Original research

Helicobacter pylori resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community

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ABSTRACT

Objective Our aim was to prospectively assess the antibiotic resistance rates in *Helicobacter pylori* strains in Europe in 2018 and to study the link between antibiotic consumption in the community and *H. pylori* resistance levels in the different countries.

Design The proportion of primary antibiotic resistance cases of *H. pylori* and their corresponding risk factors were investigated in 24 centres from 18 European countries according to a standardised protocol. Data on antibiotic consumption in the community were collected for the period 2008–2017. The link between antibiotic consumption and resistance data was assessed using generalised linear mixed models. The model with the best fit was selected by means of the Akaike Information Criterion.

Results *H. pylori* resistance rates for the 1211 adult patients included were 21.4% for clarithromycin, 15.8% for levofloxacin and 38.9% for metronidazole and were significantly higher in Central/Western and Southern than in the Northern European countries.
The best model fit was obtained for the Poisson distribution using 2013 consumption data. A significant association was found between *H. pylori* clarithromycin resistance and consumption in the community of macrolides (p=0.0003) and intermediate-acting macrolides (p=0.005), and between levofloxacin resistance and consumption of quinolones (p=0.0002) and second-generation quinolones (p=0.0003).

Conclusion This study confirms the positive correlation between macrolide and quinolone consumption in the community and corresponding *H. pylori* resistance in European countries. Hence, *H. pylori* treatment with clarithromycin and levofloxacin should not be started without susceptibility testing in most European countries.

Significance of this study

What is already known on this subject?
- Increasing *Helicobacter pylori* resistance to key antibiotics like clarithromycin and levofloxacin is leading to treatment failures or the use of quadruple therapies in Europe.

What are the new findings?
- Compared with the previous decade, clarithromycin resistance shows a limited increase and levofloxacin resistance shows a stabilisation following the decrease in the consumption of these antibiotics for other infections in the community.

How might it impact on clinical practice in the foreseeable future?
- While triple therapies without prior susceptibility testing are not yet possible in many countries, the continuation of policies to decrease antibiotic consumption should allow such a comeback in the future.

INTRODUCTION

Treatment of *Helicobacter pylori* infection remains a challenge. The standard triple therapy (STT), which consists of the association of a proton pump inhibitor and two antibiotics, one of which most commonly includes clarithromycin as a key agent, has been used empirically worldwide for two decades without antimicrobial susceptibility testing (AST) as first-line therapy. Since 2010, a marked decrease in efficacy of this treatment regimen has been observed globally, which resulted in the recommendation to prescribe quadruple therapies with or without bismuth when clarithromycin resistance was over 15% (Maastricht V).[1] The quadruple therapies have been shown to achieve higher success rates than STT, but they may cause more frequent adverse events, lead to more antimicrobial resistance in other bacteria and have a greater impact on the gut microbiota, while the WHO recommends avoiding the use of unnecessary antibiotics. In this context, regularly performed point prevalence surveys to update the resistance rates (especially to clarithromycin and other alternative antibiotics) are of great value to optimise the therapeutic choices and to continue using STT whenever possible. *H. pylori* resistance to antibiotics has been monitored at the European level every 10 years, beginning in 1998[2] and then in 2008.[3] Our aim was to perform a survey in 2018 using the same methodology as in the past, including as many centres in the European *H. pylori* resistance network involved in the past

surveys as possible and also to establish a correlation between the resistance to selected key antibiotics known to have a negative impact on *H. pylori* eradication, that is, macrolides and quinolones, and antibiotic consumption of these classes of antibiotics in the community in the different countries, to assess the current trend.

MATERIAL AND METHODS

We conducted an observational (non-interventional) multicentre study using a common standard protocol based on the protocol of the previous surveys.[2 3] The protocol was presented, discussed and approved by the participants during a plenary meeting before the start of the study.

Centres were selected on a voluntary basis in different countries in Europe, but special attention was given to recruit a number of centres proportional to the country's population (ie, one centre for each small country (in the range of 10 million inhabitants) and several centres (two to four) for larger countries (more than 10 million inhabitants). The centres that already participated in previous studies were the first to be contacted. In France, a country-wide national survey (five regions in the metropolitan areas of the country) was organised in 2018 and involved the National Reference Centre for Helicobacters, thus no other centre was contacted.

From January 2018 to February 2019, each participating centre was requested to include prospectively a minimum of 50 consecutive, non-duplicate clinical isolates of *H. pylori* obtained from gastric biopsy specimens from adult patients who attended an outpatient endoscopy clinic and had not received previous eradication treatment (that is, so-called naive patients), in order to evaluate the prevalence of primary resistance. For each case included, the participating centres had to collect and provide the following information: demographic data (age, gender, place of birth, current country and city of residency), clinical data (type of symptoms, treatment during the previous 3 months and endoscopic results), but a previously validated questionnaire was not used. The questionnaires were completed online and registered in a secure database (login and password).

Ethics

No patient informed written consent was obtained as information was collected routinely in the context of patient care. Furthermore, all of the collected data wre transmitted anonymously to the central database and analysed anonymously by a third party in accordance with data protection and privacy as per the General Data Protection Regulation European Law.

Culture and susceptibility testing

Culture and susceptibility testing of *H. pylori* to clarithromycin, levofloxacin, amoxicillin, metronidazole, tetracycline and rifampicin (as a surrogate antibiotic marker for rifabutin) were performed locally at each centre according to a standardised protocol using a quantitative agar diffusion method by means of minimal inhibitory concentration (MIC) agar diffusion gradient strips (ETEST, bioMérieux, Marcy-l'Etoile, France) for clarithromycin, levofloxacin, amoxicillin and metronidazole and by disk diffusion for tetracycline (30 µg/disk) and rifampicin (5 µg/disk). Culture and susceptibility tests were performed on fastidious Mueller Hinton agar medium (MH-F) supplemented with 5% defibrinated horse blood and 20 mg of beta-NAD (bioMérieux). Agar plates were incubated for 48–72 hour at 35°C±2°C in a microaerobic atmosphere also registered in the online database. Susceptibility test results were interpreted according to the guidelines and criteria of the European Committee of Antibiotic Susceptibility Testing (EUCAST Clinical Breakpoint Tables V.9.0, valid from 1 January 2019).[4] All strains were kept frozen at −70°C in broth cryovials with 20% glycerol in the participating centres until the completion of the study.

Quality control was performed, first by providing an *H. pylori* strain (CCUG 18742, susceptible to all antibiotics tested) to be used locally, and second on completion of the study by requesting that the local centres send a 10% random sample of the strains as well as all of the strains reported as resistant to amoxicillin, tetracycline and rifampicin, to one of the two coordinating centres (Bordeaux, France, or Yvoir, Belgium). For amoxicillin, verification of the susceptibility test result was performed by ETEST for all strains with an MIC value of 0.125 mg/L or higher. A verification of the MIC value by ETEST was also carried out on strains with a reduced diameter, less than 20 mm for tetracycline and less than 19 mm for rifampicin based on our experience. Finally, the control for clarithromycin susceptibility or resistance was performed by ETEST MIC determination as well as by a real-time PCR assay (Amplidiag *H. pylori*+ClariR, Mobidiag, Paris, France), which detects the mutations on the 23S rRNA gene known to be associated with *H. pylori* macrolide resistance.

Data on outpatient macrolide and quinolone consumption

Data on consumption of macrolides (Anatomical Therapeutic Chemical (ATC) group J01FA) and quinolones (ATC J01M) in the community from The European Surveillance System (TESSy) were released by the European Centre for Disease Prevention and Control.[5] Countries providing data to TESSy included Austria, Belgium, Bulgaria, Croatia, Denmark, France, Germany, Greece, Ireland, Italy, Latvia, the Netherlands, Norway, Poland, Portugal, Slovenia and Spain. For the period 2008–2017, data on macrolides and quinolones, aggregated at the level of the active substance, were collected in accordance with the ATC Classification System and the defined daily dose (DDD) measurement unit (WHO ATC/DDD January 2019 version)[6] and expressed in DDD per 1000 inhabitants per day (DID).

Macrolides were classified according to mean plasma-elimination half-life, subdividing macrolides into short-acting macrolides (half-life <4 hours) including erythromycin and spiramycin, intermediate-acting macrolides (half-life from 4 to 24 hours) including clarithromycin and roxithromycin and long-acting macrolides (half-life >24 hour) including azithromycin.[7] Quinolones were classified according to chemical structure and antimicrobial activity, subdividing quinolones into first-generation (eg, norfloxacin), second-generation (ofloxacin, levofloxacin and ciprofloxacin) and third-generation quinolones (moxifloxacin).[8]

These consumption data do not concern antibiotic use in animal husbandry or fish culture.

Statistical analysis

Factors associated with resistance to clarithromycin, levofloxacin and metronidazole were determined through univariable logistic regression. Age and sex were forced into the multivariable model, and additional variables were selected through univariable analysis ($p \leq 0.25$) and included in the model using backward selection ($p \leq 0.05$). Separate multivariable models were adjusted for region of birth and residence. Data analysis was performed by means of SAS software (V.9.3, SAS Institute, Cary, North Carolina, USA).

A generalised linear model with a logarithmic link function was used to model the association between consumption

Table 1 Distribution of the inclusions according to country

Countries represented*	No. of results	%
France	186	15.3
Spain	146	12
Portugal	90	7.4
Germany	85	7.0
Poland	85	7.0
Italy	69	5.7
Belgium	65	5.3
Norway	56	4.6
Bulgaria	53	4.4
Austria	52	4.3
Croatia	50	4.1
Greece	50	4.1
Slovenia	50	4.1
Lithuania	45	3.7
Latvia	44	3.6
Ireland	43	3.5
The Netherlands	21	1.7
Denmark	21	1.7

*Countries not represented in the study include the following: Czech Republic, Finland, Hungary, Romania, Sweden and the UK.

of macrolides and quinolones (total and by subgroup) in the community (independent variables) and the number of test results resistant to clarithromycin and levofloxacin, respectively, in 17 European countries. The logarithm of the total number of test results was used as the offset variable, and the best fitting model was selected based on the Akaike Information Criterion (AIC).[9] Different models included: (1) a Poisson or negative binomial distribution for the number of resistant test results and (2) yearly consumption with a time-lag from 1 to 6 years (2012–2017) or cumulative consumption of 1–10 years prior to the end of sample collection (2008–2017).

RESULTS

Twenty-four centres in 18 European countries achieved the recruitment of 1211 *H. pylori* culture-positive patients fulfilling the inclusion criteria. The distribution per country is presented on table 1 and the number of countries and centres participating in this survey in figure 1.

The patients' mean age was 51.2 years (range 17–91 years), 54.6% were women and 91.2% were born in Europe. The distribution by age was in line with the usual distribution observed among upper digestive endoscopy patients. Data on symptoms were available for 822 (67.9%) patients for whom complete microbiological data were available. Epigastric pain (n=347) (42.2%) and other symptoms of dyspepsia (n=220) (26.8%) accounted by far for the most frequent reasons for consultation and indication of upper digestive endoscopy. Alarm symptoms such as anaemia (n=35) or haemorrhage (n=7) where very rarely mentioned and altogether accounted for less than 5% of the indications for performing an upper GI endoscopy. Concerning endoscopic findings (data available for 645 patients), redness or inflammation located in one or several parts of the stomach were present in more than half of the patients (n=361, 56.0%), and gastric erosions were documented in 87 (13.5%) patients. However, an ulcer (n=68) (10.5%) and especially lesions of gastric malignancy (n=8) (1.2 %) were observed much less frequently.

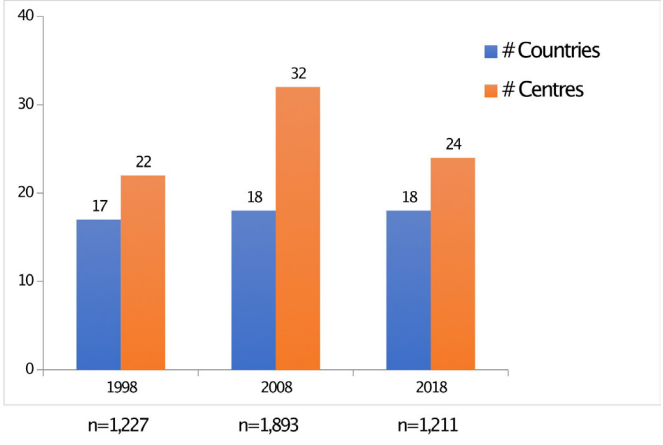

Figure 1 Participation of the centres and countries in the three surveys on *Helicobacter pylori* resistance in Europe.

Table 2 Proportion of *Helicobacter pylori* primary antimicrobial resistance in European countries in 2018

Antibiotic	No. of results	Percentage	Range
Clarithromycin	259	21.4	(4.8–36.9)
Levofloxacin	192	15.8	(0–29.2)
Metronidazole	471	38.9	(6.1–67.5)
Amoxicillin	2	0.2	(0–1.7)
Tetracycline	0	0.0	0
Rifampicin	11	0.9	(0–2.4)

The global rate of primary resistance of *H. pylori* in 2018 is presented in table 2. Overall, 521 (43.0%) *H. pylori* isolates displayed a fully susceptible phenotype (absence of in vitro resistance to any of the six antimicrobial agents tested). Resistance to one of the antibiotics classes was observed in *H. pylori* isolates from 452 (37.3%) patients. Dual resistance was present in 201 isolates (16.6%), and it most commonly involved the combination of clarithromycin and metronidazole resistance (n=96), levofloxacin and metronidazole resistance (n=63) or clarithromycin and levofloxacin resistance (n=35). Multiresistance defined as resistance occurring simultaneously in three or more classes of unrelated antimicrobial agents was observed in 26 isolates (2.1%), and among these, triple resistance to clarithromycin, levofloxacin and metronidazole occurred in 23 isolates. Of note, dual or triple drug resistance to clarithromycin and to metronidazole was observed in 119 isolates (9.7%). Resistance against the other three antibiotics tested was exceptional: 0.2% for amoxicillin, 0.9% for rifampicin and nil for tetracycline, which is in line with previous reports.[3]

The distribution of clarithromycin and levofloxacin resistance by country is presented in figures 2 and 3, respectively, and shows an important heterogeneity. For clarithromycin, the resistance rate ranged from 4.8% in Denmark to 36.9% in Italy and for levofloxacin resistance from nil in Denmark and the Netherlands to 29.2% in Italy.

A selection of 142 *H. pylori* isolates, including 115 randomly selected isolates as well as 27 *H. pylori* isolates reported as resistant to rifampicin (n=22) or to amoxicillin (n=5) by the local laboratories were retested for verification of susceptibility/resistance results at one or both central laboratories (including PCR multiplex assay for clarithromycin resistance (Mobidiag) at both central laboratories. The quality control performed at the end of the study proved excellent (>95% concordance of primary results) and allowed the correction of some errors mainly related to the recognition of double populations of clarithromycin-susceptible and clarithromycin-resistant genotypes (concomitant

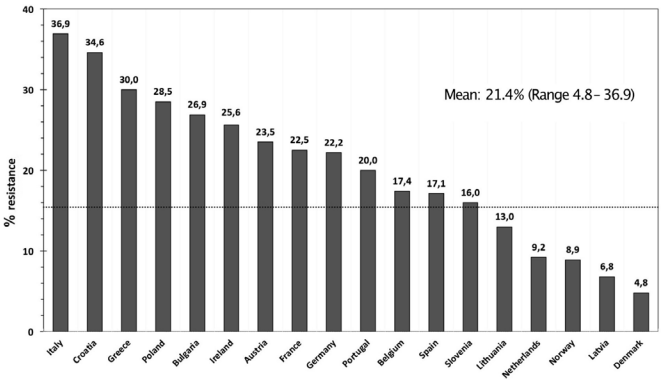

Figure 2 Distribution of primary clarithromycin resistance of *Helicobacter pylori* in the different European countries in 2018. The dotted line represents the 15% threshold of resistance recommended for susceptibility testing.[1]

presence of wild type and mutated 23S rRNA genotypes) that went undetected by phenotypical testing with the ETEST (only the clarithromycin-susceptible population was present by the ETEST), and also four corrections of transcription errors in the results were reported. Only two of the amoxicillin-resistant isolates could be confirmed, with MICs of 0.19 and 0.25 µg/mL, respectively, thus quite close to the EUCAST cut-off value of 0.125 µg/mL. For rifampicin, 11 of the isolates were confirmed with MIC ≥4 µg/mL or a zone size diameter <19 mm.

In the multivariable analyses, region of birth was significantly associated with clarithromycin, levofloxacin and metronidazole resistance. Compared with participants born in Northern Europe, those born in Southern Europe or Western/Central Europe were more likely to present clarithromycin and levofloxacin resistance, and being born outside Europe was associated with metronidazole resistance (table 3). Similar results were obtained for the region of residence (data not shown).

The link between macrolide and quinolone use and the proportions of clarithromycin-resistant and levofloxacin-resistant *H. pylori* isolates was obtained using the Poisson distribution (and log-link). Model fit improved for each additional year of antibiotic use accumulated, but the fit of a model with average consumption over the past 6 years as covariate always remained inferior to that of the model proposed to yearly consumption in 2013 (that is, consumption 5 years before) that was selected as the final model (AIC values for macrolides and quinolones:

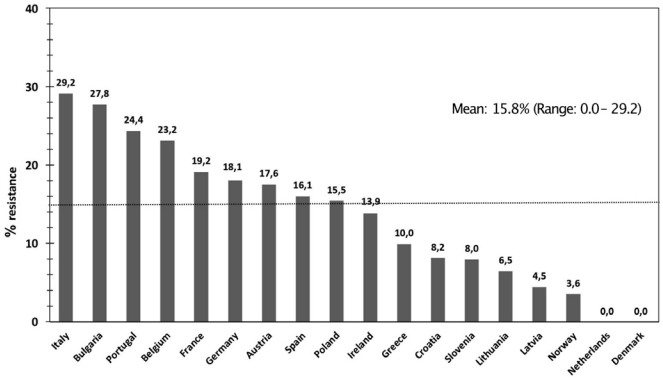

Figure 3 Distribution of primary levofloxacin resistance of *Helicobacter pylori* in the different European countries in 2018. The dotted line represents the 15% threshold of resistance recommended for susceptibility testing.[1]

Table 3 Univariable and multivariable analysis of factors associated with clarithromycin, levofloxacin and metronidazole resistance in Europe

	Clarithromycin (n=527)					Levofloxacin (n=522)					Metronidazole (n=527)				
	Univariable analysis			Multivariable analysis		Univariable analysis			Multivariable analysis		Univariable analysis			Multivariable analysis	
	OR	95% CI	P values	OR	95% CI	P values	OR	95% CI	P values	OR	95% CI	P values	OR	95% CI	P values
Gender			0.788			0.604			0.636			0.526			0.596
Men	1			1			1			1			1		
Women	0.94	0.61 to 1.45		0.89	0.57 to 1.38		1.12	0.71 to 1.75		0.87	0.56 to 1.35		1.09	0.74 to 1.59		1.11	0.76 to 1.63	
Age (years)			0.723			0.538			0.465			0.608			0.586			0.841
<50	1			1			1			1			1			1		
≥50	0.92	0.60 to 1.43		0.87	0.56 to 1.36		1.18	0.75 to 1.87		0.89	0.57 to 1.39		0.9	0.62:1.31		1.04	0.70 to 1.54	
Region of birth			0.0005						0.0007						0.012			
Northern Europe*	1			1			1			1			1			1		
Western/Central Europe†	3.22	1.14 to 9.13	0.028	3.15	1.10 to 8.97	0.032	10.46	2.37 to 46.23	0.002	2.85	0.99 to 8.18	0.051	1.78	0.88 to 3.60	0.111	1.78	0.87 to 3.64	0.112
Southern Europe‡	3.82	1.48 to 9.84	0.005	3.8	1.47 to 9.83	0.005	6.81	1.62 to 28.64	0.008	3.63	1.40 to 9.38	0.008	1.1	0.59 to 2.03	0.765	1.09	0.59 to 2.03	0.776
Outside Europe	0.83	0.19 to 3.67	0.807	0.78	0.18 to 3.49	0.746	8.47	1.76 to 40.81	0.007	0.77	0.17 to 3.43	0.73	2.75	1.23 to 6.14	0.014	2.8	1.23 to 6.38	0.014
Endoscopy finding			0.159						0.197						0.326			
Normal	1						1						1					
Ulcer/erosions	1.75	0.84 to 3.61	0.133				1.86	0.88 to 3.94	0.103				1.34	0.76 to 2.37	0.309			
Inflammation	1.85	0.95 to 3.61	0.07				1.75	0.87 to 3.51	0.114				0.97	0.58 to 1.64	0.921			

*Northern Europe: Ireland, Norway, Lithuania, Latvia, The Netherlands and Denmark.
†Western/Central Europe: Austria, Belgium, France, Germany, Bulgaria and Poland.
‡Southern Europe: Croatia, Greece, Italy, Portugal, Slovenia and Spain.

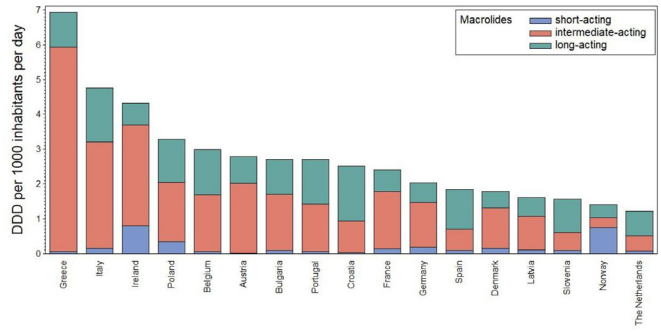

Figure 4 Consumption of the different groups of macrolides in the community in Europe in 2013 expressed in DDD per 1000 inhabitants per day. DDD, defined daily dose.

98.74 and 94.05, respectively, for DID in 2013 vs 100.33 and 98.50 for DID on average consumption over 6 years). Large variations in antibiotic consumption in the community in 2013 were observed between countries (figures 4 and 5): the use of macrolides, mostly intermediate acting macrolides, ranged from 1.22 DID in the Netherlands to 6.94 DID in Greece, and the use of quinolones, mostly second-generation quinolones, ranged from 0.54 DID in Norway and Denmark to 3.55 DID in Italy.

Clarithromycin resistance in *H. pylori* was significantly associated with 2013 community consumption of macrolides (p=0.0019, incidence rate ratio (IRR): 1.17, 95% CI 1.07 to 1.29), intermediate-acting macrolides (p=0.0050, IRR: 1.16, 95% CI 1.05 to 1.28) and long-acting macrolides (p=0.0199, IRR: 1.80, 95% CI 1.11 to 2.92). Levofloxacin resistance was significantly associated with 2013 community consumption of quinolones (p=0.0002, IRR: 1.57, 95% CI 1.29 to 1.92) and second-generation quinolones (p=0.0003, IRR: 1.63, 95% CI 1.31 to 2.03) (figures 6 and 7).

DISCUSSION

After the emergence of clarithromycin resistance in the 1990s and its continuous rise during the following decade, it was considered important to reassess the current status. Indeed, for clarithromycin, the most important antibiotic used in *H. pylori* treatment, there is still a large heterogeneity from country to country, but following the WHO recommendations for a prudent use of antibiotics, some countries took important measures to decrease the rate of antibiotic consumption including macrolides, which are now used less for respiratory infections. For example, the overall macrolide consumption decreased by 46% in France from 2000 to 2015 from 6 to 3.2 DID and was stable thereafter,

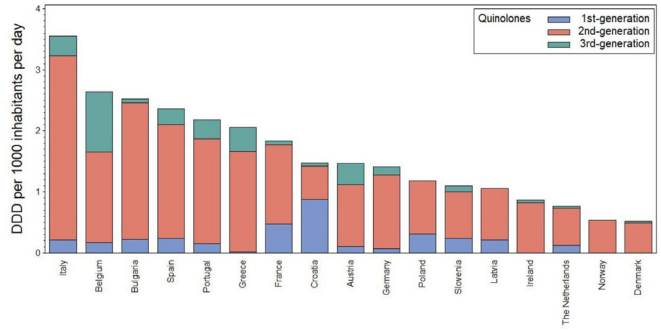

Figure 5 Consumption of the different generations of quinolones in the community in Europe in 2013 expressed in DDD per 1000 inhabitants per day. DDD, defined daily dose.

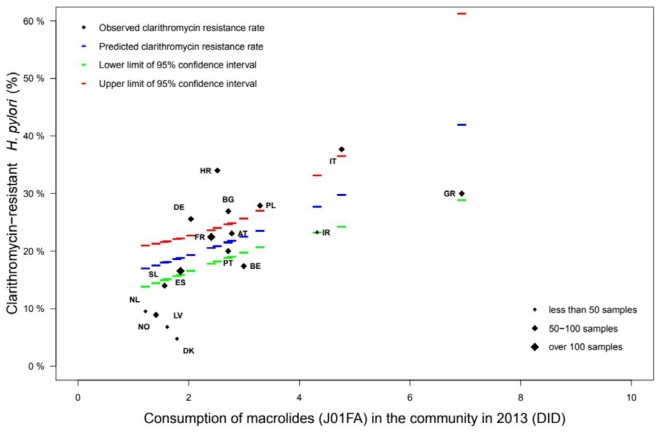

Figure 6 Correlation between the consumption of macrolides in the community in 2013 in different European countries and clarithromycin resistance in the corresponding countries (p: 0.005). AT, Austria; BE, Belgium; BG, Bulgaria; DID, defined daily dose per 1000 inhabitants per day; DE, Germany; DK, Denmark; ES, Spain; FR, France; Gr, Greece; *H. pylori*, *Helicobacter pylori*; HR, Croatia; IR, Ireland; IT, Italy; LV, Latvia; NL, The Netherlands; NO, Norway; PL, Poland; PT, Portugal; SL, Slovenia.

but the situation may not be the same in all of the different European countries. However, in comparison with the results of our previous studies in 1998 and 2008 (figure 8) for which 60% of the centres were the same as shown on figure 1, it is interesting to note that the global resistance increase between 2008 and 2018 (from 17.5% to 21.4%, p<0.05) was not as high as could have been expected based on the observations in the previous decade, that is, approximately 1% per year. With regard to the resistance mechanism, it should be noted that for clarithromycin, besides the 23S rDNA point mutations, another mechanism has been highlighted these last years, that is, the efflux pump genes.[10–12] This mechanism concerns mainly multidrug resistance and leads to a limited increase in MICs, but it could possibly have been involved in our survey in a few *H. pylori* isolates, where discordant results were observed between genotypic and phenotypic results for clarithromycin resistance.

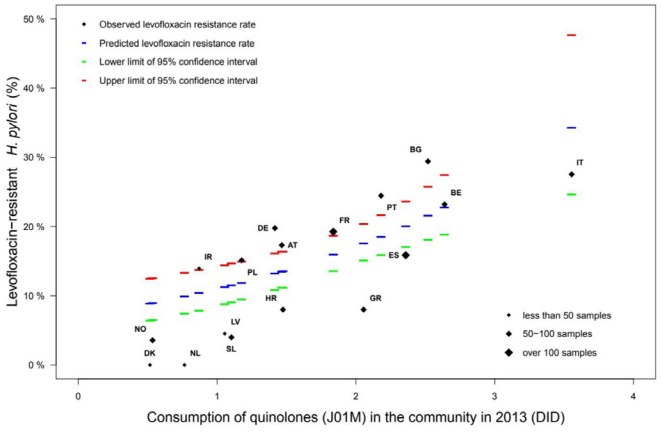

Figure 7 Correlation between the consumption of quinolones in the community in 2013 in different European countries and levofloxacin resistance in the corresponding countries. (p:0.0002). AT, Austria; BE, Belgium; BG, Bulgaria; DDD, defined daily dose per 1000 inhabitants per day; DE, Germany; DK, Denmark; ES, Spain; FR, France; Gr, Greece; *H. pylori*, *Helicobacter pylori*; HR, Croatia; IR, Ireland; IT, Italy; LV, Latvia; NL, The Netherlands; NO, Norway; PL, Poland; PT, Portugal; SL, Slovenia.

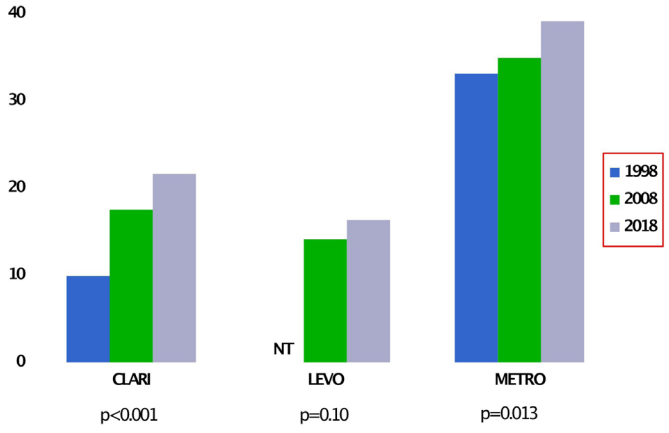

Figure 8 Evolution of *Helicobacter pylori* primary antimicrobial resistance in Europe (1998–2018). NT, not tested.

In contrast, *H. pylori* resistance to levofloxacin, which was not tested in 1998, did not increase significantly during the last 10 years. This can be explained by the fact that fluoroquinolones are used more for severe infections in some countries. Furthermore, due to potentially serious adverse events, recent recommendations tend to limit its prescription.[13 14] Metronidazole resistance is still increasing, but fortunately, its impact on *H. pylori* eradication is limited.[15] Interestingly, for tetracycline, an antibiotic component in the drug Pylera (Allergan, Dublin, Ireland), resistance remains extremely seldom, despite the continuous development of this drug in Europe these last years.[15] Amoxicillin resistance remains the exception. Most of the strains reported in this category could either not be confirmed or had an MIC very close to the threshold of 0.125 mg/L, defined by a EUCAST epidemiological cut-off 10 years ago. With regard to rifabutin and rifampicin testing, recent results show a higher resistance for rifampicin than for rifabutin.[16]

Despite being a strictly human pathogen, some studies have shown the presence of viable *H. pylori* in external environment and its persistence in biofilms suggesting that contamination could come from the environment especially, via natural or waste water systems.[17–19] Therefore, the possibility that *H. pylori* could acquire antibiotic resistance by horizontal transfer of genes during this phase cannot be totally ruled out. In addition, antibiotic residues present in food products or in aquaculture could eventually contribute to its resistance but no data are available.

Unfortunately, we could not compare our results with those of a recent systematic review and meta-analysis concerning the different WHO regions, including Europe, because in this analysis, the results were not presented in a stratified manner according to both primary/secondary resistance and year of testing.[20]

In terms of risk factors associated with resistance, performed by univariate and multivariate analysis for the first three antibiotics tested (clarithromycin, levofloxacin and metronidazole), the region of birth was the only significant factor found, that is, those born in Western/Central Europe, Southern Europe and outside Europe had a higher risk for resistance compared with those born in Northern Europe. This risk factor was also present in our previous study, while the risk factors of age over 50 years for levofloxacin and female gender for metronidazole were not found this time.

As in our previous study performed a decade ago (in 2008–2009), we observed a good correlation between *H. pylori* resistance against clarithromycin and macrolide consumption in the European countries. This confirms the recommendation that clarithromycin should not be used without prior testing in the numerous countries where its resistance rate is above 15%.[1] These data will be helpful to treat *H. pylori* infection. As said before, quadruple therapies with or without bismuth are alternative empirical treatments to STT leading to satisfactory eradication rates, but they generate more adverse events such as more resistance in bacteria other than *H. pylori*, and they have a stronger impact on the gut microbiota, which is a plea to perform AST.[21] Commercially available molecular methods,[22] detecting the presence of *H. pylori* and associated mutations conveying an eventual resistance to clarithromycin, allow a quick result and should be used as much as possible to establish a tailored treatment.[23 24]

Since our previous article on this topic, other studies have been carried out. One used a multicentre design to monitor the nationwide antibiotic resistance pattern of *H. pylori* in Korea during the years 2017–2018. They included 590 patients subjected to an upper digestive endoscopy in 15 centres but could only recover 349 *H. pylori* strains using a central laboratory. The main difference was the type of patients since 53% of them suffered from a gastric cancer, and therefore, they may not have had a current infection. The levels of clarithromycin, fluoroquinolone, metronidazole, amoxicillin and tetracycline resistance were 17.8%, 37%, 29.5%, 9,5% and 0%, respectively.[25]

Other studies used a sentinel surveillance system. In Indonesia, a monitoring was performed from 2012 to 2017 on 849 adult patients in 11 centres on seven islands. As expected in this population known to be rarely infected, they found only 77 strains from the patients who had undergone endoscopy.[26] In China, 18 hospitals in 13 provinces were involved in a survey from 2010 to 2016 using a centralised laboratory for AST, and 960 cases were included.[27] In Alaska, USA, four centres were involved in a survey. From 2000 to 2016, they obtained results for 763 patients including children.[28] When it was not possible to organise a study, systematic reviews of data available in the literature were carried out. In Latin America, 56 studies from 12 out of the 20 countries of the region were analysed,[29] and in the Asian-Pacific region, 174 articles from 24 countries could be found from 2006 to 2015.[30]

All of these studies had in common to look for the so-called primary resistance in adults and were based on similar methodology. They show that global clarithromycin resistance is above the 15% threshold except in Indonesia (9%), metronidazole is in the range of 45%–55% except in China (78%) and levofloxacin resistance is in the range of 14%–20% except in Indonesia (31%), with a trend towards an increase over time and variability among the regions. Resistance to amoxicillin and tetracycline remains very low everywhere without evolution.

Only one study from Taiwan also included antibiotic consumption data as in our study.[31] Following a law restricting antibiotic use in 2001, there was a reduction in consumption and a stabilisation or slight decrease in *H. pylori* resistance for clarithromycin and metronidazole observed but not for fluoroquinolones for which the consumption increased.

Our study has, however, some limits. First, we had difficulty in finding participating centres in some countries, either because the frequency of infected patients is very low, such as in Sweden,[32] or because of regulatory problems such as in the UK, and the general motivation for the topic was not as great as before. Second, the sample representativeness and the extrapolation of the results of one region to the whole country was not always satisfactory as was the case for Italy where only centres from the southern part participated and the observed rate was

not in line with the expected rate (figure 6). Such variability in a given country has already been observed in other countries like Belgium where the West Flanders were compared with the Brussels area (with a significantly lower resistance rate of *H. pylori* to clarithromycin and to metronidazole in the former region), and a possible explanation was the proportion of migrants, which was more important in the Brussels area together with the rate of antibiotic resistance.[33] In addition, the protocol for France was slightly different since it was possible to receive gastric biopsies from volunteer gastroenterologists distributed throughout the country.[34] Third, despite strict criteria to include clinical isolates from patients without previous *H. pylori* eradication treatment and to perform the control a posteriori, we cannot ensure that they were always respected. Finally, we could not follow the outcome of the treatment of the included patients. However, given previous results showing that clarithromycin resistance decreases the success rate of the standard triple therapy by 70%,[35] we can anticipate that it would also have been the case in this study. In addition, our survey did not include children in contrast to the previous one, the reason being that in most countries, they are treated in different departments than adults and therefore we had a poor representativeness of this age group.

In conclusion, for clarithromycin, the resistance rate has increased since 2008 and continues to do so but to a lesser extent than between 1998 and 2008, indicating that there is still a need to test before prescribing this antibiotic in many countries. A significant increase is also observed for metronidazole, while levofloxacin resistance appears to be stable. In addition, the positive correlation between *H. pylori* resistance and the consumption of the corresponding antibiotics in the community was confirmed.

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Contributors FM and YG designed the protocol, organised the study, interpreted susceptibility results and wrote the manuscript. RB SC and HG established the correlation with antibiotic consumption. LW studied the risk factors for *Helicobacter pylori* resistance. T-DH, MH, LB and PL performed the strain control. All made a critical review of the manuscript. FM and YG are the guarantors of the article.

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