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Antimicrobial Resistance of *Helicobacter pylori* Isolates from Cow Feces, Raw Milk and Drinking Water in Iran

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Abstract: *Helicobacter pylori* is a Gram-negative microaerophilic bacterium that has colonized the human gastric mucosa. This infection is very common and affects more than half of the human population. The present study was conducted to determine antimicrobial resistance of *H. pylori* isolates from cow face, cow milk, sheep milk and drinking water in Iran. From March 2009 to May 2011, a total of 438 samples from cow feces (n = 90), cow milk (n = 90), sheep milk (n=60) and drinking water (n = 198), were purchased from Isfahan and Chaharmahal va and Bakhtyari provinces, Iran. A total 22 isolates of *H. pylori* was obtained from cow face, cow milk, sheep milk and drinking water samples. Susceptibilities of 22 *H. pylori* isolates were determined for five antimicrobial drugs (metronidazole, clarithromycin, amoxicillin, tetracycline and furazolidone) using the disk diffusion assay. Out of the 22 *H. pylori* isolates, 11 (50.0%) were resistant to at least one antibiotic. The highest rate of resistance belonged to metronidozaled (36.4%). The resistance rates to clarithromycin, amoxicillin, furazolidone and tetracycline were 0.9%, 0.0%, 0.5% and 18.2%, respectively. Therefore, the major antibiotic resistance would be associated with metronidazole in Iran.

Key words: *Helicobacter pylori* · Antimicrobial resistance · Cow feces · Cow milk · Sheep milk · Drinking water

INTRODUCTION

Helicobacter pylori is a microaerophilic gram negative spiral bacillus that infects the gastric mucosa in 20-80 % of humans throughout the world [1, 2]. Although most *H pylori* infections are clinically silent, this microorganism is associated with substantial morbidity and mortality, causing duodenal and gastric ulcers as well as gastric cancers [3, 4]. There are certain virulence factors important in the pathogenesis of *H. pylori*: first, a large amount of urease production which helps protect organisms from gastric acid; second, penetration across the mucus layer via rapid motility; and, third, the possession of certain toxins such as vacuolating cytotoxin and cytotoxin - associated antigen (Cag A) [5, 6]. The prevalence is however unbalanced between rural developing areas (more than 80%) and urban developed areas (less than 40%) [7-9].

The route of transmission of *H. pylori* is not completely understood. The only known reservoir of *H. pylori* is the human stomach [10] and since *H. pylori* appears to have a narrow host range; new infections are thought to occur because of direct human - to - human transmission or environmental contamination. There is evidence supporting a gastro - oral, oral - oral and faecal - oral transmission, but no conclusive data addressing the predominance of transmission via any of these routes exist [11, 12].

Amoxicillin, tetracycline, metronidazole and clarithromycin are frequently used, combined with proton pump inhibitors or bismuth salts, for the treatment of *H. pylori* infections [13]. However, side effects, poor

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compliance and resistance to antibiotics are causes of treatment failure [14, 15]. Resistance to metronidazole and clarithromycin is population dependent and several studies suggest that clarithromycin resistance is higher in strains obtained from children than in those from adults [16].

Currently, there is limited information regarding the prevalence and antimicrobial susceptibility patterns of *H. pylori* cow face, cow milk and drinking water in Iran. The present study was conducted to determine antimicrobial resistance of *Helicobacter pylori* isolates from cow face, cow milk, sheep milk and drinking water in Iran.

MATERIALS AND METHODS

Sample Collection and Isolation of *Helicobacter Pylori*: From March 2009 to May 2011, a total of 438 samples from cow feces (n = 90), cow milk (n = 90), sheep milk (n=60) and drinking water (n = 198), were purchased from Isfahan and Caharmahal and Bakhtyari provinces, Iran. Bacterial isolation was begun within 6 h of sample collection. Samples were cultured directly on Columbia agar medium supplemented with 10% fetal calf serum, 5% blood and 10 µg/ml Trimethoprim, 6 µg/ml cefsulodin and 5 µg/ml vancomycin and followed by incubation for 3-5 days at 37°C under microaerophilic conditions. Suspected colonies identified as *H. pylori* with catalase, oxidase, urease positive tests and the appearance of Gram-negative curved bacilli. In this study, 7 of 90 cow fecal samples (6.9%), 4 of 90 cow milk samples, 1 of 60 sheep samples and 8 of 198 dinking water samples were found to be contaminated with *H. pylori*.

Antimicrobial Susceptibility Testing: Pure cultures of *H. pylori* isolates were used for antibiotic susceptibility test. One strain from each *H. pylori* -positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood and 7% fetal calf serum, according to the Clinical Laboratory Standards Institute [17]. The following antimicrobial impregnated disks (HiMedia Laboratories, Mumbai, India) were used: metronidazole (5 µg), clarithromycin (2 µg), erythromycin (5 µg), tetracycline (30 µg), amoxicillin (10 µg) and furazolidone (1 µg). After incubation at 37°C for 48 h in a microaerophilic atmosphere, the susceptibility of the *H. pylori* to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI (2006).

RESULTS

Over the 22 months of the study period from March 2009 to May 2011 a total 22 isolates of *H. pylori* was obtained from cow face, cow milk, sheep milk and drinking water. The resistance pattern of *H. pylori* isolates to five antimicrobial agents tested in this study is shown in Table 1. Out of the 22 *H. pylori* isolates, 11 (50.0%) were resistant to at least one antibiotic. The highest rate of resistance belonged to metronidozaled (36.4%). The resistance rates to clarithromycin, amoxicillin, furazolidone and tetracycline were 0.9%, 0.0%, 0.5% and 18.2%, respectively. No resistance to amoxicillin was observed.

Of the two isolates resistant to two antibiotics, one was resistant to metronidazol and clarithromycin and one was resistant to metronidazole and tetracycline. One isolate was resistant to three antibiotics inclouding metronidozale, furazolidon and tetracycline.

DISCUSSION

Antimicrobial resistance of *H. pylori* is a challenging problem and an important factor in determining treatment outcome. The prevalence of antimicrobial resistance varies in different regions [18-20]. Culture for the diagnosis of *H. pylori* infection has not been applied for a long time, though many studies have shown that better management result are obtained when antibiotics are selected based on susceptibility testing versus choosing empirically [19, 21].

This study shows a high level of the prevalence of metronidazole and tetracycline resistance in our region. Treatment with this antibiotic can lead to eradication failure. Other antimicrobial drugs used including clarithromycin, amoxicillin and furazolidone are appropriate choices for treatment.

Considering the three previous studies in our country, rate of resistance reported 35, 54.2 and 51.1% for metronidazole and also 2.4, 4.2 and 0.0 % for clarithromycin and 2.5, 8.3 and 0.0% for amoxicillin. In these studies, no resistance to tetrancycline and furazolidone was reported [20, 22, 23]. Therefore, the major antibiotic resistance would be associated with metronidazole in Iran.

Table 1: Antibiotic resistance profiles of *H. pylori* isolated from cow feces, cow milk, sheep milk and drinking water

	Rates of resistance (%)				
Antimicrobial	Isolates from cow feces	Isolates from cow milk	Isolates from sheep milk	Isolated from drinking water	Total
Metronidazole	3.7 (42.9)	2.4 (50.0)	0.1 (0.0)	3.8 (37.5)	8.22 (36.4)
Clarithromycin	1.7 (14.3)	0.4 (0.0)	0.1 (0.0)	1.8 (12.5)	2.22 (0.9)
Amoxicillin	0.7 (0.0)	0.4 (0.0)	0.1 (0.0)	0.8 (0.0)	0.22 (0.0)
Tetracycline	1.7 (14.3)	2.4 (50.0)	1.1 (100)	1.8 (12.5)	4.22 (1/8)
Furazolidone	0.7 (0.0)	0.4 (0.0)	0.1 (0.0)	1.8 (12.5)	1.22 (4.5)

Therapy of *H. pylori* infection has become the undisputed first option for all patients with peptic ulcers and is advised for several other associated clinical conditions [24]. Eradication treatment usually consists of a proton pump inhibitor in combination with several antimicrobial agents. Those commonly used for the treatment of *H. pylori* infections include clarithromycin, amoxicillin, metronidazole and tetracycline. An increase in resistance rates to these antimicrobial agents is to be expected because of the increasing numbers of patients treated, imperfect patient compliance and increasing consumption of antibiotics in recent years [18, 25]. The principal reason for failure to eradicate infection is believed to be the combination of resistance of *H. pylori* with poor patient compliance. Reported prevalence's of resistance to clarithromycin, amoxicillin and metronidazole vary widely among geographic areas [25, 26]. Knowledge of antibiotic resistance rates is important because treatment of *H. pylori* infection is started with a standard therapeutic regimen. Primary resistance decreases the success of *H. pylori* eradication; hence, the local prevalence of resistance should be monitored [27].

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