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Primary antibiotic resistance of *Helicobacter pylori* isolates is twofold more frequent in HIV-positive than HIV-negative individuals: A descriptive observational study

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Abstract

The antimicrobial susceptibility of *Helicobacter pylori* strains isolated from HIV-positive individuals is not well characterized. This study aimed to measure the prevalence and long-term trends associated with primary *H. pylori* antibiotic resistance, evaluate correlations with antibiotic consumption, and compare predictors for *H. pylori* antibiotic resistance between HIV-positive and HIV-negative individuals. In this longitudinal registry study, we evaluated consecutive adults with and without HIV infection, naïve to *H. pylori* treatment, who underwent upper gastrointestinal endoscopy and had a positive *H. pylori* culture, with susceptibility testing available, between 2004 and 2015. Outpatient antibiotic consumption data were based on nationwide aggregated numbers. *H. pylori* was isolated from gastric biopsies of 3008/8321 patients, 181/477 (37.9%) were HIV-positive and 2827/7844 (36.0%) HIV-negative. Overall cohort mean prevalence of *H. pylori* primary antibiotic resistance was 11.1% for clarithromycin, 17.8% levofloxacin, and 39.4% metronidazole. The prevalence of *H. pylori* primary resistance was significantly higher for these three drugs in HIV-positive individuals across the study period. Linear regression showed that the prevalence of clarithromycin and levofloxacin resistance correlated with the country aggregate daily dose consumption of macrolides and quinolones, respectively. Multivariable regression analysis showed that HIV infection is a strong independent risk factor for multiple *H. pylori* antibiotic resistance. In summary, HIV infection is a risk factor for carrying multi-resistant *H. pylori* strains and this is correlated with antibiotic consumption. Empirical therapies should be avoided in HIV-positive individuals. These data highlight the need to implement ongoing monitoring of *H. pylori* antimicrobial susceptibility among HIV-positive individuals. The study is registered at ISRCTN registry, number 13466428: https://www.isrctn.com/ISRCTN13466428.

KEYWORDS
anti-bacterial agents, cohort studies, drug resistance, *Helicobacter pylori*, HIV, prevalence, risk factors

1 | INTRODUCTION

Helicobacter pylori (*H. pylori)* and human immunodeficiency virus (HIV) infections are common worldwide, but the frequency of *H. pylori* infection among HIV-positive individuals varies according to the region and population studied (Nevin et al., 2014). As an example, reported *H. pylori* infection prevalence rates are 23% and 51% among HIV-positive individuals in Germany and Ghana, respectively (Eidt et al., 1995; Sarfo et al., 2015). Complications related to *H. pylori* infection, notably gastro-duodenal ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma, found in both HIV-positive and HIV-negative patients, could be avoided by treating *H. pylori* infection (Crowe, 2019). For decades, the standard of care for treating *H. pylori* infection has been triple therapy (TT) which combines a proton pump inhibitor (PPI) with two antibiotics (any two of clarithromycin [CLR], metronidazole [MET], amoxicillin [AMX], levofloxacin [LEV], or tetracycline [TET]), twice daily for at least 7–14 days (Malfertheiner et al., 2017; Thung et al., 2016). However, the failure rate for TT is rising in many parts of the world (Gisbert & Pajares, 2002; Glupczynski et al., 2001; Graham & Shiotani, 2008; Malfertheiner et al., 2017). The main reasons for this appear to be non-compliance and antimicrobial resistance of *H. pylori* (Malfertheiner et al., 2002, 2017; Vakil & Megraud, 2007). Resistance to CLR has led to a 70% decline in TT eradication rates, while resistance to LEV and MET are responsible for 50% and 33% declines, respectively (Wong et al., 2003). The Maastricht V/Florence consensus conference has recommended that empirical TT containing CLR should be avoided when the prevalence threshold for CLR resistance is greater than or equal to 15% (Malfertheiner et al., 2017). Primary CLR resistance among adult patients is variable across countries. For example, CLR resistance rates were 5.6% in The Netherlands, 22% in Belgium, and 31.5% in Portugal in 2008–2009 (Thung et al., 2016). *Helicobacter pylori* antibiotic resistance rates are increasing globally and, at the same time, global antibiotic consumption increased by 36% between 2000 and 2010, mainly in developing countries (Thung et al., 2016).

In this regard, HIV-positive individuals are a model of interest. Indeed, HIV, by chronically destroying CD4 T lymphocytes (CD4+ T), leads to a decline and impairment in immune function (Pantaleo, & Fauci, 1996). For this reason, HIV-positive individuals are more susceptible to infections and, consequently, are exposed to antibiotic therapy for the treatment of acute infections as well as for chemoprevention against opportunistic infections (Panel on Opportunistic Infections in HIV-Infected Adults & Adolescents, 2020). The extent to which this antibiotic exposure affects microbial susceptibility in HIV-positive individuals is understudied. In a previous short-term study from our center, we observed that *H. pylori* primary antibiotic-resistant strains were more common among HIV-positive compared to HIV-negative individuals. Currently, there are few data available concerning recent levels and trends in *H. pylori* antibiotic resistance among HIV-positive individuals (Gill et al., 2008). The goals of our study were to compare prevalence rates and long-term trends in primary *H. pylori* antibiotic resistance among HIV-positive and HIV-negative individuals who underwent an upper gastrointestinal (GI) endoscopy for any medical reason in our hospital, to evaluate correlations with aggregate antibiotic consumption in the country, and to identify predictors for primary *H. pylori* antibiotic resistance among HIV-positive individuals. This information may provide valuable data for decision-making concerning the prescription of appropriate anti-*H. pylori* drugs for *H. pylori*-HIV-co-infected patients. The interim results of this work were published already in 2015 (Nkuize et al., 2015).

2 | MATERIALS AND METHODS

This descriptive observational study was carried out at CHU Saint-Pierre in Brussels, a general hospital that currently monitors more than 3000 HIV-positive patients regularly and performs more than 3500 UGI endoscopies per annum.

We prospectively collected patient data in a registry from 1 January 2004 through 31 December 2015. Inclusion criteria were as follows: all consecutive HIV-positive and HIV-negative individuals, naïve to *H. pylori* treatment, aged ≥18 years of age that underwent upper GI endoscopy for any reason and for which *H. pylori* antimicrobial susceptibility test results were available. Exclusion criteria were as follows: denial or withdrawal of consent, coagulation disorders, and gastrectomy. Data collected on the day of inclusion were demographics (age, gender, and ethnicity); HIV status and HIV-related parameters (duration of HIV infection, Center for Disease Control (CDC) stage, viral load, antiretroviral treatment, CD4 T lymphocyte cell count); and *Toxoplasma gondii* or *Pneumocystis jiroveci* chemoprevention or antimalarial drug use (including mefloquine, atovaquone, chloroquine, and primaquine) within 12 months before endoscopy because cross-resistance has been described between trimethoprim-sulfamethoxazole and sulfamides, and chloroquine and quinolones (Davidson et al., 2008; Gill et al., 2008). Bismuth compounds are rarely used in Belgium and were not recorded. The history of *H. pylori* treatment was obtained at the time of upper GI endoscopy, and at inclusion by anamnesis and, if not, from the patient's medical chart and the family medical doctor for all HIV-positive and HIV-negative individuals with antibiotic-resistant strains of *H. pylori*. To take into account differences in the prevalence of antibiotic resistance between ethnicities and regions, region of origin was recorded, based on the patient's self-identified ethnicity and geographic area of origin. The regions of origin were Eastern Europe, Western Europe, North Africa, Sub-Saharan Africa, and Other, which included Latin and North America, Asia, and the Middle East. Similarly, a region of birth was classified following the same definition as a region of origin. Details are presented in Appendix 1.

Patients underwent upper GI endoscopy after fasting for 12 h or overnight. At least four gastric biopsy samples were taken (one from the angulus, one from the antrum, and two from the body). Samples were immediately transferred to Amies Agar Gel Collection and Transport Swabs (Copan Diagnostic Inc.) and placed in a refrigerator

at −5 to +5°C before being sent within 24 h to the Department of Microbiology (Nkuize et al., 2015).

Susceptibility to CLR, MET, LEV, AMX, and TET was assessed under routine conditions using disk diffusion methods (Neo-Sensitabs; Rosco, Taastrup, Denmark), and the minimum inhibitory concentration (MIC) determined by an agar dilution method. Isolates were classified as resistant with cut-off values of ≥1 mg/L for CLR, >8 mg/L for MTZ, and >1 mg/L for LEV (Clinical & Laboratory Standards Institute, 2009; Miendje Deyi et al., 2011).

Data on outpatient antibiotic use were extracted from the database of the Agence Intermutualiste, which has compiled the health data of insured individuals for the full population in Belgium since 2002. Drug use data are registered following the Anatomical Therapeutic Chemical (ATC) classification. For this study, we first used ATC classification to select individuals with ambulatory consumption of antiretroviral (ARV) drugs (See details in Appendix 2). Patients were categorized according to their use of ARV (HIV-positive subgroup) or no-use of ARV (HIV-negative subgroup); these subgroups were compared for macrolide (J01FA) and quinolone (J0MA) aggregated consumption expressed as defined daily dose per 1000 inhabitants per day/year for the whole insured Belgian population (Coenen et al., 2014) Metronidazole consumption and its correlation with MET resistance have not been studied given (i) there is currently some controversy regarding the reproducibility and benefit of the *H. pylori* metronidazole susceptibility test (Filipec Kanizaj et al., 2009; Malfertheiner et al., 2017), (b) it is taken by oral, rectal, or vaginal administration and, while oral dosing is consistent, rectal, or vaginal dose may vary.

2.1 | Statistical analysis

Descriptive statistics are expressed as a median and interquartile range for continuous variables and as frequencies or proportions with 95% confidence intervals for categorical variables. Wilcoxon rank-sum test was used for median comparisons. Fisher's exact test (2 groups) and Pearson's chi-square test (>2 groups) were used for statistical analysis of frequency and proportion distributions. In univariable analysis, the strength of association was measured as odds ratio (OR) with a 95% confidence interval.

Risk factors for primary *H. pylori* resistance to one antibiotic and ≥two antibiotics in HIV-positive patients and the whole cohort, respectively, were included in multivariable analysis (logistic regression) for any parameter with a p-value < 0.1.

The following variables were included in the HIV-positive subgroup model: age, sex, region of origin, region of birth, HIV duration, and treatment with antiretroviral or treatment with trimethoprim-sulfamethoxazole, CDC stage, and CD4 nadir.

The following parameters were included in the whole cohort model: age, gender, ethnicity, country of birth, and HIV status.

To measure the period effect on *H. pylori* antibiotic resistance, the OR of frequency of *H. pylori* antibiotic resistance according to HIV status was determined within each period. The global effect of OR adjusted for the period was determined with the Mantel-Hanszel method.

To study correlations between macrolide and quinolone consumption and primary *H. pylori* CLR and LEV resistance in the cohort, simple linear regression was used.

For the parameters studied, missing data were specified accordingly.

Analyses were performed using IBM SPSS Statistical software (v25 - 08/2018 - IBM Corporation). A bilateral p-value of <0.05 was considered statistically significant.

3 | RESULTS

The study population (Table 1) included 8321 patients (477 HIV-positive and 7844 HIV-negative), naïve for *H. pylori* treatment, and for whom an *H. pylori* culture was available between 2004 and 2015. *Helicobacter pylori* culture was positive in 3008 patients (36.1%): 181 (37.9%) HIV-positive and 2827 (36.0%) HIV-negative. See details in Appendix 4. The proportion of resistance to at least one antibiotic (CLR, LEV, or MET) was markedly greater in the HIV-positive group (76.8%) than in the HIV-negative group (52.4%) (OR = 3.01). A proportionally similar difference was observed for these three antibiotics analyzed separately (see Appendix 4). Data were incomplete in 127 individuals: 13/181(7.1%) and 114/2827 (4.0%) among HIV-positive and HIV-negative, respectively. When the analysis was restricted to individuals in the *H. pylori*-positive subcohort for whom all three antibiotic tests were jointly available, proportionally similar differences and odds ratios were obtained.

Figure 1 shows that the prevalence of *H. pylori* resistance to at least one of the three antibiotics (CLA, LEV, or MET) in HIV-negative subjects was 50.4%. This proportion increased significantly to 71.3% in HIV-positive AIDS-free individuals and increased further to 92.6% in HIV-positive AIDS individuals.

Appendix 5 compares demographic and endoscopic data in both the HIV-positive and HIV-negative groups of the *H. pylori*-positive cohort. HIV-positive patients were significantly older and originated more frequently from sub-Saharan Africa. The gender ratio was similar in both groups. Oesophageal candidiasis was more frequent (OR = 22.3) while gastric and duodenal ulcers were less frequent in the HIV-positive group (OR = 0.52 and 0.50). Appendix 6 shows the demographics of HIV-positive patients.

Table 2 compares the proportion of combined resistance to two or three antibiotics (CLR ± LEV ± MET) in the HIV-positive and HIV-negative groups. The proportion of resistance to any combination of two antibiotics was significantly greater in HIV-positive versus HIV-negative individuals (CLR-MET: 8.2% vs. 4.7%, LEV-MET: 15.1% vs. 8.5%, CLR-LEV: 5.7% vs. 2.9%) with similar odds ratios of 1.82, 1.90, and 1.97, respectively. Triple antibiotic resistance was observed in 2.3% versus 1.5% (not significant). Finally, quadruple resistance was observed in 0.5% versus 0.0%.

Univariable analysis of the characteristics of the HIV-positive group in relation to *H. pylori* resistance to antibiotics (Table 3) showed

TABLE 1 Study population stratified according to HIV infection, *Helicobacter pylori* infection, and *H. pylori* susceptibility testing

Parameters	HIV-negative	HIV-positive	Odds Ratio (95%CI)	Probability
Initial cohort: patients with *H. pylori* culture, total no.	7844	477	—	
H. pylori cohort: positive *H. pylori* culture and at least one antibiotic tested, no. (% of initial cohort)	2827 (36.0%)	181 (37.9%)	1.09 (0.90–1.31)	0.7
Resistance to ≥1 antibiotic, no. (% of *H. pylori* + cohort)	1481 (52.4%)	139 (76.8%)	3.01 (2.1–4.3)	<0.0001
Clarithromycin, no./no. tests (% resistant)	294/2720 (10.8%)	29/176 (16.4%)	1.62 (1.0–2.4)	0.02
Levofloxacin, no./no. tests (% resistant)	480/2825 (16.9%)	57/180 (31.6%)	2.26 (1.6–3.1)	<0.0001
Metronidazole, no./no. tests (% resistant)	1083/2815 (38.4%)	93/173 (54.9%)	1.94 (1.4–2.6)	<0.0001
H. pylori subcohort: subgroup of *H. pylori* + cohort with joint testing of clarithromycin, levofloxacin, and metronidazole, no. (% of initial cohort)	2713 (34.6%)	168 (35.2%)	1.03 (0.8–1.2)	0.7
Resistance to ≥1 antibiotic, no. (% of *H. pylori* + subcohort)	1367 (50.4%)	126 (75.0%)	2.95 (2.0–4.2)	<0.0001
Clarithromycin, no./no. tests (% resistant)	290/2713 (10.7%)	26/168 (15.5%)	1.52 (0.9–2.3)	0.07
Levofloxacin, no./no. tests (% resistant)	438/2713 (16.1%)	49/168 (29.2%)	2.14 (1.5–3.0)	<0.0001
Metronidazole, no./no. tests (% resistant)	1025/2713 (37.8%)	92/168 (54.8%)	1.99 (1.4–2.7)	<0.0001
≥2 antibiotics, no. (% of *HP* + subcohort)	345/2713 (12.7%)	37 /168 (22.0)	1.93 (1.3–2.8)	<0.0001

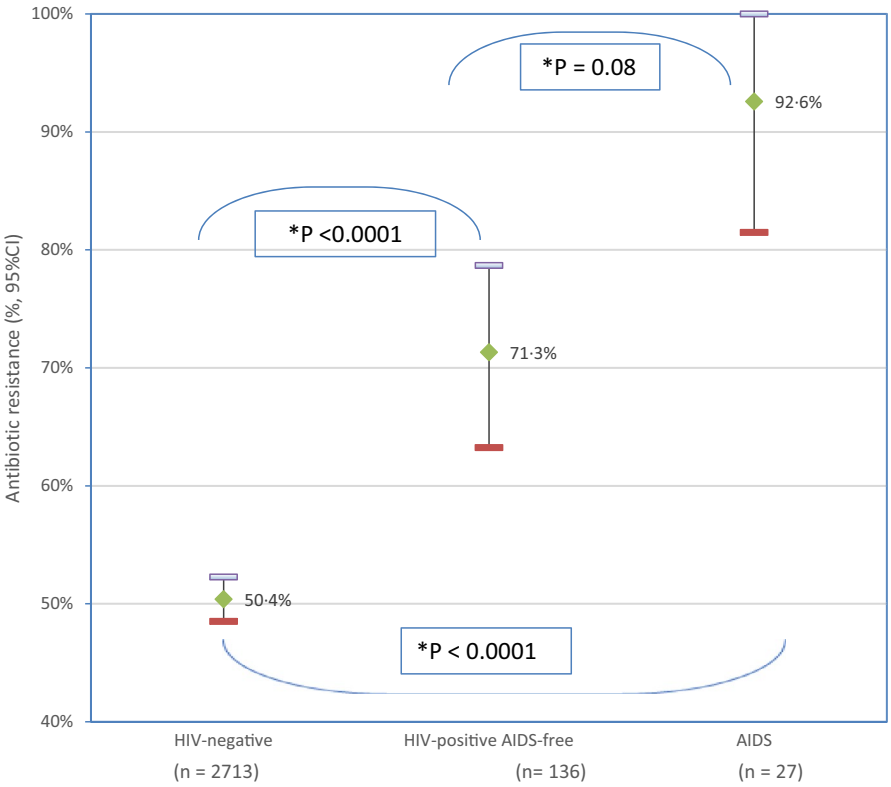

FIGURE 1 Prevalence and 95% confidence interval of primary *Helicobacter pylori* resistance to at least one antibiotic among HIV-positive individuals with and without AIDS, and HIV-uninfected patients. AIDS-f, AIDS-free. *p-value after Bonferroni correction: $p_1 = 0.08$, $p_2 < 0.0001$, $p_3 < 0.0001$. The figure represents comparison according to AIDS status of the prevalence of *H. pylori* primary antibiotic resistance to at least one antibiotic among HIV-negative and HIV-positive individuals. The bars represent the statistic for each subgroup studied; the center representing the mean percentage, the top and bottom are the upper and lower boundary of the 95% confident interval. The frequency of *H. pylori* primary antibiotic resistance is higher in HIV-positive individuals having AIDS than those who are AIDS-free (P1), significantly higher than HIV-negative individuals (P2). HIV-positive AIDS-free individuals carry significantly more *H. pylori* strains with primary antibiotic-resistant than HIV-negative individuals (P3)

that CDC stage C had an important independent effect (OR = 5.02), that persisted after adjustment for all other potential confounding variables in logistic regression (OR = 4.34). The effect of HIV duration in univariable analysis (OR = 0.53) (p = 0.09) remained at the same level of significance in multivariable analysis (adjusted OR = 0.56) (p = 0.10). The other characteristics had no significant effect in univariable analysis (p > 0.10 to be included in the logistic analysis).

TABLE 2 *Helicobacter pylori* primary resistance to two or more antibiotics in HIV-positive and -negative individuals

Antibiotic resistance	HIV-negative *n* (%)	HIV-positive OR 95% CI *n* (%)
CLR-MET	128/2714 (4.7)	14/169 (8.2) 1.82 (1.0–3.2)*
LEV-MET	241/2813 (8.5)	26/172 (15.1) 1.90 (1.2–2.9)**
CLR-LEV	81/2719 (2.9)	10/175 (5.7) 1.97 (1.0–3.8)***
CLR-LEV-MET	41/2713 (1.5)	4/168 (2.3) 1.58 (0.5–4.4)****
Two or more	345/2713 (12.7)	37/168 (22.0) 1.93 (1.3–2.8)*****

*p-value 0.04.; **p-value 0.005.; ***p-value 0.06.; ****p-value 0.3.; *****p-value 0.001.

Appendix 7 shows that, in univariable analysis, the OR of *H. pylori* resistance to at least one antibiotic in HIV-positive versus HIV-negative individuals was significantly associated with age, gender, ethnicity, country of birth, and HIV and these associations all remained significant after adjustment for confounding variables when logistic regression was performed. See Appendix 8 (-A, -B, and -C) for data variations according to ethnicity, gender, and age.

Figure 2 shows the percentage of individuals carrying *H. pylori* strains that have primary resistance to at least one antibiotic in HIV-negative and HIV-positive individuals across four consecutive 3-year periods from 2004 until 2015. Across each of the four periods, the percentages of *H. pylori* resistance to at least one antibiotic were statistically significantly greater in HIV-positive individuals than in HIV-negative patients; Fisher's exact test: $p < 0.05$. When all four periods were analyzed, the statistical significance by Pearson's chi-square test was $p < 0.0001$. In HIV-negative individuals, the percentages of variation of *H. pylori* primary resistance to at least one antibiotic during the four periods (21% and 24%) were not statistically significant (Pearson's chi-square, $p > 0.20$). By contrast, in HIV-positive individuals, these percentage variation differences were statistically significant across the four periods (Pearson's chi-square, $p = 0.019$).

In a complementary analysis by linear regression for HIV-positive individuals to define the variance due to the period, linear regression in HIV-positive individuals was based on a percentage of *H. pylori* resistance to antibiotics (Y-axis), and the year of observation (X-axis). If we considered 1/1/2004 as the intercept (zero time), the linear regression equation was as follows: $Y = -0.0022X + 0.3741$.

TABLE 3 Risk factors for primary *H. pylori* antibiotic resistance in 168 HIV-positive individuals

Parameters	Category (number)	Number (%)	Unadj. OR, 95% CI	*p*-value	Adj OR	95% CI	*p*-value
Age (years) (n = 168)	<50 versus >50 (n = 135) (n = 33)	100 (74.0) versus 26 (78.7)		0.6			
Gender (n = 168)	F versus M (n = 83) (n = 85)	66 (79.5) versus 60 (70.5)		0.2			
Region of origin (n = 163)	not-SS versus SS (n = 66) (n = 97)	50 (75.7) versus 72 (74.2)		0.8			
Region of birth (n = 168)	not-SS versus SS (n = 71) (n = 97)	53 (74.6) versus 73 (75.2)		1.0			
HIV duration (years) (n = 153)	<10 versus >10 (n = 102) (n = 51)	79 (77.4) versus 33 (64.7)	0.53 (0.2–1.1)	0.09	1.79	0.8–3.7	0.1
ARV treat. duration (years) (n = 130)	<10 versus >10 (n = 102) (n = 42)	78 (76.4) versus 20 (71.4)		0.6			
On ARV (n = 163)	yes versus no (n = 121) (n = 42)	91 (75.2) versus 31 (73.8)		0.8			
On TMT-SFX (n = 168)	No versus yes (n = 123) (n = 45)	90 (71.3) versus 36 (80.0)		0.4			
CDC stage C (n = 163)	No versus Yes (n = 136) (n = 27)	97 (71.3) versus 25 (92.5)	5.02 (1.1–22.2)	0.02	0.23	0.06–0.9	0.04
CD4 nadir at HIV diagnosis (n = 146)	≥200 versus <200 (n = 91) (n = 55)	67 (73.6) versus 42 (76.3)		0.8			

Abbreviations: 95% CI, 95% confidence interval; Adj OR, adjusted odds ratio; ARV antiretroviral; CDC, centers for disease control; F, female; M, male; not-SS, not-sub-saharan; SS, sub-Saharan; TMT-SFX trimethoprim-sulfamethoxazole; Unadj. OD, Unadjusted odds ratio.

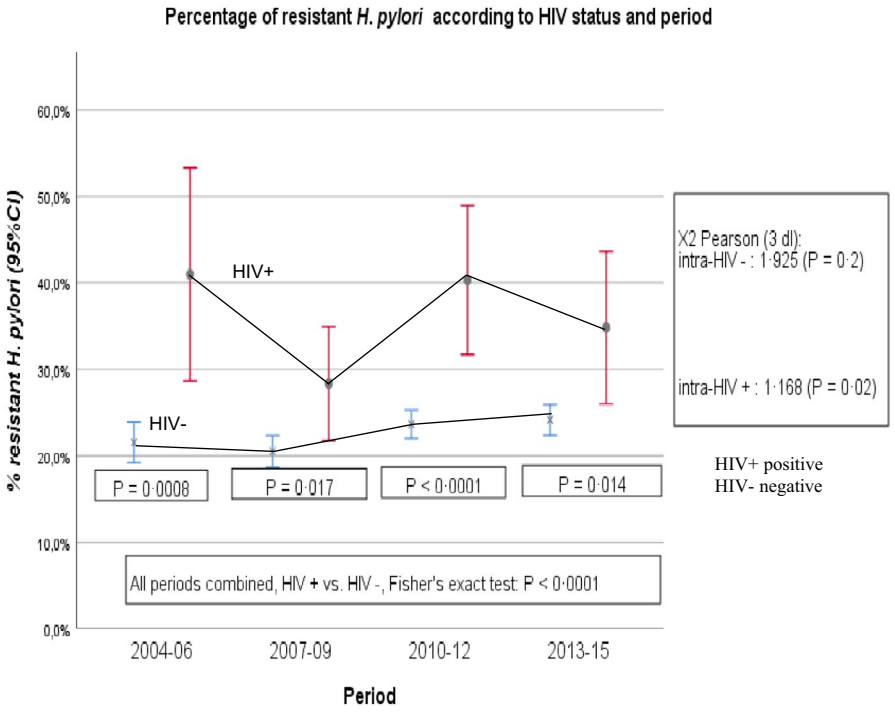

FIGURE 2 Evolution over time of the cohort prevalence and 95% confidence interval of primary *Helicobacterpylori* antibiotic resistance according to HIV status from 2004 to 2015

The R^2 coefficient of Pearson's determinations (0.0204) and the slope coefficient (−0.0022±...) were not significant ($p > 0.10$). The slight decrease in the percentage of *H. pylori* resistance (−2.2% over 10 years) was not significant. The variation of the percentage of *H. pylori* resistance to an antibiotic in HIV-positive patients is due to the absence of homogeneity of percentages (residual random variation), and not to a period effect. Supplemental data are presented in Appendix 9.

Figure 3 shows the relationship between *H. pylori* resistance in our cohort of HIV-negative and HIV-positive individuals as a dependent variable and defined daily dose (DDD) per 1000 inhabitants of antibiotics in the Belgian population and the Belgian HIV population as independent variables for both fluoroquinolone and macrolide antibiotic classes. For fluoroquinolones (Figure 3-a), mean comparison by Student's t-test was 9.122 ($p < 0.0001$) for DDD and 3.974 ($p = 0.0003$) for percent *H. pylori* resistance. For macrolides (Figure 3-b), *t*-test was 9.694 ($p < 0.0001$) for DDD and 3.401 ($p = 0.001$) for percent *H. pylori* resistance. No overlap of values was observed for DDD between the Belgian population and the Belgian HIV population. Slope coefficients of the linear regression between percent *H. pylori* resistance and DDD (Figure 3-a,b) were significant for both antibiotics: fluoroquinolones: $p = 0.002$; macrolides: $p < 0.0001$. Details are presented in Appendix 10 (10-A and 10-B).

Appendix 3 shows the ecologic association of aggregate variables of national antibiotic consumption from 2004 to 2015 in HIV-positive patients and HIV-negative individuals. The evolution of yearly antibiotic consumption (fluoroquinolone, macrolide) for the Belgian HIV-negative and -positive population is illustrated. For both classes of antibiotics, the DDD in the Belgian population was much higher in the HIV-positive population than in the HIV-negative Belgian population. Details are presented in Appendix 10 (10-A and 10-B).

4 | DISCUSSION

This study provides insight into the prevalence of, and risk factors for, primary antibiotic resistance among *H. pylori* strains infecting HIV patients as well as correlations with aggregate antibiotic consumption, and the evolution of these over time. Primary antibiotic resistance was almost twofold more common among *H. pylori* strains isolated from HIV-positive individuals over 12 years of observation, indicating that *H. pylori* strains isolated from HIV-positive individuals can be classified as "usually antimicrobial-resistant," according to European Medicines Agency (EMA) recommendations and in agreement with our preliminary data (Malfertheiner et al., 2017; Nkuize et al., 2015). They were inconsistently susceptible to LEV, "usually resistant" to MET, and inconsistently susceptible to CLA. One major reason for this increased prevalence in primary *H. pylori* antibiotic resistance in HIV-positive individuals is probably increased antibiotic exposure during antibiotherapy (Bell et al., 2014; Krucke et al., 2009; Megraud et al., 2013; Ngalani et al., 2019; Sarfo et al., 2015). Hence, we demonstrate (Appendix 10 and Appendix 3) that aggregate macrolide and quinolone consumption was two times more common among HIV-positive individuals than their HIV-negative counterparts from the national database of outpatient antibiotic use in Belgium during the period from 2004 to 2015. Furthermore, we found, for the whole cohort (Figure 3, -a and -b), a strong correlation between the prevalence of *H. pylori* primary resistance to clarithromycin and levofloxacin and the consumption of macrolides and quinolones, respectively, within the country. These data extend the known association between *H. pylori* primary resistance and antibiotic consumption in European countries to HIV-positive individuals (Malfertheiner et al., 2017; Megraud et al., 2013). In previous studies, as well as in the Maastricht V/Florence consensus conference,

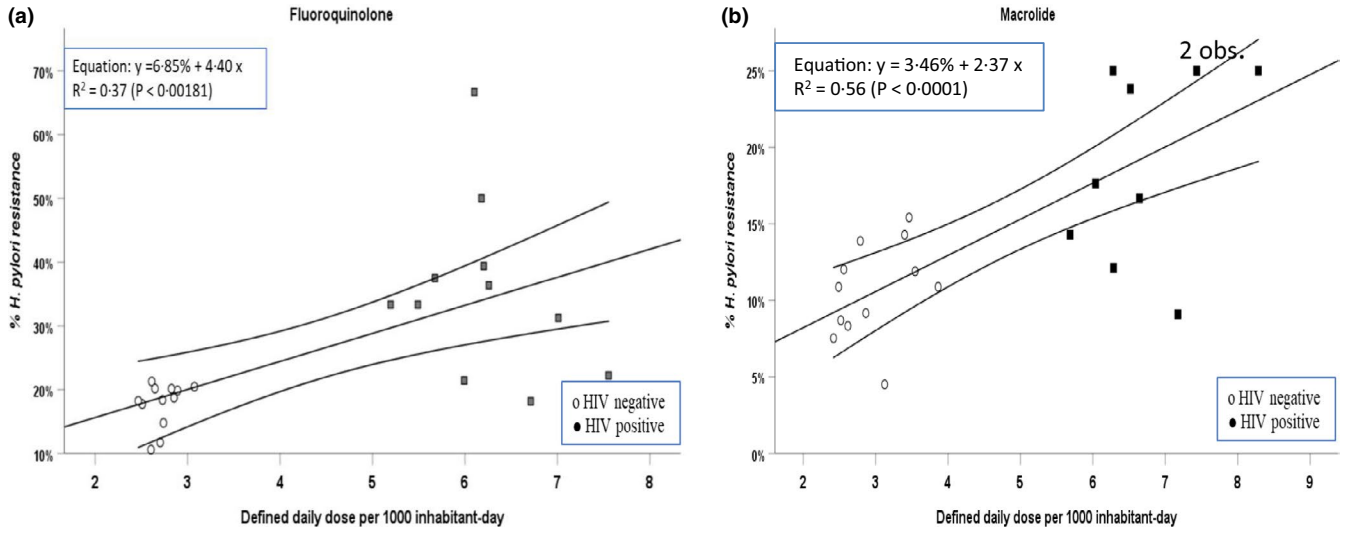

FIGURE 3 Yearly aggregated data for percent *Helicobacterpylori* resistance to antibiotics in local hospital HIV-negative (white circles) and HIV-positive individuals (gray and black squares) in relation to yearly aggregated data for defined daily dose (DDD) in the Belgian HIV-negative and HIV-positive population for fluoroquinolones (a) and macrolides (b). Each point represents yearly aggregated DDD per 1000 inhabitants from 2004 to 2015. Missing data for percent *H. pylori* resistance: fluoroquinolones [local hospital HIV-positive individuals: 2012]; macrolides [local hospital HIV-positive individuals: 2005 and 2007]. The linear regression model with 95% confidence intervals is illustrated

there was no analysis of HIV-positive individuals (Malfertheiner et al., 2017; Megraud et al., 2013).

In line with the above findings, we suggest that, in HIV-positive individuals, CLR-, LEV-, and MET-containing empirical first-line and rescue treatments should be avoided because their resistance rate is over the threshold proposed by the Maastricht V/Florence Consensus Report. It has also been shown that primary LEV, CLA, and MET resistance undermines the efficacy of LEV-, CLA-, and MET-containing triple therapy against *H. pylori* by 52%, 30%, and 70%, respectively. In this situation, with *H. pylori* primary resistance to multiple antibiotics, bismuth-containing therapy has been proposed as the best empiric alternative treatment for the general population, although it is not widely available. Each of the above suggestions on LEV, MET, and CLR is well supported by studies in HIV-negative patients. The magnitude of the impact of this resistance among HIV-positive individuals is expected to be similar to that found among HIV-negative patients but this hypothesis still needs to be confirmed (Bogaerts et al., 2006; Chey et al., 2017; Malfertheiner et al., 2017; Filipec Kanizaj et al., 2009; Glupczynski et al., 2001; Kouitcheu Mabeku et al., 2019; Malfertheiner et al., 2017; Raymond et al., 2010; Sugano et al., 2015; Thung et al., 2016; Wong et al., 2003). Our team is evaluating bismuth-containing therapy in a subgroup of *H. pylori* -HIV co-infected patients (In press).

Primary AMX and TET resistance were uncommon, <1% for each, in HIV-positive as well as in HIV-negative individuals, as shown in other European studies (Thung et al., 2016). In Africa and Latin America, both AMX and TET resistance seem to be more frequent in the general population. Data for *H. pylori* antimicrobial resistance among HIV-positive individuals from other regions to compare with our findings are lacking (Kouitcheu Mabeku et al., 2019; Thung et al., 2016). Even so, HIV status can be added to the region or country of birth, ethnicity, age, and gender, which are known independent risk factors of *H. pylori* antibiotic resistance in the general population (Miendje Deyi et al., 2011; Thung et al., 2016).

Over the entire study period, there was a stable and persistent higher overall prevalence of primary *H. pylori* resistance among HIV-positive individuals compared to HIV-negative individuals (Figure 2; Appendix 9). The reason for this could be, in part, as outlined above, the persistent need for, or consumption of, antibiotics by HIV-positive individuals. For instance, we demonstrate that aggregate antibiotic consumption remained high throughout the study periods among HIV-positive individuals, but as a consequence of enormous therapeutic progress in HIV-infection management (Saag et al., 2018), it started to decrease (Appendix 3) in the more recent period compared with the earlier period.

The reason why HIV-positive AIDS-free patients are also at risk of primary resistance is unclear (Figure 1). One hypothesis is that HIV and *H. pylori* generate oxidative stress within gastric mucosa (Butcher et al., 2017; Conway et al., 2014; Mouery et al., 2006; O'Rourke et al., 2003; Wells & Gaynor, 2006) and that this oxidative stress might be involved in the emergence of *H. pylori* antibiotic resistance (e.g., via DNA mutations or DNA transformation) (Lin et al., 2009). This might also explain why *H. pylori* infection is less frequently found in AIDS patients (e.g., by inducing a lower bacterial load or by triggering more coccoid forms) (Connolly et al., 2007; Lin et al., 2009; Roe et al., 1999). There are limitations to our study. First, the size of the ethnic subgroups was not balanced. Second, molecularly based methods rather than subculture could have provided additional information, such as that for bacterial load and strain diversity (Malfertheiner et al., 2017; Thung et al., 2016). The emerging test for *H. pylori* is costly, often limited to CLR and LEV resistance, and is not currently used in daily practice

in many countries. Finally, around twenty percent of people living with HIV did not receive ARV in the early HAART era, and, therefore, had not been selected for nationwide antibiotic consumption analysis. Nevertheless, this weakness is overcome by the very large number of patients analyzed.

The strengths of the study include the long period of observation of an adult population from diverse ethnic origins, residing in the same geographic region, and the fact that our findings may have a direct clinical impact. Current recommendations state that AST should be done in case of second-line treatment failure (Malfertheiner et al., 2017; Sugano et al., 2015). By contrast, given the finding of a high prevalence of multiple primary resistances to antibiotics among *H. pylori* strains isolated in HIV-positive individuals in our setting, we would recommend performing an *H. pylori* diagnosis with an antimicrobial susceptibility test by culture or polymerase chain reaction from gastric biopsy, or, if available, by a fecal bacterial DNA investigation (THD fecal test) to guide first-line *H. pylori* treatment in HIV-positive individuals (Malfertheiner et al., 2017; Pero et al., 2019; Sugano et al., 2015). This may help to avoid the use of several *H. pylori* treatment courses and subsequent increases in resistance and dysbiosis of gut microbiota (Malfertheiner et al., 2017). Both HIV and *H. pylori* infections already impair the gut microbiota and unnecessary treatment increases life-threatening primary antibiotic (AMX, CLR, and LEV) resistance for bacteria other than *H. pylori* (Iannone et al., 2018; Malfertheiner et al., 2017; Pero et al., 2019; Saxena et al., 2012). Furthermore, dysbiosis is associated with the development of numerous diseases, including neoplasia (Ferreira et al., 2018). Taken together, enhancing awareness of the medical community to the issue of *H. pylori* antibiotic resistance among HIV-positive individuals is crucial and a subject of public health interest. This study also highlights that there is a need for guidelines and consensus focused on *H. pylori* care in HIV-positive individuals.

In conclusion, our study demonstrates that HIV infection is a risk factor for carrying *H. pylori* strains with multiple antimicrobial resistances that persist over time, and correlate, in part, with antibiotic consumption in HIV-positive individuals while other mechanisms might also be involved. *Helicobacter pylori* diagnostics using tools that also provide AST from gastric samples should be implemented to guide first-line *H. pylori* infection treatment by avoiding empirical therapies in HIV-positive individuals. Locally establishing the prevalence of antimicrobial resistance of *H. pylori* strains in HIV-positive individuals may provide useful information. The distinction between individuals with and without HIV infection is mandatory in future epidemiological studies and consensus on *H. pylori* antibiotic resistance in a given population.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Marcel Nkuize: Conceptualization (lead); Data curation (lead); Formal analysis (equal); Funding acquisition (equal); Investigation (lead); Methodology (lead); Project administration (lead); Resources (lead); Software (equal); Supervision (lead); Validation (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead). **Jean Vanderpas:** Data curation (equal); Formal analysis (lead); Methodology (supporting); Software (equal); Supervision (supporting); Validation (equal); Visualization (equal); Writing-original draft (supporting); Writing-review & editing (equal). **Michel Buset:** Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Funding acquisition (supporting); Methodology (supporting); Project administration (supporting); Supervision (supporting); Visualization (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **Maria Gomez-Galdon:** Data curation (equal); Investigation (supporting); Project administration (supporting); Software (supporting); Validation (supporting); Visualization (supporting); Writing-original draft (supporting); Writing-review & editing (equal). **Marc Delforge:** Data curation (equal); Formal analysis (equal); Methodology (supporting); Resources (equal); Software (equal); Validation (equal); Visualization (equal); Writing-review & editing (supporting). **Véronique Yvette Miendje-Deyi:** Data curation (supporting); Investigation (equal); Project administration (supporting); Resources (equal); Supervision (supporting); Validation (supporting); Visualization (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **Vinciane Muls:** Data curation (supporting); Formal analysis (supporting); Resources (supporting); Supervision (supporting); Validation (supporting); Visualization (supporting); Writing-review & editing (supporting). **Stephane De Wit:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (supporting); Methodology (equal); Project administration (supporting); Resources (supporting); Software (supporting); Supervision (equal); Validation (supporting); Visualization (equal); Writing-original draft (equal); Writing-review & editing (equal).

ETHICS STATEMENT

The study was conducted following the Declaration of Helsinki and was approved by our local hospital ethics committee. All procedures described in the study were performed for routine medical purposes. Oral or written consent was obtained, following the evolution of the law throughout the study period.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article. Antibiotic consumption data analyzed during

this study are the property of the Agence InterMutualiste (www.aim-ima.be). The study is registered at ISRCTN registry, number 13466428: https://www.isrctn.com/ISRCTN13466428

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APPENDIX 1

Ethnicity

Eastern European countries included Austria, Belgium, Cyprus, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, and Northern Ireland.

Western European countries included Albania, Armenia, Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Georgia, Hungary, Kosovo, Latvia, Lithuania, Macedonia, Moldova, Montenegro, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, and Ukraine.

Sub-Saharan African countries included Angola, Benin, Burkina Faso, Burundi, Cameroon, The Central African Republic, Chad, Republic of the Congo, The Democratic Republic of the Congo, Cote d'Ivoire, Djibouti, Equatorial Guinea, Ethiopia, Gabon, The Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Liberia, Madagascar, Mali, Mauritania, Mauritius, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, Sudan, Tanzania, Togo, Uganda, and Zimbabwe.

North African countries included Algeria, Libya, Morocco, Tunisia, Egypt, Mauritania, and Somalia.

Other countries included Asian countries (Bangladesh, Cambodia, China, India, Indonesia, Malaysia, Mongolia, Nepal, Philippines, Sri Lanka, Thailand, and Vietnam), Middle Eastern countries included (Afghanistan, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Pakistan, Qatar, Saudi Arabia, Syria, and Turkey), and Latin and North American countries (Argentina, Brazil, Chile, Colombia, Cuba, Dominican Republic, Ecuador, Mexico, Paraguay, Peru, Uruguay, and Venezuela, and Canada, the United States of America, and Haïti).

APPENDIX 2

ATC codes

The ATC classifications used to select individuals with ambulatory prescriptions of antiretrovirals ["J05AE," "J05AF," "J05AG," "J05AR," and "J05AX," and excluding CNK: 3054137, 1411354, 1411362, 767657, 776070, 3054137, and "J05AE11," "J05AE12," "J05AE14," "J05AF10," "J05AF08," "J05AX02," "J05AX05," "J05AX15," "J05AX30," as well as 5AF11 and 5AF12, which are not commercialized in Belgium].

The ATC classifications were used to select individuals with ambulatory prescriptions of antibiotics [including J01FA (macrolides), J01FA09 (CLA), and J01 M (quinolones), and J01MA02 (ciprofloxacin)].

APPENDIX 3

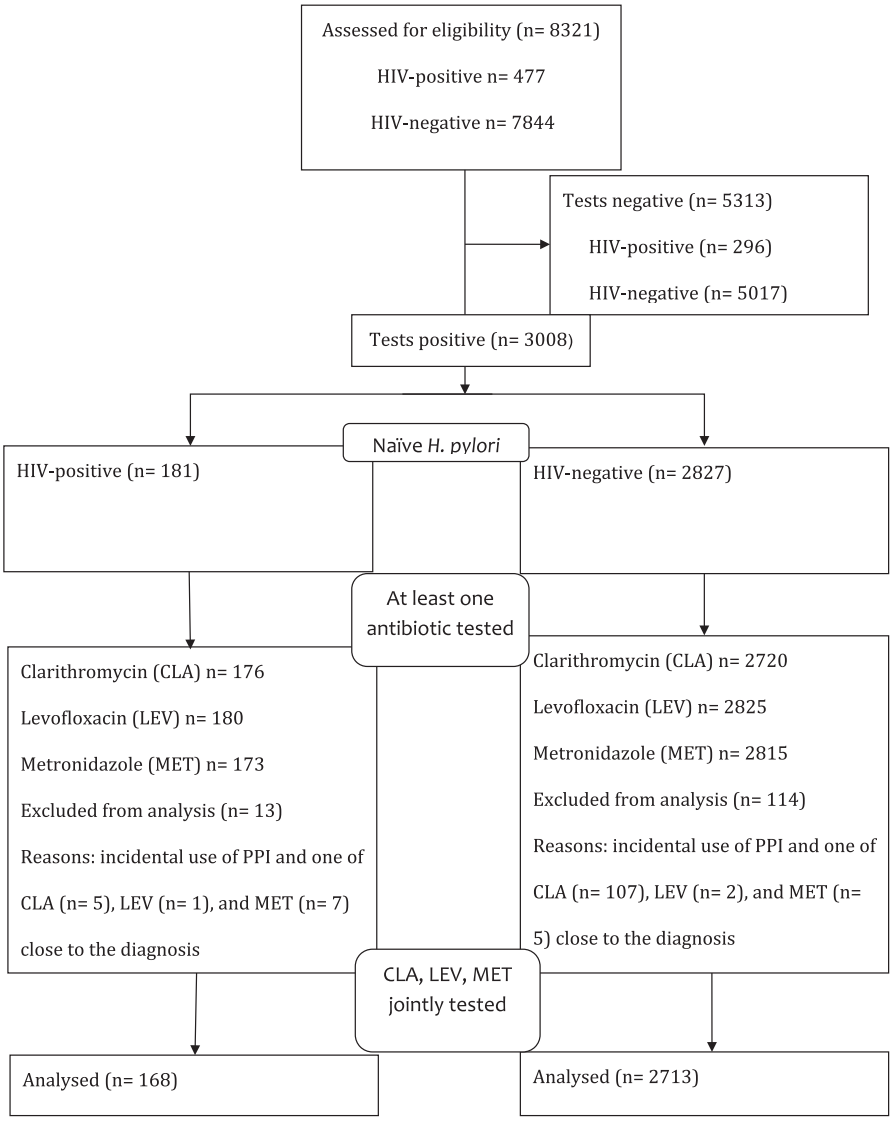


FIGURE A1 Yearly aggregated data for antibiotics consumption in HIV-negative and HIV-positive individuals. Antibiotic consumption as defined daily dose (DDD) in the Belgian HIV-negative and HIV-positive population for fluoroquinolones and macrolides. Each point represents yearly aggregated antibiotic consumption per 1000 inhabitants from 2004 to 2016

APPENDIX 4

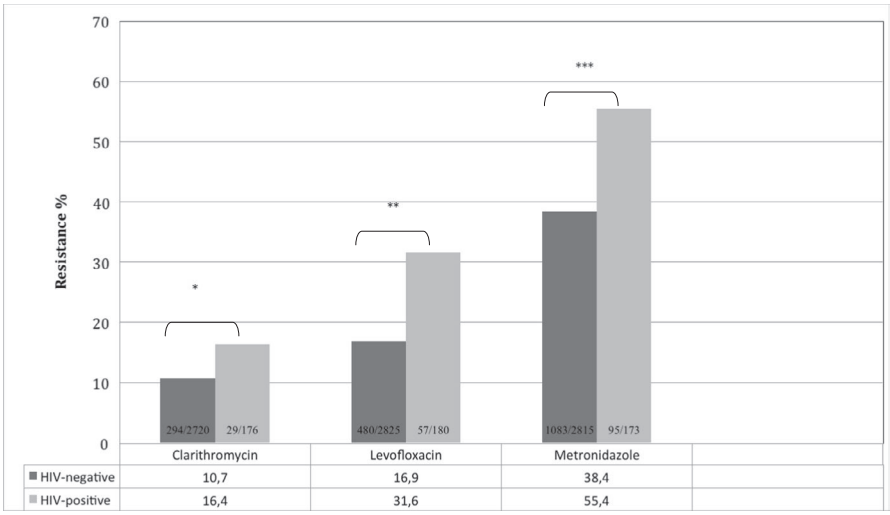


FIGURE A2 Cohort prevalence of *H. pylori* primary resistance to clarithromycin, levofloxacin, and metronidazole according to HIV status from 2004 to 2015. *p = 0.02, odds ratio and 95% confidence interval 1.62 (1.0–2.4). **p < 0.0001, odds ratio and 95% confidence interval 2.26 (1.6–3.1). ***p < 0.0001 odds ratio and 95% confidence interval 1.94 (1.4–2.6).

APPENDIX 5

TABLE A1 Cohort demographics according to HIV status

Parameters	Overall (*n* = 3008)	HIV-negative (*n* = 2827)	HIV-positive (*n* = 181)	95% CI	*p*-value
Age median	38	38	41.3	—	0.002
(Q1–Q3)	28.0–48.0	28.0–48.0	33.0–49.0		
Gender					
Female	1672 (55.5)	1581 (55.9)	91 (50.2)		0.1
Male	1336 (44.4)	1246 (44.0)	90 (49.7)		
Region of origin					
Western Europe	800 (26.6)	757 (26.8)	43 (23.7)		<0.0001
Eastern Europe	244 (8.1)	241 (8.5)	3 (1.6)		
North Africa	929 (30.9)	914 (32.4)	15 (8.2)		
Sub-Saharan Africa	665 (22.1)	559 (19.8)	106 (58.5)		
Other	363 (12.0)	349 (12.3)	14 (7.7)		
Unknown	7 (0.2)	7 (0.2)	0 (0.0)		
Region of birth					
Not-SS	2343 (77.9)	2268 (80.2)	75 (41.4)	5.73 (4.2–7.8)	<0.0001
SS	665 (22.1)	559 (19.7)	106 (58.5)		
Endoscopic findings	*n* = 795	*n* = 627	*n* = 168		
Oesophageal candidiasis	25	4 (0.6)	21 (12.5)	22.3 (7.5–66)	<0.0001
Gastric ulcer	128	111 (17.7)	17 (10.1)	0.52 (0.3–0.9)	0.01
Duodenal ulcer	139	121 (19.3)	18 (10.7)	0.50 (0.2–0.8)	0.008

APPENDIX 6

TABLE A2 Characteristics of HIV-positive individuals

Parameters
Age at HIV diagnosis (years) 32.0 (27.2–38.5) (median) (Q_1–Q_3)
HIV duration (years) 6.5 (2.0–11.8) (median) (Q_1–Q_3)
Ethnicity
Not Sub-Saharan 66 (40.4)
Sub-Saharan 97 (59.6)
Risk factor *n* (%)
Men sex men 41 (24.4)
Heterosexual 103 (61.3)
PWID 7 (4.1)
Other 17 (10.2)
$CD4^+$ T count (cells/μl)
Median (Q_1–Q_3) 482.0 (322.0–648.0)
>350 110 (65.4)
<350 42 (25.0)
Unknown 16 (9.5)
Nadir (Q_1–Q_3) 229.0 (134.0–357.0)
Viral load (Q_1–Q_3) (copies/ml) 50 (20.–1110.0)
CDC stage *n* (%)
Stage C 27 (16.5)
Not stage C 136 (83.4)
Unknown 5

Abbreviations: PWID, people who inject drugs; Q_1–Q_3, first–third interquartile.

APPENDIX 7

TABLE A3 Cohort (n = 2881) baseline risk factors for carrying primary *Helicobacter pylori* strains resistant to two or more antibiotics

Parameters	Category, number	Number (%)	Unadj. OR, 95% CI	*p*-value	Adj. OR	95% CI	*p*-value
Age	<50 versus >50 *n* = 2267 *n* = 614	262 (11.5) versus 120 (19.5)	1.85 (1.4–2.3)	<0.0001	2.06	1.6–2.6	<0.0001
Gender	Female versus Male *n* = 1595 *n* = 1286	251 (15.7) versus 131 (10.1)	0.60 (0.4–0.7)	<0.0001	0.6	0.4–0.7	<0.0001
Region of origin							
	Western Europe (*n* = 764)	– 85 (11.1)		<0.0001	0.52	0.4–0.6	<0.0001
	Eastern Europe (*n* = 238)	35 (14.7)					
	North Africa (*n* = 889)	77 (8.6)					
	Sub-Saharan Africa (*n* = 637)	130 (20.4)					
	Other (*n* = 346)	54 (15.6)					
Region of birth	not-SS versus SS *n* = 2244 *n* = 637	252 (11.2) versus 130 (20.4)	2.20 (1.6–2.5)	<0.0001	1.97	1.0–2.3	<0.0001
HIV status	Neg versus Pos *n* = 2713 *n* = 168	345 (12.7) versus 37 (22.0)	1.93 (1.3–2.8)	0.001	1.57	1.0–2.3	0.02

Abbreviations: 95% CI, 95% confidence interval; Adj OR, adjusted odds ratio; Adj, adjusted; CI, confidence interval; Neg negative; not-SS, not-sub-Saharan; OR, odds ratio; Pos positive; SS, sub-Saharan; Unadj. OD, Unadjusted odd ratio.

APPENDIX 8

TABLE A4 Prevalence of *Helicobacter pylori* primary clarithromycin (8A), levofloxacin (8B), or metronidazole (8C) resistance according to HIV status, age, gender, and ethnicity

	HIV-negative	HIV-positive	OR, 95% CI	
Parameters	***N* (%)**	***N* (%)**	***N* (%)**	***p*-value**
8A. Clarithromycin resistance[a]				
Age				**0.04**
<50 years	274/2170 (9.8)	22/140 (15.7)	1.70 (1.0–2.7)	0.4
≥50 years	79/549 (14.3)	7/36 (19.4)	—	
Gender				
Female	191/1517 (12.5)	12/87 (13.7)	—	0.7
Male	102/1202 (8.4)	17/89 (19.1)	2.54 (1.4–4.4)	**0.003**
Ethnicity				
East European	22/236 (9.3)	1/3 (33.3)	—	0.2
West European	97/725 (13.3)	12/42 (28.5)	2.59 (1.2–5.2)	**0.01**
Maghrebian	92/876 (10.5)	1/15 (6.6)	—	1
Sub-Saharan	49/543 (9.4)	11/102 (10.7)	—	0.5
Other	32/332 (9.6)	4/14 (28.5)	3.75 (1.1–12.6)	**0.04**
8B. Levofloxacin resistance[a]				
Age				
<50 years	320/2242 (14.2)	41/142 (28.8)	2.43 (1.6–3.5)	**<0.0001**
≥50 years	160/583 (27.4)	16/38 (42.1)	1.92 (0.9–3.7)	**0.06**
Gender				
Female	305/1580 (19.3)	25/91 (27.4)	1.58 (0.9–2.5)	**0.07**
Male	175/1245 (14.0)	32/89 (35.9)	3.43 (2.1–5.4)	**<0.0001**
Ethnicity				
East European	34/241 (14.1)	1/3 (33.3)	—	0.3
West European	132/757 (17.4)	13/43 (30.2)	2.05 (1.0–4.0)	**0.04**
Maghrebian	124/913 (13.5)	4/14 (28.5)	—	0.1
Sub-Saharan	125/559 (22.3)	36/106 (33.9)	1.70 (1.0–2.6)	**0.01**
Other	65/348 (18.6)	3/14 (21.4)	—	0.7
8C. Metronidazole resistance[a]				
Age				
<50 years	855/2233 (38.2)	78/136 (57.6)	2.16 (1.5–3.0)	**<0.0001**
≥50 years	226/582 (38.8)	18/37 (48.6)	—	0.2
Gender				
Female	635/1575 (40.3)	56/86 (65.1)	2.76 (1.7–4.3)	**<0.0001**
Male	446/1240 (35.9)	40/87 (45.9)	1.51 (0.9–2.3)	0.06
Ethnicity				
East European	108/241 (44.8)	1/2 (50.0)	—	1
West European	227/754 (30.1)	17/42 (40.4)	—	0.1
Maghrebian	232/911 (25.4)	8/15 (53.3)	3.3 (1.1–9.3)	**0.01**
Sub-Saharan	318/555 (57.3)	62/100 (62.0)	—	0.4
Other	194/347 (55.9)	8/14 (57.1)	—	1

Abbreviations: 95% CI, 95% confidence interval; *N*, number; OR, odds ratio.

[a]For the parameters age, gender, and ethnicity, given that there is no homogeneity of odds ratios between these parameters, the adjusted Mantel-Haenszel odds ratio cannot be used.

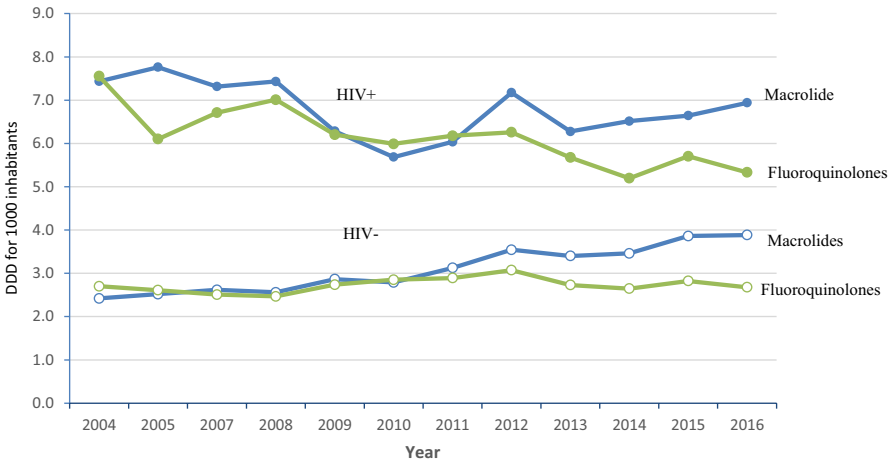
APPENDIX 9

TABLE A5 Data for the study of the evolution over time of the *Helicobacter pylori* primary antibiotic resistance according to HIV status

Parameter	2004–2006	2007–2009	2010–2012	2013–2015	*p*-value
HIV-negative					
(*n*)	253/1175	378/1845	623/2637	528/2187	0.1
Mean, 95% CI	21.5 (19.2–23.9)	20.5 (18.6–22.3)	23.6 (22.0–25.3)	24.1 (22.3–25.9)	
HIV-positive					
(*n*)	25/61	51/180	50/124	39/112	0.01
Mean, 95% CI	41.0 (28.6–53.3)	28.3 (21.7–34.9)	40.3 (31.7–49.0)	34.8 (26.0–43.6)	
p-value	0.0008	0.01	0.00003	0.01	

APPENDIX 10

Figure A11 Fluoroquinolones and macrolides consumption, according to HIV status, in Belgium from 2004 until 2016



APPENDIX 11

TABLE A6 Data for fluoroquinolone and macrolide consumption in the Belgian population from 2004 to 2016

Year	DDD 1000 inhabitants-year in the Belgian general population without HIV infection	DDD 1000 inhabitants-year in the Belgian population living with HIV infection
10-A Fluoroquinolones		
2004	2.70	7.55
2005	2.61	6.10
2006	2.60	5.49
2007	2.50	6.71
2008	2.46	7.00
2009	2.73	6.20
2010	2.85	5.99
2011	2.89	6.17
2012	3.07	6.25
2013	2.72	5.67
2014	2.64	5.19
2015	2.82	5.70
2016	2.67	5.33
10-B Macrolides		
2004	2.41	7.43
2005	2.51	7.76
2006	2.48	8.28
2007	2.61	7.31
2008	2.56	7.43
2009	2.86	6.28
2010	2.78	5.68
2011	3.12	6.03
2012	3.54	7.17
2013	3.39	6.27
2014	3.46	6.51
2015	3.86	6.64
2016	3.88	6.94

Abbreviation: DDD, defined daily dose per.