Short title: Not applicable

Title: PHARE study: An observational multicenter study on Antibiotic Resistance of *Helicobacter pylori* in France

Study ID: PYR-12-01

Sponsor: Allergan Inc.
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Study phase: Observational

First Patient First Visit: Feb 2014

Last Patient Last Visit: Feb 2019

Date of final version of study report: 10-Dec-2019

ABSTRACT

Title: PHARE study: An observational multicenter study on antibiotic resistance of *Helicobacter pylori* in France

Keywords: PYLERA®, antibiotic, resistance, observational, bismuth subcitrate potassium, metronidazole, tetracycline hydrochloride, *Helicobacter pylori*

Rationale and background: In countries where it is available, the quadruple bismuth therapy of a proton pump inhibitor (PPI) (omeprazole) + PYLERA® (bismuth subcitrate potassium, metronidazole, and tetracycline hydrochloride) has been proposed as first-line treatment for the eradication of *H. pylori* infections. PYLERA® has been available in France since 10 April 2013. Antibiotic (ATB) resistance is the main factor for treatment failure to eradicate *H. pylori* [1]. This observational, national, multicenter 5-year study on *H. pylori* resistance to ATBs was requested by the "Agence Nationale de Sécurité du Médicament et des Produits de Santé"– French Health Authorities) as part of the European Risk Management Plan for PYLERA®. It is intended to address the French regulatory agency requirements for the surveillance over 5 years of *H. pylori* resistance to ATBs following the marketing authorization of PYLERA® in France.

Research question and objectives: The primary objective was to assess the resistance rate in clinical isolates of *H. pylori* to ATBs (amoxicillin, clarithromycin, levofloxacin, metronidazole, rifampicin, and tetracycline). The secondary objectives were to monitor the trend of strain resistance to ATBs over the 5-year period following the commercial launch of PYLERA® and to evaluate the impact of trend of strain resistance to ATBs on the efficacy of PYLERA® treatment.

Study design: This is an observational, multicenter study in France. The participation of each subject in the study involved 1 visit, which took place during a scheduled endoscopy with biopsies by a gastroenterologist as part of a regular medical investigation. Biopsies collected by all gastroenterologists were sent to a central bacteriology laboratory where the detection of *H. pylori* and the resistance to ATBs were determined.

Setting: This study was conducted exclusively in metropolitan France using a representative sample of 100 gastroenterologists distributed in 5 regions of France: North East, North West, South East, South West, and Paris area. These gastroenterologists were randomly selected to participate in the study. Participating gastroenterologists included patients, based on inclusion criteria, on 1 specific day per month.

Patients and study size, including dropouts: The study population was defined as being comprised of patients 18 years of age and older who had a scheduled or planned endoscopy at one of the centers involved in the study, who exhibited a pathology potentially associated with an infection by *H. pylori* or who had failure of a previous treatment for eradication, and who signed the information consent.

The study included 2908 patients recruited during 3 collection periods: collection period 1 (1st trimester 2014 to 1st trimester 2015: 987 patients), collection period 2 (1st trimester 2016 to 1st trimester 2017: 970 patients), and collection period 3 (1st trimester 2018 to 1st trimester 2019: 951 patients), for a total of 2908 participating patients over 5 years.

Variables and data sources: Gastroenterologists collected gastric biopsies in accordance with their center's usual practices. The required number of biopsies were collected for the usual pathology evaluation by the center and 2 additional biopsies (1 antral and 1 corpus) were collected for antimicrobial susceptibility testing in the context of the study.

Safety variables: Not applicable.

Data to be collected:

- Endoscopy date and sample collection time
- Sociodemographic data: age, gender, country of birth, current place of residence, and education level
- Reason for consultation: epigastric pain, other signs of dyspepsia, anemia, and others
- Use of ATBs in the 30 days before the endoscopy including name, duration and end date
- Use of a PPI in the 7 days before the endoscopy including name, duration, and end date
- History of *H. pylori* infection: previous eradication attempts including number of attempts, and nature and duration of treatment prescribed, such as the following:
    - Bismuth quadruple therapy: PPI (omeprazole or other name and dosage) + PYLERA® (10 days)
    - Triple therapy: PPI + amoxicillin + clarithromycin (7, 10, or 14 days)
    - Triple therapy: PPI + amoxicillin + metronidazole (7, 10, or 14 days)
    - Sequential treatment: PPI + amoxicillin (5 days) followed by PPI + clarithromycin + metronidazole (5 days)
    - PPI + amoxicillin + levofloxacin (10 days)
    - PPI + amoxicillin + rifabutin (10 days)
    - Others
- Biopsies for bacteriology: localization (antral, corpus) and number
- Results of endoscopy: rashes, superficial erosions, ulcers, esophageal diseases, others
- Antimicrobial resistance results from central laboratory

Biopsy analysis: The resistance of *H. pylori* to 6 different ATBs was determined by agar diffusion using 2 different tests: disk diffusion for tetracycline, rifampicin and levofloxacin, and Epsilometer test (E-test) for clarithromycin, metronidazole and amoxicillin.

During the last recruitment period, the testing method for levofloxacin was modified. The

resistance of *H. pylori* to levofloxacin was determined by E-Test.

Real-time polymerase chain reaction (PCR) was performed on all biopsy DNA extracts for detection of *H. pylori* and clarithromycin resistance.

Data analysis: The number and percentage of patients who were considered as positive or negative for the presence of *H. pylori* were calculated. The number and percentage of *H. pylori*-positive patients (and the 95% CI) with *H. pylori* resistant to each ATB were calculated in each of the 5 geographic regions (North East, North West, South East, South West, and the Paris area) and for all regions combined.

Milestones: The following milestones were projected for the study:

- First period of inclusion, collection period 1: 1st trimester 2014 to 1st trimester 2015
- Interim report for collection period 1: 2nd trimester 2015
- Second period of inclusion, collection period 2: 1st trimester 2016 to 1st trimester 2017
- Interim report for collection period year 2: 2nd trimester 2017
- Third period of inclusion, collection period 3: 1st trimester 2018 to 1st trimester 2019
- Closure of the database: 2nd trimester 2019
- Final data analysis: 2nd trimester 2019
- Final clinical study report: 3rd trimester 2019

Results: This report covers the results Collection Period 3 (26-FEB-2018 to 26-FEB-2019). The results of the first (24-FEB-2014 to 24-FEB-2015) and the second (24-FEB-2016 to 23-FEB-2017) time periods were covered in detail in the interim reports dated 19 June 2015 and 25 August 2017 respectively. Comparison of the results from the three Collection Periods are also presented.

The following results were observed:

- In the 3rd collection period, 62 sites participated in this study, enrolling 951 patients of whom 359 were culture positive for *H. pylori*. The most common reason for consultation was epigastric pain in 592 patients (62.2%).
- Most patients (746 [78.6 %]) had no previous eradication attempts and 188 patients (19.8 %) had previous eradication attempts; of these 122 (65.2 %) had one eradication attempt. Among the 951 samples tested, 366 samples (38.5 %) showed positive PCR result for *H. pylori*, 359 samples (37.7 %) were culture positive for *H. pylori*, and 359 samples (37.7 %) were positive by both methods.
- Among the 359 H. pylori positive patients, 244 were naive patients, 110 had already received eradication treatment and for 5 the information was missing

Resistance rates determined on the 359 positive isolates of *H. pylori* from all culture positive patients are, as follows:

- amoxicillin 0.0 %, 0 patient,
- clarithromycin 32.1 %, 115 patients,

- levofloxacin 18.9 %, 68 patients,
- metronidazole 66.0 %, 244 patients,
- rifampicin 1.1 %, 4 patients,
- tetracycline, 0.0 %, 0 patient,
- at least 1 of the 6 ATBs, 78.8 %, 283 patients.

For patients (366) who were *H. pylori* positive by PCR test:

- 250 (68.3 %) were susceptible to clarithromycin,
- 116 (31.7%) were resistant:
    - 79 (21.6%) were resistant with mutation A2142G/A2143G,
    - 35 (9.6%) were resistant with a dual population of wild type and mutation A2142G/A2143G,
    - 1 (0.3 %) were resistant with mutation A2142C,
    - 1 (0.3 %) was resistant with a dual population of wild type and mutation A2142T.

When results were assessed for isolates from patients with prior treatment for *H. pylori*, the following was observed for the 110 isolates:

- 0 (0.0%) was resistant to amoxicillin,
- 63 (57.3 %), were resistant to clarithromycin,
- 25 (22.7 %), were resistant to levofloxacin,
- 96 (87.3%) were resistant to metronidazole,
- 1 (0.9%), was resistant to rifampicin,
- None were resistant to tetracycline,
- 104 (94.5%) were resistant to at least 1 of the 6 ATBs.

When results were assessed for 73 isolates from patients who had received PYLERA® as prior treatment for *H. pylori*:

- No isolates of *H. pylori* were resistant to amoxicillin
- 38 (52.1 %) isolates of *H. pylori* were resistant to clarithromycin,
- 18 (24.7 %) isolates of *H. pylori* were resistant to levofloxacin,
- 66 (90.4%) isolates of *H. pylori* were resistant to metronidazole,
- 1 (1.4 %) isolates of *H. pylori* were resistant to rifampicin,
- No isolate of *H. pylori* was resistant to tetracycline,
- 70 (95.9%) were resistant to at least 1 of the 6 ATBs.

When results were assessed for the 244 isolates from patients who did not receive a previous eradication treatment

- No isolate was resistant to amoxicillin or tetracycline
- 51 (20.9%) isolates were resistant to clarithromycin
- 43 (17.6%) isolates were resistant to levofloxacin
- 143 (58.6%) isolates were resistant to metronidazole
- 1 (0.4%) isolate was resistant to rifampicin
- 30 (12.3%) isolates were resistant to both clarithromycin and metronidazole

Regarding the secondary objectives of the study:

- Comparison of resistance rates of the first, second and third collection periods was performed. Statistically significant changes were found for the resistance rate of *H. pylori* to clarithromycin and metronidazole in the total population who received prior treatment for *H. pylori*.
- It should be noted that no statistically significant changes for levofloxacin and rifampicin were found in patients who received prior treatment with PYLERA®.
- Subgroup analyses of resistance rates of the first, second and third collection periods did not reveal any statistically significant differences.

Discussion: This 5-year, national, observational study was performed in metropolitan France using a representative sample of approximately 100 gastroenterologists distributed in 5 regions of France during each recruitment period. Each participating gastroenterologist was to include up to 20 patients during each of the 3 recruitment periods. Overall enrollment was not to exceed 1000 patients per recruitment period. When susceptibility results were assessed for isolates from patients who were either *H. pylori* positive (overall), or who were *H. pylori* positive, but never received prior treatment for *H. pylori*, or had any *H. pylori* eradication attempt, or had *H. pylori* eradication attempt with PYLERA®, the following was observed (table 1 and figure 1):

Table 1. Resistance Rate Across the Three Collection Periods

	Resistance* Rate, %											
	Overall *H. pylori* Positive Population			Treatment-naive Population			Any *H. pylori* Eradication Therapy Attempted			PYLERA® Eradication Therapy Attempted		
	Y1 N=386	Y2 N=359	Y3 N=359	Y1 N=266	Y2 N=231	Y3 N=244	Y1 N=115	Y2 N=125	Y3 N=110	Y1 N=34	Y2 N=73	Y3 N=73
Amoxicillin	0.5	0.8	0.0	0.8	0.9	0.0	0	0.8	0.0	0	0	0.0
Clarithromycin	37.6	34.4	32.1	22.2	20.3	20.9	73.9	59.7	57.3	61.8	54.2	52.1
Levofloxacin	15.3	17.9	18.9	15.4	14.7	17.6	15.7	23.4	22.7	11.8	25	24.7
Metronidazole	56	62.3	66.0	45.9	52.4	58.6	78.3	80.6	87.3	91.2	83.3	90.4
Rifampicin	0.8	0.6	1.1	0.8	0.4	1.2	0.9	0.8	0.9	2.9	1.4	1.4
Tetracycline	0	0	0.3	0	0	0	0	0	0	0	0	0
At least 1 of the 6 antibiotics	71	74	78.8	60.9	64.9	71.3	93.9	90.3	94.5	97.1	90.3	95.9

*Resistance or Intermediate resistance

Figure 1. **Resistance Rate Across the Three Collection Periods**

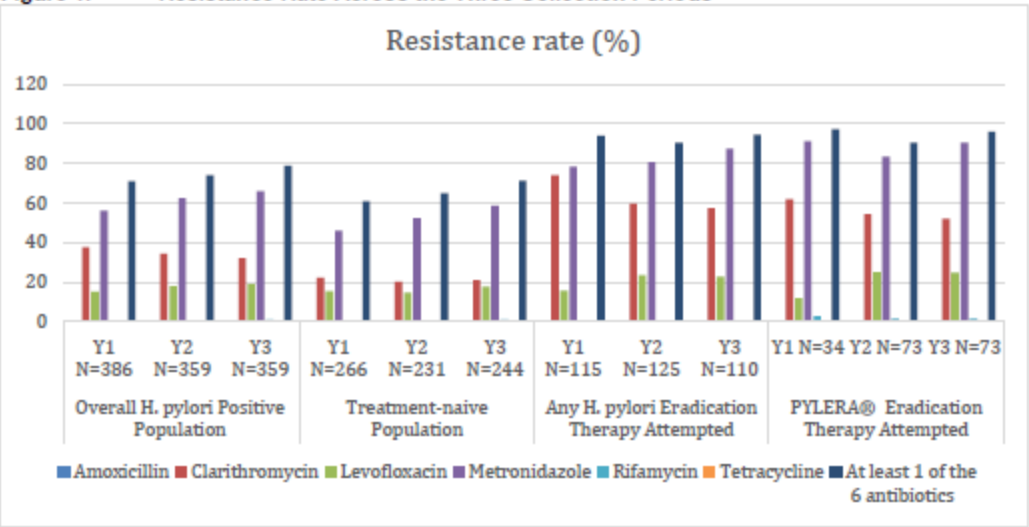
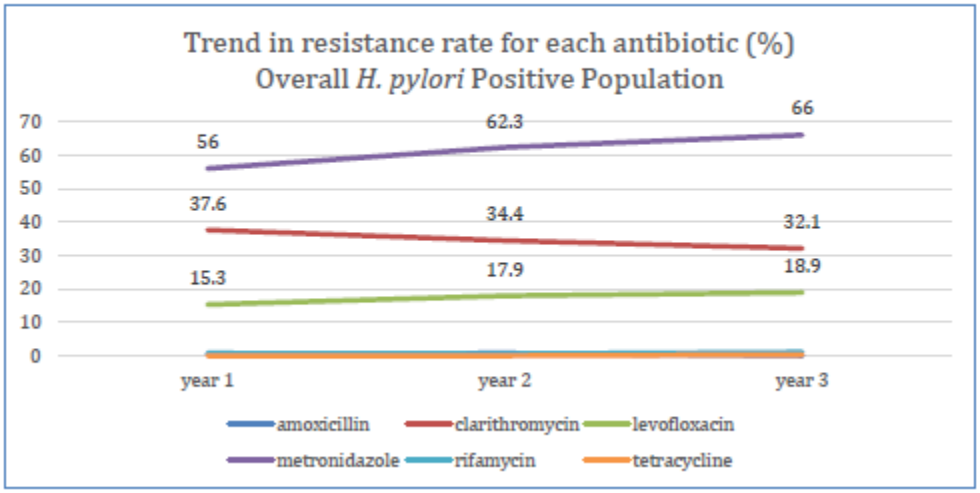


Figure 2. **Trend in Resistance Rate Across the Three Collection Periods**



PYLERA® contains bismuth subcitrate potassium, metronidazole and tetracycline hydrochloride. Bismuth subcitrate potassium resistance is not expected. Therefore, resistance to metronidazole and tetracycline is of main interest.

For Collection Period 1, 30.5 % (57/187) of patients who received prior treatment were treated with PYLERA® compared to 53.2% (100/189) for Collection Period 2 and 63.3% (119/188) for Collection Period 3.

As seen during Collection Periods 1, 2 and 3 the percentage of *H. pylori* isolates collected from culture positive patients that were resistant to metronidazole was higher for patients who received any treatment for *H. pylori* and for patients who reported treatment with PYLERA®.

During the 3 collection periods none of the *H. pylori* isolate was resistant to tetracycline.

Regarding the trend of strain resistance, the only statistically significant change was for the resistance rate of *H. pylori* to clarithromycin in the total population who received prior treatment for *H. pylori*. There was a significant reduction in the resistance rate of *Pylori* to clarithromycin in that population.

The wide distribution of patients studied and the absence of stringent admission criteria for the study suggest that the results of this study are probably generalizable to the French general population.

Marketing authorization Holder: ALLERGAN PHARMACEUTICALS INTERNATIONAL LIMITED