Antibiotic susceptibility of Helicobacter pylori isolates in Dakar, Senegal.

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Brief Original Article

Antibiotic susceptibility of *Helicobacter pylori* isolates in Dakar, Senegal

Abdoulaye Seck[1], Mouhamadou Mbengue[2], Amy Gassama-Sow[3], Lamine Diouf[2], Mouhamadou Mourtalla Ka[4], Cheikh Saad-Bouh Boye[5]

[1] *Laboratoire de Biologie Médicale, Institut Pasteur, Dakar Sénégal*
[2] *Service de Gastro-entérologie, Hôpital Arsitide Le Dantec, Dakar Sénégal*
[3] *Laboratoire de Bactériologie Expérimentale, Institut Pasteur de Dakar, Dakar Sénégal*
[4] *Service de Médecine Interne, Hôpital Arsitide Le Dantec, Dakar Sénégal*
[5] *Laboratoire de Bactériologie-virologie, Hôpital Arsitide Le Dantec, Dakar Sénégal*

Abstract

Background: *Helicobacter pylori* is the primary cause of peptic ulcer disease and an etiologic agent in the development of gastric cancer. A high frequency of *H. pylori* infection has been reported from resource-poor regions. *H. pylori* infection is curable with regimens of multiple antimicrobial agents. However, antibiotic resistance is a leading cause of treatment failure. In Africa, there are very little data concerning the susceptibility of *H. pylori* isolates to antibiotics.

Methodology: *H. pylori* isolates from gastric biopsies from outpatients ≥ 18 years old affected by a gastro-duodenal ulcer were used in this study. Susceptibility testing was performed for amoxicillin, ciprofloxacin and metronidazole by using the Epsilometer test (E-test) method.

Results: *H. pylori* strains were isolated from 40 patients of whom 36 were diagnosed as having duodenal ulcer, two with gastric ulcer, and two with gastro-duodenal ulcer. Thirty-six (90%) of the isolates were resistant to metronidazole (MICs ≥ 8 µg/l), whereas all isolates were susceptible to amoxicillin (MICs ≤ 0. 5 µg/ml) and ciprofloxacin (MICs ≤ 1µg/ml).

Conclusion: These data suggest that metronidazole should not be used therapeutically among Senegalese patients in first-line therapy, while ciprofloxacin could be recommended in association with amoxicillin and a proton pump inhibitor in Senegal.

Keywords: *Helicobacter pylori*; antimicrobial susceptibility; Senegal

J Infect Developing Countries 2009; 3(2):137-140.

Received 29 February 2008 - Accepted 3 September 2008

Introduction

The frequency of *H. pylori* infection is rising worldwide [1]. The problem is more acute in the resource-poor regions, particularly in Africa [2]. The hospital prevalence rate of *H. pylori* infection in Senegal is as high as 82% [3]. To date, the eradication of *H. pylori* infection is clearly indicated for healing gastro-duodenal ulcerous disease, not only because it modifies the disease's natural course [4], but because it also prevents long-term ulcer relapse [5]. The successful treatment for eradication of *H. pylori* infection requires a proton pump inhibitor (PPI) and a combination of two or more antibiotics, depending on the susceptibility of isolates to antibiotics [6]. As the treatment of the infection starts with a standard therapeutic regimen, it is therefore important to know in advance the resistance rates of isolates to antibiotics commonly used in the treatment. In Africa, there are very little data concerning the susceptibility of *H. pylori* isolates to antibiotics, even considering that the resistance rates should be high [7].

The aim of this study was to assess the susceptibility of *H. pylori* strains isolated from gastric biopsies of patients with ulcer to antibiotics commonly used in therapeutic procedures.

Materials and methods

Patients and Bacterial isolates

This is a prospective study including outpatients ≥ 18 years old selected between January 1999 and June 2000, and consulting at the gastroenterology department of the major teaching hospital Aristide Le Dantec in Dakar. This study involved patients with clinical symptoms which required a digestive endoscopy. Written and informed consent was obtained from all patients, and all aspects of the study

were approved by the ad hoc Ethical Committee. No patient had taken antibiotics, proton pump inhibitors (PPIs), or non-steroidal anti-inflammatory drugs a month before their inclusion in the study. During the digestive endoscopy, in the presence of duodenal ulcer or gastric ulcer or gastro-duodenal ulcer, gastric biopsies were conducted. Four biopsy specimens (two specimens from different sites of the antrum gastric and two specimens from different sites of the fundus gastric) were obtained from each patient after a digestive endoscopy. Biopsy specimens were stored and transported in tubes containing 0.5 ml of PBS (phosphate buffered saline). Samples were crushed in 0.5 ml of PBS and cultured in Wilkins Chalgren Agar (Becton Dickinson, Heidelberg, Germany) containing 5% to 10 % horse blood, vancomycin (10 mg/l) (Sigma-Aldrich, Steinheim, Germany), and trimethoprim (5 mg/l) (Sigma-Aldrich, Steinheim, Germany). We used only one isolate from each patient for our analysis. Culture media were incubated for a period of up to 12 days in a microaerophilic atmosphere (5% O_2, 10% CO_2, and 85% N_2) at 37°C, and were daily examined for bacterial growth.

Thus, forty strains isolated from gastric biopsies from adult patients affected by an ulcer were used in this study. Isolates were stored at -80°C in 20% glycerol-brain hearth infusion.

Identification of H. pylori *isolates*

H. pylori isolates were identified by colonial morphology, characteristic spiral morphology on Gram staining, and positive findings for catalase, urease and oxidase tests.

Antimicrobial susceptibility testing

Susceptibility to antibiotics testing was performed via minimum inhibitory concentration (MIC) determination by E-test® (AB biodisk, Solna, Sweden) for amoxicillin, metronidazole, and ciprofloxacin according to the recommendations of Megraud *et al.* [8]. The tested inoculum was adjusted to a turbidity of 3 to 4 MacFarland standard. Sterile swabs were used to inoculate plates of Wilkins Chalgren Agar (Becton Dickinson, Heidelberg, Germany) containing 5% to 10 % horse blood. After 72 hours of incubation at 37°C in microaerophilic atmosphere (5% O_2, 10% CO_2, and 85% N_2), the MIC of each antibiotic was determined. Quality control was performed using *H. pylori* ATCC 43504. The breakpoints used to define the resistance were as follows: metronidazole (> 8 µg/ml), amoxicillin (> 0.5 µg/ml) and ciprofloxacin (> 1 µg/ml) [8,9]. Data processing was performed using WHONET V software.

Results

Forty *H. pylori* strains were isolated from 40 patients of whom 36 were diagnosed as having duodenal ulcer, two with gastric ulcer and two with gastro-duodenal ulcer. All tested isolates were susceptible to amoxicillin (MIC_{90} 0.25 µg/ml) and ciprofloxacin (MIC_{90} 0.38 µg/ml). MIC values varied from 0.023 to 0.5 µg/ml for amoxicillin, and from 0.004 to 0.5 µg/ml for ciprofloxacin.

Metronidazole had a reduced activity (MIC_{50} 32 µg/ml); 90% of the isolates (n = 36) were resistant to this compound; and MIC values varied from 0.125 to > 256 µg/ml. The MIC_{50} and MIC_{90} of each antibiotic are shown in Table 1.

Table 1. MIC_{50} and MIC_{90} (µg/ml) of amoxicillin, ciprofloxacin, and metronidazole for *H. pylori* isolates.

Antibiotics	MIC (µg/ml)		
	MICs range	MIC_{50}	MIC_{90}
Amoxicillin	0.023 – 0.5	0.125	0.25
Ciprofloxacin	0.004 – 0.5	0.125	0.38
Metronidazole	0.125 – > 256	32	> 256

Discussion

In this study**,** MICs of three antibiotics (amoxicillin, ciprofloxacin, and metronidazole) were determined for *H. pylori* isolates from Senegal. The study showed that all isolates were susceptible to amoxicillin and ciprofloxacin, whereas the metronidazole resistance rate was high (90% of the isolates).

No resistance to amoxicillin was found, and MICs ranged from 0.003 to 0.5 µg/ml.

Usually, MICs of amoxicillin for *H. pylori* are very low (0.03 µg/ml); nevertheless, in our study, we found some sensitive isolates with a reduced susceptibility (MICs: 0.25 – 0.50 µg/ml), as described by Megraud *et al*. [8]. In many countries, no amoxicillin-resistant *H. pylori* has been detected until now **[**10,11]. However, there have been recent reports of resistance to this antibiotic. In Europe and in the USA, resistance rates are less than 1%, indicating that it is not yet a problem and antimicrobial susceptibility testing for this drug is not currently needed clinically [12, 13]. By contrast, high resistance rates have been reported in some studies

from other parts of the world: 18.5% in South Korea [14], 19.4% in Indonesia [15], 32.8% in India [16], and 38% in Brazilia [17]. In a study conducted in Ile-Ife, southwest of Nigeria, 100% of 32 isolates were resistant [18].

In our study, MIC of ciprofloxacin was averaging 0.38 µg/ml; this value is comparable to the one found by Glocker *et al.* in Germany in 2005 [19]. Quinolone resistance has been described with variable resistance rates. Indeed, low resistance rates were reported in some countries: 1% in India [16], 2% in Egypt [20], 5% in the south-west of Nigeria [18], 6% in Bulgaria [9], and 6.9% in Indonesia [15]. In Germany, ciprofloxacin resistance rate reached 11.2% in 2003, increased to 16.6% in 2004, and further increased to 22.1% in 2005 [19]. In South Korea, a high resistance rate of 33.8% has been reported in strains isolated from adult patients [14]. The good susceptibility of *H. pylori* to ciprofloxacin suggests that this antibiotic might be an alternative drug in the regimen treatment for eradication of *H. pylori* in gastro-duodenal ulcerous disease in Senegal.

Ninety percent (90%) of isolates were resistant to metronidazole with a MIC > 8 µg/ml. A high level of resistance (100%) was found in Egypt in paediatric patients [20], in the southwest of Nigeria [18], and in Indonesia [15]. Resistance rates of 77.9%, 70% and 66.2% were reported in India, in Kuwait, and in South Korea respectively [11,14,16]. Elsewhere, variable resistance rates were described: 15.8% in Bulgaria [9], 25.1% in the USA [13], and 55% in Brazilia [17]. However, resistance rates to metronidazole may vary within a country. For example, in India, the resistance rate to metronidazole was high in Lucknow, Chennai and Hyderabad (68%, 88.2% and 100%, respectively), whereas a moderate rate was observed in Delhi (37.5%) and Chandigarh (38.2%) [16]. In Europe, according to the studies conducted between 1989-2001 and 1990-2002 respectively, the resistance rates varied between 16.0% and 43% in paediatric patients and between 14.9% and 40.3% in adults patients [12]. Moreover, it seems that the primary resistance to nitro-imidazole is related to the frequency of these compounds used in the treatment of other infections. In developing countries, high estimated resistance rates (more than 60%) might be connected to the frequency of treatment by nitro-imidazole in the digestive and genital parasitoses [7]. The use or abuse of this inexpensive drug may contribute to the increase of metronidazole resistance. In addition, testing conditions, including choice of medium, age of the colonies, incubation period and conditions, and inoculum size, have been shown to influence the results of susceptibility testing for metronidazole [12]. Overall, none of the methods was found reliable for testing the susceptibility of *H. pylori* to metronidazole because there is a lack of reproducibility [21].

Our results showed a high resistance rate to metronidazole; however, no amoxicillin and ciprofloxacin resistance were observed. On the basis of our findings, metronidazole should not be used therapeutically among Senegalese patients in first-line therapy. Amoxicillin should be the first choice antibiotic and ciprofloxacin should replace metronidazole in the eradication treatment of gastro-duodenal ulcers with *H. pylori* infection in Senegal. Thus amoxicillin should be recommended in association with ciprofloxacin and a proton pump inhibitor in Senegal. Considering the increasing resistance rate in many countries, monitoring of susceptibility of *H. pylori* to antibiotics appears to be necessary in the choice of effective therapy in order to eradicate *H. pylori* infections and to optimize the regimen in case of treatment failure.

Acknowledgments

We thank Ibrahima Bahsoun and Alfred Dieudonne Kinana for critical reading of the manuscript. This work was financed from own funds by the Service de Gastro-entérologie and the Laboratoire de Bactériologie-virologie, Hôpital Arsitide Le Dantec, Dakar Sénégal. This work was carried out in the Laboratoire de Bactériologie-virologie, Hôpital Arsitide Le Dantec, Dakar Sénégal

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Corresponding Author

Abdoulaye Seck, Laboratoire de Biologie Médicale, Institut Pasteur de Dakar, BP: 220, Dakar Sénégal
Tel: +221 33 839 92 00; Fax: +221 33 822 70 52
Email: seck@pasteur.sn

Conflict of interest: No conflict of interest is declared.