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ORIGINAL ARTICLE

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Survey of the antimicrobial resistance of *Helicobacter pylori* in France in 2018 and evolution during the previous 5 years

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Abstract

Background and Objectives: Surveillance of *Helicobacter pylori* resistance to antibiotics was carried out in France in 2014, 2016, and 2018. We report here the results of the 2018 survey as well as the evolution over the 5-year period.

Materials and Methods: In this observational study, gastric biopsies were obtained by 62 gastroenterologists randomly selected in 5 regions of France and sent to a central laboratory where culture, antimicrobial susceptibility testing, and a real-time PCR were performed in order to detect *H pylori* and its mutations associated with clarithromycin resistance.

Results and Conclusion: During the year 2018, 951 patients were included: 55.3% women, mean age: 52.4 years ± 15.7, 71.6% born in France. Among them, 359 patients were *H pylori* positive by both culture and real-time PCR, and 7 more by PCR only. There were 244 naive patients, 110 previously treated patients, and unknown for 5. Primary resistance to clarithromycin was 20.9% [16.3-26.4], to levofloxacin 17.6% [13.4-22.9], and to metronidazole 58.6% [52.3%-64.6%]. Secondary resistance for these antibiotics was 56.4%, 22.7%, and 87.3%, respectively. There was no resistance to amoxicillin and tetracycline and very low resistance to rifampicin (1.2%) in both naive and treated patients. Primary resistance to clarithromycin decreased from 22.2% to 20.3% between 2014 and 2016, and appears to be stable since then. This can be linked to a stable consumption of macrolides over the 3-year time period. Primary levofloxacin resistance was relatively stable while metronidazole resistance increased. Interestingly, in both naive and treated patients, amoxicillin and rifampicin resistance were rare.

KEYWORDS

clarithromycin, culture, levofloxacin, primary resistance, real-time PCR

1 | INTRODUCTION

Despite the decrease in prevalence, *Helicobacter pylori* infection is still common in France. The main challenge concerns its increase in antibiotic resistance which jeopardizes the treatment success. An effective treatment consisting of a triple therapy with two antibiotics (amoxicillin and clarithromycin) and a proton pump inhibitor was designed in the early 1990s[1] and confirmed as excellent in large clinical trials.[2] However, in the following years a progressive increase in clarithromycin resistance was observed, leading to a decrease in the

TABLE 1 Distribution of patients and *Helicobacter pylori*-positive cases according to the regions of residence in 2018

		Northeast	Northwest	Southeast	Southwest	Paris area	Total
Patients enrolled	N	249	90	260	177	175	951
H pylori +	N	73	36	114	47	89	259
	%	20.3	10.0	31.8	13.1	24.8	37.7

Note: No significant difference was found.

eradication rate. Quadruple therapies were recommended in countries like France where the prevalence of clarithromycin resistance exceeded 15%.[3] While these regiments are efficacious, the regulatory authorities recommended the introduction of a surveillance of *H pylori* resistance in the country when the bismuth quadruple therapy was launched. Surveys were organized in 2014, 2016, and 2018 in France. The results of the first survey have been published.[4] We report here the results of the 2018 survey and the evolution of *H pylori* resistance since 2014.

2 | MATERIALS AND METHODS

2.1 | Type of study

An observational multicenter study was designed. Gastroenterologists were randomly selected in five regions in France, representing the metropolitan area of the country: Northeast, Northwest, Southeast, Southwest, and the Paris area, with the goal of recruiting 75 gastroenterologists who were to include a maximum of 20 patients each, with an end to the recruitment when a maximum of 1000 patients was obtained.

2.2 | Inclusion criteria

The inclusion criteria were the following: adult patients (18 years and older) for whom an upper digestive endoscopy was scheduled in one of the selected centers, for symptoms which could be linked to *H pylori* infection or failure of an eradication treatment, and who gave a signed consent to participate. Information concerning demography, symptoms, and endoscopy diagnosis was obtained.

During the endoscopy, two gastric biopsies (antrum and corpus) were obtained and introduced into a transport medium (Portagerm pylori, bioMérieux, Marcy l'Etoile, France). They were then maintained and transported at 4°C by a special courier to a central laboratory in Bordeaux within 24-48 hours. In the laboratory, the gastric biopsies were ground together in 1 mL of in-house Brucella broth using a disposable grinder. Part of the suspension was used to plate two agar media, a Pylori agar (bioMérieux) and an in-house blood agar containing antibiotics.[5] The media were then incubated in a microaerobic atmosphere in a special workstation (Baker Ruskinn, Concept Ruskinn, Bridget, UK) at 36°C for 10 days. After 2 days, the plates were observed every day and suspicious colonies were tested for oxidase, catalase, urease, and morphological observation. When *H pylori* was identified, an antimicrobial susceptibility testing (AST) was performed on Mueller Hinton agar containing 10% sheep blood and globular extract prepared every week, using Etest® strips (bioMérieux) for clarithromycin, amoxicillin, metronidazole, and levofloxacin and disks for rifampicin and tetracycline. For these last two antibiotics, in the case of a reduced diameter, less than 20 mm for tetracycline and less than 19 mm for rifampicin, an Etest control was carried out. The cutoff values used were those proposed by EUCAST.[6] For clarithromycin, the values indicating intermediary susceptibility were considered resistant. All strains were maintained frozen at − 80°C in broth with 20% glycerol.

In addition, another part of the suspension was used to perform a real-time PCR targeting the 23S rRNA gene of *H pylori* according to a protocol previously described and detecting the mutations associated with clarithromycin resistance: A2642/3G and A2642C.[7] Primary resistance was defined for patients who did not receive previous eradication treatment and secondary resistance for those who already received one or more eradication treatment.

2.3 | Statistics

The descriptive statistics are presented according to the nature of the variable.

Data analysis was conducted with SAS® software (SAS Institute, Version 9.2, NC, USA).

This study was approved by the ethical committees under No. 13.470 in September 2013.

3 | RESULTS

3.1 | Patient characteristics

From March 2018 until February 2019, 62 gastroenterologists were recruited and they included a total of 951 patients from 55 administrative departments of the country. The distribution according to their regions of residence is presented on Table 1.

The patients were comprised of 55.3% women and 44.7% men. The mean age was 52.4 years ± 15.7. There were 681 patients (71.6%) born in France, and 270 born outside of the country. The latter were born in Africa: 145 (including 51 in Morocco, 45 in Algeria, and 13 in Tunisia); other countries in Europe: 73 (including 37 in Portugal); Asia: 41 (including 16 in Turkey); and 11 from America.

The symptoms observed and the endoscopic observation are presented on Table 2. The gastric mucosa was normal in 356 cases (37.4%).

3.2 | Results of *Helicobacter pylori* detection and antimicrobial susceptibility testing

There were a total of 359 *H pylori*-positive patients by both culture and PCR. Their distribution according to a previous treatment or lack thereof is presented on Table 3. In addition, seven patients were found to be positive by PCR only: 4 untreated (naïve) (one was clarithromycin resistant) and three previously treated (two were found to be clarithromycin resistant).

The results of the primary antibiotic resistance obtained by culture for the naive patients are presented on Table 4 with the evolution since the 2014 and 2016 surveys, and the results of secondary resistance also over the same time period on Table 5. The first, third, and fifth years there were 34, 73, and 73 patients, respectively, who received Pylera®-omeprazole.

The distribution of mutations associated with clarithromycin resistance was as follows: A2142/3G: 114, A2142C: 2, all corresponding to the phenotypic results. There were 38 cases of double population with a wild type and a mutated strain, and 2 cases with two different mutations. It is interesting to note that in one case we obtained a melting curve with a temperature (Tm) close to that observed with the A2142C mutation (−2°C), while the strain was phenotypically susceptible and sequencing indicated a G2132A mutation.

The primary resistance shows a relative stability over these years except for metronidazole which is increasing. A double resistance to clarithromycin and metronidazole was found in 30 cases (12.3%) and resistance to both clarithromycin and levofloxacin in 12 cases (5.1%). Resistance to at least one antibiotic tested was found in 174 cases (71.3%).

The secondary resistance exhibited a decrease in clarithromycin resistance over the years, which can be explained by the switch from a clarithromycin-based triple therapy to quadruple therapies as a first-line treatment, and a slight increase in levofloxacin resistance which is given as an alternative second line.

4 | DISCUSSION

The evolution over the last 5 years (2014-2018) of *H pylori* resistance to the antibiotics used to treat this infection is presented in this article. It is important to note that antibiotic resistance in *H pylori* is essentially linked to mutations which occur randomly in the genome[8,9] and are selected by the antibiotics used. The main interest concerns resistance to macrolides because clarithromycin is a key antibiotic in this respect and has been listed on the WHO list for research and development of new antibiotics as Priority 2: high.[10] Indeed, in France the rate of primary clarithromycin resistance appears to be stable. As has been shown, there is a good correlation between macrolide consumption in the community and *H pylori* primary resistance to clarithromycin three to 4 years later, especially for long-acting macrolides.[11] We can therefore hypothesize that the reason is the decrease in macrolide consumption. Data show that the daily dose per day per 1,000 inhabitants (ddd) decreased from 6 to 3.2 between 2000 and 2015, that is, a 46.5% decrease, while it was stable over the last 3 years of this period.[12] However, the resistance rate stands above the 15% threshold and it is still recommended to test before prescribing this antibiotic.[13]

Primary resistance to levofloxacin is also stable at a level around 15%, and it must also be due to a decreased consumption of quinolones in the community which evolved from 2.1 to 1.6 ddd between 2000 and 2015 (25.3% decrease); however, in contrast with macrolides, it occurred essentially after 2010.[12] Globally the high rate

TABLE 2 Distribution of symptoms and endoscopic observations among the patients included in 2018

	Naive	Treated	Total
Symptoms	**N = 746**	**N = 188**	**N = 951[a]**
Epigastric pain	472 (63.2%)	106 (56.3%)	578 (60.7%)
Other signs of dyspepsia	200 (26.8%)	29 (15.4%)	229 (24.1%)
Anemia	24 (3.2%)	3 (1.6%)	77 (2.8%)
Others (Include gastroesophageal symptoms)	182 (24.3%)	73 (38.8%)	255 (26.8%)
Endoscopic observations			
Inflammation	77 (10.3%)	22 (11.7%)	99 (10.4%)
Erosion	90 (12.1%)	24 (12.8%)	114 (12%)
Ulcer	110 (14.7%)	24 (12.8%)	134 (14.1%)
Esophageal disease	62 (8.3%)	27 (14.4%)	89 (9.3%)
Others	113 (15.1%)	46 (24.5%)	159 (16.7%)

[a]Previous treatment unknown in 17 cases.

TABLE 3 Distribution of *Helicobacter pylori*-positive patients by culture according to treatment status in 2018

		Naive	Treated	Unknown	Total
Patients enrolled	N	746	188	17	951
H pylori +	N	244[a]	110[b]	5	359[c]
	%	32.7	58.5	29.4	37.7

[a]plus 4 by PCR.
[b]plus 3 by PCR.
[c]plus 7 by PCR.

of secondary resistance is a plea to eradicate *H pylori* on the first attempt.

The situation is different for metronidazole for which primary resistance increased from 45.9% to 58.6% during these 5 years. We do not have data on the prescriptions in the community which usually concern mainly gynecological and dental infections. However, the result for metronidazole must be treated with caution because it has been reported that in vitro testing is not reproducible[14] and could be due to a lack of control of the redox potential of the media[15]; this could be overcome in vivo by increasing the length of the treatment.[16] It was even considered not to be necessary to routinely perform AST for this antibiotic.[3]

The good news is that resistance is very low or nil for two antibiotics: amoxicillin and tetracycline for which the consumption in the community is stable. For amoxicillin, there are reports of resistant strains, results which usually cannot be reproduced after subculture. Furthermore, the cutoff value for resistance has been defined by analogy with other bacteria, which may explain why supposedly resistant strains have MICs just above the threshold.

For tetracycline, resistance is due to a triplet of mutations in position 965-967 on the 16S rRNA gene (AGA965-967TTC).[17] The occurrence of three contiguous mutations is a very rare event. When one occurs, the MIC remains low; with two, MICs are clearly increased while with three mutations, resistance is at a high level. For rifampicin which is tested for rifabutin, the antibiotic used against *H pylori*, there is also a very limited number of cases with *rpo*B mutations.[18]

Looking again at clarithromycin, there is a quasi-perfect correlation between resistance observed phenotypically and the associated mutations previously described, detected by PCR. As usual, the A2142/3G mutations, which we did not differentiate, is the most frequent compared to the mutation A2142C. However, it is important to look carefully at the Tm on the melting curve and to sequence the amplicons in the event of a difference with what expected. It allowed us to identify in one case a G2132A mutation, which indeed, did not have any impact on the strain MIC.

This excellent correlation between genotypic and phenotypic results for clarithromycin allows us to use PCR as a first diagnosis approach. Commercial tools based on real-time PCR are now available allowing within a few hours the detection of *H pylori* in gastric biopsies as well as the mutations associated with its resistance.[19] This technique also offers the possibility to detect *H pylori* in stools when an endoscopy is not mandatory, for example,. in young patients without alarm symptoms. Until recently, results were impaired by the difficulty in obtaining sufficient amounts of *H pylori* DNA in the special environment of feces, but extraction methods have improved and currently allow satisfactory results.[20]

The French High Authority in Health (HAS) is now recommending such an approach, that is, testing for clarithromycin resistance in order to prescribe an optimized triple therapy if the strain is clarithromycin susceptible instead of a quadruple therapy, a situation which occurs in approximately 80% of the cases, and

TABLE 4 Evolution of *Helicobacter pylori* primary resistance to antibiotics in France over a 5-year period (2014-2018) (% with confidence interval)

	2014	2016	2018
	N = 266	N = 231	N = 244
Clarithromycin	22.2 [17.3-27.7]	20.3 [15.3-26.1]	20.9 [16.3-26.4]
Levofloxacin	15.4 [11.3-20.3]	14.7 [10.4-20]	17.6 [13.4-22.9]
Amoxicillin	0.7 [0.1-2.0]	0.9 [0.1-3.1]	0
Metronidazole	45.9 [39.8-52.1]	52.4 [47.5-59]	58.6 [52.3-64.6]
Rifampicin	0.7 [0.1-2.7]	0	1.2 [0.4-3.6]
Tetracycline	0	0	0

TABLE 5 Evolution of *Helicobacter pylori* secondary resistance to antibiotics in France over a 5-year period (2014-2018) (% with confidence interval)

	2014	2016	2018
	N = 115	N = 125	N = 110
Clarithromycin	73.9 [64.9-81.7]	59.7 [50.5-68.4]	56.4 [47-65.3]
Levofloxacin	14.8 [8.9-22.6]	23.4 [16.3-31.8]	22.7 [15.9-31.4]
Amoxicillin	0	0.8 [0-4.4]	0
Metronidazole	78.3 [69.6-85.4]	80.6 [72.6-87.2]	87.3 [79.8-92.3]
Rifampicin	0.9 [0-4.7]	0.8 [0-4.4]	1.2 [0.4-3.6]
Tetracycline	0	0	0

prescribing for the remaining 20% either a levofloxacin-based triple therapy or a quadruple therapy.[21] Such a protocol has been applied in Annecy, France, and led to 93% success for those receiving the optimized triple therapy (72 cases) and 86% success for those receiving a bismuth-containing quadruple therapy (BQT) (21 cases).[22]

More antibiotics prescribed also increase the resistance of bacteria other than *H pylori*, and, as was recently shown, the gut microbiota is more strongly impacted by quadruple therapies than by triple therapies with the risk linked to dysbiosis.[23] Such a strategy also follows the WHO recommendation of a prudent use of antibiotics.[24]

This study had the advantage of recruiting patients throughout the entire country, not from tertiary centers, and using a central facility. However, it has certain limits such as the low number of cases per region which impairs the possibility of comparing the prevalence of the infection and the prevalence of resistance by regions of the country. Also, concerning an eventual previous *H pylori* eradication, we did not rely on a national treatment register but only on the gastroenterologist's record and the patient's memory which may be erroneous in some cases.

In conclusion, this study shows a stabilization of *H pylori* resistance to the main antibiotics concerned in France, that is, clarithromycin and levofloxacin, and a lack of emergence of resistance to amoxicillin as well as tetracycline despite the launch of the Pylera®-omeprazole BQT in 2013.

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DISCLOSURE

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

Francis Mégraud supervised the study and wrote the manuscript. Chloé Alix, Paul Charron, Lucie Bénéjat, and Astrid Ducournau carried out the analyses during the different years and reviewed the manuscript. Emilie Bessède and Philippe Lehours discussed the data and critically reviewed the manuscript.

DATA AVAILABILITY STATEMENT

The data are subject to third party restriction.

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