies, strains were considered "resistance" to CIP when the MIC values were $\geqslant$ 1 μg/mL[24,25].

Statistical analysis

All statistical analyses were performed by SPSS version 19 (SPSS Inc., Chicago, IL, United States). The univariate association between each group was quantified using the unpaired *t* test, Mann-Whitney *U* test, Fisher's exact test, and χ^2 test. A two-tailed *P* value of < 0.05 was considered statistically significant.

RESULTS

We isolated 111 strains of *H. pylori* from *H. pylori*-positive Bhutanese patients; the identity of these strains was microbiologically confirmed. The patient group included 51 men and 60 women, with an average age of 36.8 ± 13.9 years. Seventy strains were isolated from patients with gastritis, 11 from patients with peptic ulcer, and 1 from a patient with GC. Nineteen strains were susceptible to all the antibiotics tested. The greatest proportion of isolated strains was resistance to MNZ (92/111, 82.9%, Table 1). The resistance rate was 84.2% (48/57) in the strains isolated from patients younger than 34 years of age, 82.4% (28/34) in the strains isolated from patients aged 35-49 years, 71.4% (10/14) in the strains isolated from patients aged 50-64 years, and 100% (6/6) in the strains isolated from patients above 65 years of age. There was no relationship between the age of the patient and the rate of resistance of the isolated strain to MNZ ($P = 0.45$). Gender was not associated with MNZ resistance as well

Table 1 Antibiotic susceptibility of 111 *Helicobacter pylori* strains isolated in Bhutan *n* (%)

Total	*n* = 111
Amoxicillin	0 (0.0)
Clarithromycin	0 (0.0)
Metronidazole	92 (82.9)
Levofloxacin	3 (2.7)
Ciprofloxacin	3 (2.7)
Tetracycline	0 (0.0)

(P = 0.71). All 11 strains of *H. pylori* from patients with peptic ulcer showed resistance to MNZ; however, it did not statistically differ from the resistance rates of strains isolated from gastritis patients (P = 0.11). The distribution of MIC values for MNZ: among 92 MNZ-resistant strains, 74 strains (80.4%) showed high-level resistance (MIC ⩾ 256 μg/mL). Three strains (2.7%), from patients with gastritis, were resistant to LVX. These strains were also resistance to CIP. The MIC values for LVX and CIP were 32 and 32 μg/mL, respectively. These 3 strains were also resistance to MNZ. None of the strains showed resistance to CLR, AMX, and TET. Resistance to multiple antibiotics was observed in 3 strains (2.7%), where the bacteria were resistant to MNZ, LVX, and CIP. The strain isolated from the GC patient was susceptible to all the antibiotics tested.

DISCUSSION

This is the first study exploring the antibiotic resistance pattern of *H. pylori* strains isolated from Bhutanese population. In Bhutan, no domestic guidelines are available for the treatment of *H. pylori* because of insufficient domestic data. At present, the European, Asia-Pacific, and American guidelines on the treatment of *H. pylori* infection recommend a combination of 1 PPI and 2 antibiotics, AMX plus CLR or MNZ, as the first-line therapy[7,11,12]. Although lack of patient compliance, inadequate length of therapy, or high bacterial burden are conditions that may contribute to loss of efficacy, antimicrobial resistance is regarded as the leading factor responsible for the failure of eradication of infection. This issue is of particular relevance with regard to CLR, where there can be up to 70% loss of antibiotic effectiveness, depending on macrolide susceptibility *in vitro*[16]. Meta-analysis showed that triple therapy consisting of PPI, AMX, and CLR in CLR-resistant infections decreased the treatment efficacy by 66%[26]. In fact, the Maastricht Ⅲ guidelines on *H. pylori* infection management recommend that CLR should not be used when resistance to the antibiotic exceeds 15%-20%[7]. However, surprisingly, none of the strains isolated in Bhutan showed resistance to CLR, suggesting that CLR-based triple therapy can still be used to eradicate *H. pylori* in Bhutan. However, resistance to CLR is increasing worldwide with the increase in the use of CLR[27-29], and it is imperative to examine the CLR resistance rate in the *H. pylori* strains in Bhutan.

Resistance to MNZ is extremely high in Bhutan. Recently, the rate of resistance to MNZ has been reported to increase; this can be considered a major factor leading to reduced efficacy of the standard triple therapy in most countries[11]. Most MNZ-resistant strains in this study showed a high MIC value (⩾ 256 μg/mL). Regimens including MNZ are not a preferred choice in populations with an MNZ resistance rate of > 40%[19,30]. Therefore, if CLR resistance increases in future, MNZ cannot be used as a substitute for CLR in the first-line regimen in Bhutan.

MNZ is frequently used to treat not only *H. pylori* infections, but also other infections such as intestinal parasite infections and periodontal and gynecological diseases, which are common in developing countries[16,31]. National Statistics Bureau of Bhutan also showed that infectious diarrhea is one of the major causes of mortality in the country, which suggests that MNZ can be often used for its treatment (http://www.nsb.gov.bt/).

Recently, LVX has been prescribed as a rescue drug to eradicate infection in case of failure of the first-line therapy[32,33]. However, the prevalence of LVX resistance seems to be increasing worldwide and this may reduce the efficacy of treatment with LVX-based regimens[34-39]. Therefore, according to the European, Asia-Pacific, and American guidelines, LVX should be used in salvage therapy based on antibiotic susceptibility testing[7,11,12]. In Bhutan, LVX is rarely used for the treatment of other infectious diseases and LVX resistance was found only in 3 strains. These strains were also resistant to CIP, suggesting cross-resistance among the fluoroquinolone drugs. TET resistance was not noted in any of the strains tested, consistent with the findings of previous studies from other countries[25,40,41]. TET is not often used for the treatment of infectious diseases in Bhutan. Therefore, TET-based or quadruple therapy including TET can be a useful alternative to the first-line regimen, as recommended in the European and Asia-Pacific guidelines[7,11]. Likewise, all strains in this study were susceptible to AMX, which is consistent with previous findings[40,42,43].

However, we should be cautious about implementing the eradication therapy in Bhutan. Despite the success of the *H. pylori* eradication therapy, the infection does frequently recur in patients in developing countries where there is a high prevalence of *H. pylori* infection[44]. Such repeat infection is either a recurrence of the original infection or reinfection with a new strain. Environmental factors, including poor living conditions, are related to high rates of *H. pylori* infection[45,46]. In rural areas of the country, river or pond water can be used as the source of drinking water (information from National Statistics Bureau, http://www.nsb.gov.bt/); furthermore, unsanitary pit latrines are widely used in this country. It is necessary to improve sanitary conditions to decrease the prevalence of *H. pylori*.

In conclusion, CLR-based triple therapy can still be used to eradicate *H. pylori* in Bhutan. However, because of high resistance rates, MNZ-based triple therapy is not useful as the first-line therapy. It is necessary to have

current and reliable information on the prevalence of antibiotic resistance to *H. pylori,* in particular, in Bhutan. Careful consideration is required for formulating national therapeutic guidelines for the first-line and second-line therapies for *H. pylori* infection, considering factors such as disease prevalence, access to health care centers, diagnostic facilities, and the burden of health care costs borne by the government.

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COMMENTS

Background

Eradication of *Helicobacter pylori* (*H. pylori*) reduces the risk of developing gastric cancer (GC). Antibiotic resistance is the most common factor causing the failure of the treatment. Prevalence of antibiotic resistance is now increasing worldwide and varies in geographic area. The incidence of GC in Bhutan is reported to be quite high comparing with neighbor area. Therefore, effective eradication therapy can contribute to the decrease of incidence of GC in Bhutan. However, the prevalence of drug resistant *H. pylori* in Bhutan has not been elucidated.

Research frontiers

Triple therapy regimens including one proton pump inhibitor and two antimicrobial agents such as amoxicillin (AMX), clarithromycin (CLR), metronidazole (MNZ), levofloxacin (LVX), ciprofloxacin (CIP), and tetracycline have been widely used to eradicate *H. pylori*. Prevalence of antibiotic resistance is now increasing worldwide and varies in geographic area. Although the success of the treatment depends on several factors such as smoking and patient compliance, antibiotic resistance is the most common factor causing the failure of the treatment. Therefore, it is necessary to examine the recent drug resistance rates to select the proper eradication regimens.

Innovations and breakthroughs

Although the incidence of GC in Bhutan is quite high comparing with neighbor area, the prevalence of drug resistant *H. pylori* in Bhutan has not been elucidated. The author's findings can contribute to the decrease of incidence of GC in Bhutan.

Applications

CLR-based triple therapy can be used to eradicate *H. pylori* whereas MNZ-based triple therapy is not suitable for *H. pylori* eradication in Bhutan.

Peer review

The manuscript reports on the pattern of *H. pylori* resistance to antibiotics in Bhutan. The strains of *H. pylori*, cultured from antral mucosal biopsies 111 patients, were assessed for susceptibility to GC, clarithromycin, metronidazole, levofloxacin, ciprofloxacin, and tetracycline, using Epsilometer test. The results revealed the highest resistance rate (83%) to MNZ followed by LVX and CIP, both about 2.7%. This manuscript of limited importance, and of interest to those studying the health status of Bhutanese.

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