Molecular Detection of *Helicobacter pylori* and its Antimicrobial Resistance in Brazzaville, Congo

Esther Nina Ontsira Ngoyi,* Blaise Irénée Atipo Ibara,† Rachelle Moyen,‡ Philistine Clausina Ahoui Apendi,† Jean Maurisse Obar,‡ O. Obengui,* Roland Bienvenu Ossibi Ibara,† Etienne Ngumbi,† Rock Fabien Niama,‡ Jean Maurice Ouamba,‡‡ Fédele Yala,* Ange Antoine Abena,§ Jamuna Vadivelu,†† Khean Lee Goh,†† Armelle Menard,* Lucie Benejat,** Elodie Sifre,** Philippe Lehours††† and Francis Megraud†††

*Microbiology and Haematology Department, Health Sciences Faculty, Brazzaville, Congo, †Medicine Department, Health Sciences Faculty, Brazzaville, Congo, ‡Molecular and Cellular Biology Department, Technical and Sciences Faculty, Brazzaville, Congo, §Biochemistry Department, Health Sciences Faculty, Brazzaville, Congo, ††INSERM U853, Bordeaux, France, †††French National Reference Center for Campylobacters and Helicobacters, University of Bordeaux, Bordeaux, France, ‡‡University of Malaya, Kuala Lumpur, Malaysia, ‡‡Chemistry plant and life unit, Technical and Sciences Faculty, Brazzaville, Congo

**Keywords**

Macrolides, fluoroquinolones, tetracycline, real-time PCR, gastric biopsies.

Reprint requests to: Esther Nina Ontsira Ngoyi, B.P 15342, Brazzaville Congo. E-mail: esther_muller2003@yahoo.fr

Abstract

**Background:** *Helicobacter pylori* infection is involved in several gastro-duodenal diseases which can be cured by antimicrobial treatment. The aim of this study was to determine the prevalence of *H. pylori* infection and its bacterial resistance to clarithromycin, fluoroquinolones, and tetracycline in Brazzaville, Congo, by using molecular methods.

**Material and Methods:** A cross-sectional study was carried out between September 2013 and April 2014. Biopsy specimens were obtained from patients scheduled for an upper gastrointestinal endoscopy and were sent to the French National Reference Center for Campylobacters and Helicobacters where they were tested by molecular methods for detection of *H. pylori* and clarithromycin resistance by real-time PCR using a fluorescence resonance energy transfer-melting curve analysis (FRET-MCA) protocol, for detection of tetracycline resistance by real-time PCR on 16S rRNA genes (*rrnA* and *rrnB*), for detection of point mutations in the quinolone resistance-determining regions (QRDR) of *H. pylori gyrA* gene, associated with resistance to quinolones, by PCR and sequencing.

**Results:** This study showed a high *H. pylori* prevalence (89%), low rates of clarithromycin and tetracycline resistance (1.7% and 2.5%, respectively), and a high rate of quinolone resistance (50%).

**Conclusion:** Therefore, the use of standard clarithromycin-based triple therapy is still possible as an empiric first-line treatment as well as prescription of bismuth-based quadruple therapy, which includes tetracycline, but not a levofloxacin-based triple therapy because of the high rate of resistance to fluoroquinolones.

The role of *Helicobacter pylori* infection in several gastroduodenal diseases such as gastritis, peptic ulcer, MALT lymphoma, and gastric cancer is well established [1]. There are several invasive and noninvasive methods to diagnose *H. pylori*. With regard to culture, the difficulty lies in the susceptibility of the bacterium to drying and changes in temperature and its requirement for a microaerobic atmosphere for growth [2]. It is difficult to obtain these conditions in developing countries due to limited technical platforms. Molecular methods are rapid methods to detect pathogens, and they can also be used for the determination of resistance to antibiotics such as macrolides, quinolones, and tetracyclines [3–5]. Clarithromycin is a macrolide commonly used in first-line treatment of *H. pylori* infection [6]. Resistance to clarithromycin is due to point mutations within the peptidyltransferase-encoding region of the 23S rRNA gene and was described before [7,8]. Increasing resistance against clarithromycin is currently compromising *H. pylori* eradication therapies [9]. Therefore, other antibacterial drugs, such as fluoroquinolones (levofloxacin or moxifloxacin), have been proposed. *H. pylori* resistance against quinolones, which exert their antimicrobial effects by affecting a subunit of the *H. pylori*
DNA gyrase, is caused by point mutations in the so-called quinolone resistance-determining region (QRDR) of the gyrA [10–12]. Concerning tetracyclines, they exert their antimicrobial effects by affecting the 30S subunit of the ribosome and block the binding of aminoacyl-tRNA, resulting in impaired protein biosynthesis [13,14]. *H. pylori* resistance to tetracyclines is also reported to be caused by mutations in the 16S rDNA [5,13,15].

In Congo Brazzaville, a serologic study of *H. pylori* was performed and showed that almost the entire population is infected by *H. pylori* and that infection arises early in life. However, the study of *H. pylori* resistance to antibiotics was never performed. The aim of this study was to determine the prevalence of *H. pylori* infection and the bacterial resistance to clarithromycin, fluoroquinolones, and tetracycline by using molecular methods.

**Materials and Methods**

**Obtention of Gastric Biopsies and DNA Extraction**

A cross-sectional study was carried out between September 2013 and April 2014. Biopsy specimens were obtained from patients who were never treated for *H. pylori* eradication, scheduled for an upper gastrointestinal endoscopy, in Schnell clinic (a private medical clinic in Brazzaville, Congo). Informed consent was obtained beforehand. Gastric biopsies were obtained and sent to the National Reference Center for Campylobacters and Helicobacters in Bordeaux, France where they were ground in 1 mL of Brucella broth for molecular study. A small fragment was digested in 20 μL of proteinase K (Qiagen SA, Courtaboeuf, France) with 180 μL of lysis buffer (Qiagen). DNA extraction was performed by using a MagNA Pure LC DNA isolation kit 1 (Qiagen).

**Detection of *H. pylori* and of Point Mutations Associated with Clarithromycin Resistance, Quinolones Resistance, and Tetracycline Resistance**

Detection of *H. pylori* and of point mutations in the 23S rRNA gene associated with clarithromycin resistance was performed by real-time PCR as previously described [3]. The method included amplification of a fragment of the *H. pylori* 23S rRNA gene coupled with a simultaneous detection of the amplicon by probe hybridization, followed by a melting curve analysis [16,17]. Point mutations associated with quinolones resistance detection, in the QRDR of *H. pylori* gyrA gene, were performed by classical PCR and sequencing. They were carried out as described by Rimbara et al. [4]. Detection of point mutations in the *H. pylori* 16S rRNA gene associated with tetracycline resistance was carried out by real-time PCR as previously described [5].

**Statistical Analysis**

The data were treated using Epi Info 3.5 statistical software.

**Results**

**Characteristics of Patients**

Sixty three patients were included. Thirty one patients (49.2%) were male and 32 (50.8%) female (sex ratio = 1). Fifty seven patients (90.47%) were outpatients, and six (9.53%) were hospitalized. The patients’ age ranged from 17 to 76 years, with a mean age of 43.9 ± 15.3 years.

The clinical symptoms and endoscopy results are presented on Tables 1 and 2.

**Detection of *H. pylori* and the Mutations Associated with Resistance to Antibiotics**

Of the 63 patients tested, 56 (89%) were positive for *H. pylori*. The prevalence in the age group 17–37 years was 95.8% (23+/24), in the age group 38–58 years: 85.1% (23+/27), and in the age group 59–76 years: 83.3% (10+/12) (NS). The results of detection of mutations associated with antimicrobial resistance are presented on Table 3. For clarithromycin, only one strain presented a resistance profile which corresponded to a mutation A2142/3G while 55 strains had a wild-type

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epigastralgia</td>
<td>42</td>
<td>66.7</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>4</td>
<td>6.3</td>
</tr>
<tr>
<td>Pyrosis</td>
<td>3</td>
<td>4.8</td>
</tr>
<tr>
<td>Ascitis</td>
<td>3</td>
<td>4.8</td>
</tr>
<tr>
<td>Ulcer follow-up</td>
<td>3</td>
<td>4.8</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>2</td>
<td>3.0</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Foreign body sensation</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Odynophagy</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Digestive hemorrhage</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 Distribution of patients according to clinical symptoms
profile (susceptible). Only 36 DNA could be amplified and sequenced for the QRDR; 18 of them (50%) had a wild-type profile and 18 had other profiles, 16 with single mutations and 2 with double mutations. The polymorphism Asn87Thr which does not lead to resistance was detected 5 times. Details of the mutations found are presented on Table 4.

Concerning tetracycline, we could amplify DNA from 40 strains; 39 had a wild-type profile (susceptible) with melting temperatures of 61 °C and exhibiting an AGA 926-928 sequence. One strain presented a single mutation AGC 926-928, (melting temperature 56.2 °C), corresponding to a decreased susceptibility profile.

**Discussion**

As expected, *H. pylori* prevalence in Brazzaville, Congo, is quite high (89%), acquired early in childhood, as there is no statistically significant difference between the age groups. It confirms an early report concerning children from the same city [18] and is in line with other studies from Western Africa: Ghana [19], and Senegal [20], and Eastern Africa: Kenya [21], as well as the general knowledge on epidemiology of the infection.

But the main interest of this study is to bring us important information on the status of *H. pylori* resistance against the antibiotics used in treating this infection.

In contrast to what is observed in many countries of the world, clarithromycin resistance is almost nonexistent in the Congo. In Europe, a positive correlation was established between long-acting macrolide consumption and *H. pylori* resistance to clarithromycin [22]. In China, *H. pylori* clarithromycin resistance increased from 12.8% to 23.8% and in Japan, from 7 to 15.2% [23]. Indeed, macrolides are not frequently prescribed in the Congo apart from *H. pylori* eradication. So the Congo is among the countries where it is still possible to use the standard triple therapy as an empiric first-line treatment for this infection. Given that tetracycline is part of the bismuth-based quadruple therapy, actually used in many countries, it was interesting to look to tetracycline resistance of *H. pylori*. Only one strain harbored a single mutation and none the triple mutation leading to high level of resistance. Indeed, this is the most frequent situation around the world, resistant strains being reported essentially in Korea and Brazil.

There are no data on the consumption of macrolides and tetracyclines in Congo Brazzaville, but these results let think that it is low.

A large proportion of strains possessed mutations associated with fluoroquinolone resistance (50%), including two (5.5%) with double mutation.

The level of resistance appears extremely high compared to other countries, including countries from Africa. It was 15% in Senegal [20], and 26% in Portugal, country with the highest rate of resistance to fluoroquinolones in Europe. Already in 2013, a study on urinary tract infection pathogens showed high rate resistance to quinolones (50–60%) in Brazzaville [24]. This is certainly due to the high prescription of these antibiotics, although there is no available data. The most frequent mutation was Asn87Ileu (seven cases) as
found in Senegal [20] and also in France [25]. Mutations in position 91 were less frequent. These mutations have been associated with levofloxacin resistance, but it is possible that new fluoroquinolones such as sitafloxacin or gemifloxacin would have lower MICs and overcome this resistance [26].

The absence of culture which did not allow us to get MICs for the antibiotic considered is a consequence of limited infrastructure. Indeed, if point mutations allow a good prediction of resistance data, it is not perfect as other mechanisms such as efflux may also be responsible, especially for tetracycline resistance [27]. Furthermore, we could not amplify 100% of the target DNAs. It also eliminates the possibility of testing metronidazole resistance. Indeed, given the low reproducibility of in vitro data and the lack of correlation with in vivo results, it does not turn out to be a major problem.

It is not a problem either for amoxicillin given that resistance is seldom encountered.

**Conclusion**

In conclusion, in this country of high *H. pylori* prevalence, the use of standard clarithromycin-based triple therapy is still possible as an empiric first-line treatment, given the very low rate of resistance to this antibiotic. But for an empiric second-line treatment, given the limited resistance to tetracycline, it is possible to prescribe a bismuth-based quadruple therapy, which includes tetracycline, but not a levofloxacin-based triple therapy because of the high rate of resistance to fluoroquinolones. In this study, genotypic susceptibility testing shows how molecular genetic techniques can be referred to if culture is not available. Furthermore, molecular genetic testing is easier to standardize than phenotypic testing.

**Acknowledgements and Disclosures**

This study was supported by Grant No. UM.C/625/1/HIR/MoE/CHAN/13/5 H50001-00-A000032 (University of Malaya).

**Competing interests:** the authors have no competing interests.

**References**

20. Seck A, Burucoa C, Dia D, Mbengue M, Onambele M, Raymon J, et al. Primary antibiotic resistance and associated...


