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Antimicrobial Susceptibility Study

Update of primary *Helicobacter pylori* resistance to antimicrobials in Brussels, Belgium☆

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ABSTRACT

This study aimed to follow the trend of primary antimicrobial resistance in *Helicobacter pylori* isolates obtained from several centers in Brussels.
We observed increasing rates of primary resistance to macrolides (10.5% to 18%) to nitro-imidazoles (28% to 40%) and to fluoroquinolones (12.4% to 22.8%), respectively, from 2008/2009 to 2016.

Helicobacter pylori colonizes the stomach of approximately one-half of human beings, although its prevalence is declining, particularly in industrialized countries. Although *H. pylori*–induced chronic gastritis is a generally asymptomatic infectious disease, it can progress to severe diseases among which are peptic ulcer, gastric cancer, and MALT lymphoma (Sugano et al. 2015; Malfertheiner et al. 2017).

Susceptibility to antimicrobial agents and patients' adherence are the main determinants for the success of *H. pylori* eradication regimen. Since *H. pylori* may develop resistance to many of commonly used antibiotics, awareness of the local and national resistance rates is essential to optimize the choice of effective treatment strategies.

Owing to the increasing *H. pylori* resistance rate to antimicrobials coupled with the declining eradication rates in most parts of the world (Savoldi et al. 2018), Maastricht V/Florence consensus report recommends to choose local therapeutic strategy on the basis of the rates of resistance to antibiotics and particularly clarithromycin and secondarily metronidazole or dual clarithromycin–metronidazole (Malfertheiner et al. 2017).

The aim of this *in vitro* antimicrobial resistance survey was 1) to follow the primary resistance rates in clinical isolates of *H. pylori* against currently used antimicrobials in several gastrointestinal disease centers in the region of Brussels and 2) to evaluate the trend of resistance compared to our previous large survey (Miendje Deyi et al. 2011).

The study included patients, without history of previous *H. pylori* therapy, who have attended several endoscopy clinics in the region of Brussels from January 1, 2015, to December 31, 2016.

Analyses have been carried out in a central lab (LHUB-ULB; Laboratoire Hospitalier Universitaire de Bruxelles–Universitair Laboratorium Brussel) which is the result of a partnership between 5 University Hospitals located in the Region of Bruxelles-Capitale [CHU Saint-Pierre, Institut Jules Bordet, Hôpital Universitaire des Enfants Reine Fabiola (HUDERF), CHU Brugmann, CUB Hôpital Erasme], representing close to 3000 beds and covering a population of 700,000 inhabitants.

Upon arrival at the laboratory, fresh gastric biopsies were either frozen at −70 °C until required or immediately processed. Each specimen was ground and inoculated onto *Helicobacter* agar plates (Becton Dickinson, USA). Plates were incubated for 3 to 14 days at 37 °C under a

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humid, microaerophilic atmosphere using an incubator (Binder [serial no. 08-51907], Germany.

Systematic susceptibility testing was performed by disc diffusion method (Rosco, Taastrup, Denmark) and/or E-test (AB Biodisk, Solna, Sweden). A fresh 3 McFarland inoculum was inoculated onto a BD Columbia horse blood plate (Becton Dickinson, USA). Etest strips or Neo-sensitabs™ were applied. The plates were incubated for 2 to 3 days. Owing to the important gap between MICs for susceptible and resistant strains in the context of large routine activity (around 5000 cultures per year), MICs are determined (using EUCAST criteria) if the inhibition zone is under the susceptibility breakpoints of the manufacturer: ≥30 mm for clarithromycin (CLA), levofloxacin (LEV), and tetracycline (TET); ≥26 mm for metronidazole (MET); and ≥25 mm for ampicillin (for amoxicillin (AMO) resistance (Rosco, Taastrup, Denmark).

Primary resistance rates were analyzed annually and compared to those obtained in our previous study (Miendje Deyi et al. 2011).

A total of 408 in 2015 and 438 in 2016 naïve patients with positive *H. pylori* culture were included in the study.

Primary resistance rates of *H. pylori* against CLA, MET, AMO, and LEV were, respectively, 15.2%, 33.1%, 1%, and 16% in 2015 and 18%, 40%, 0%, and 22.8% in 2016. No resistance to TET was observed. The dual clarithromycin–metronidazole resistance occurred in 6.14% and 8% of the isolates, respectively, in 2015 and 2016, compared to 3.2% and 6.5% for dual clarithromycin–levofloxacin.

Our last survey spanning 20 years (1990 to 2009) on clinical isolates of *H. pylori* from the same digestive disease centers in Brussels region included 1001 naïve patients from 1 January 2008 to 31 December 2009. These last 2 years of the survey showed primary resistance rates of 10.5% for CLA, 28% for MET, and 12.4% for LEV. No resistance was observed for TET and AMO (Miendje Deyi et al. 2011). Hence, resistance to CLA, MET, and LEV have dramatically increased since 2009 as illustrated by Fig. 1. However, the resistance trend is different for these 3 antibiotics.

CLA, the most effective agent against *H. pylori*, showed a variable resistance rate over time in our population: 1) primary clarithromycin resistance increased significantly, reaching peaks in 2003 (19.2%); 2) a subsequent decrease to 9.9% in 2009 followed in parallel with a decrease in macrolide consumption during the same period (Miendje Deyi et al. 2011); and 3) we are now observing an important increase of clarithromycin resistance (28% in 2016).

We are clearly facing an increase of *H. pylori* resistance to 3 antibiotics classes without any significant increase in the Belgian community consumption of those antibiotics (ECDC reports 2017).

Primary MET resistance remained stable over the years until 2009 (average rate of 30.6% over 20 years); then, we observed an important increase of resistance rate up to 40% in 2016.

It is probably related to the increasing number of immigrants from sub-Saharan Africa (18.7% of increase from 2010 to 2015; Schoumaker and Schoonvaere 2014; Demart et al. 2017) who could have used metronidazole to cure parasitological infections. Indeed, the highest *H. pylori* resistance to MET is observed in Africa (Jaka et al. 2018), and our last study confirmed the highest prevalence of *H. pylori* infection in patients with African ethnic background (Mana et al. 2013). Compared to other European countries, metronidazole resistance seems to be low (Savoldi et al. 2018). This is probably related to the inclusion of a high number of pediatric patients in our study. In fact, in 2016, the primary metronidazole resistance reported in a children's hospital (HUDERF) was 26.5 compared to 45.7% for CHU Saint-Pierre, which is a general hospital.

H. pylori resistance to fluoroquinolones increased gradually (rare in the early 90s, 11.6% in 2009, and 22.8% in 2016). It is probably related to the increasing use of these molecules to treat various infections.

Increasing primary resistance rates to *H. pylori* against currently used antimicrobials have also been recently reported in several areas of the world (Bouihat et al. 2017, Boyanova et al. 2017, Butenko et al. 2017, Kuo et al. 2017, Liu et al. 2018, Macías-García et al. 2017).

The empirical treatment should be continuously adapted since the resistance to antimicrobials jeopardizes *H. pylori* eradication. Hence, according to the last Maastricht V/Florence consensus report (Malfertheiner et al. 2017), bismuth quadruple or concomitant non–bismuth-containing quadruple therapy is currently the appropriate empiric treatment in our population, and antimicrobial susceptibility testing is needed prior to clarithromycin and levofloxacin use in standard triple therapy.

In conclusion, this study clearly shows increasing rates of primary resistance of *H. pylori* to macrolides, to nitro-imidazoles, and to fluoroquinolones, while resistance rates to amoxicillin and tetracycline remained low. In contrast to our previous recommendations, the empirical clarithromycin-based triple therapy should no more be considered in our population. This study highlights the relevance of monitoring the epidemiology of *H. pylori* resistance to antimicrobials in order to provide appropriate treatment options to physicians.

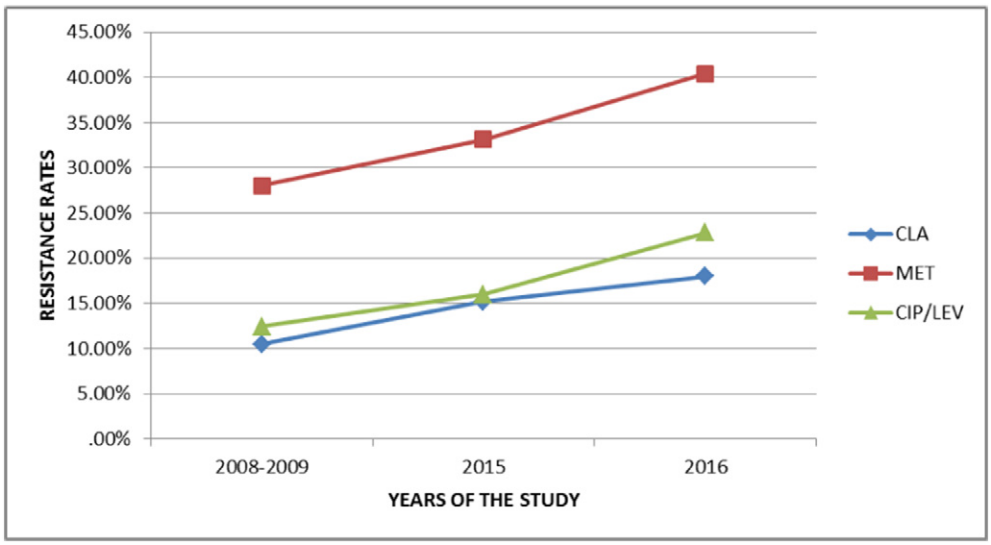

Fig. 1. Evolution of primary resistance of *H. pylori* to clarithromycin metronidazole and fluoroquinolones in Brussels, Belgium. CLA = clarithromycin, MET = metronidazole, CIP/LEV = fluoroquinolones: ciprofloxacin followed by levofloxacin.

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