ORIGINAL PAPER

Available from:http://www.jgld.ro/wp/archive/y2017/n2/a8/
DOI: http://dx.doi.org/10.15403/jgld.2014.1121.262.itl

Eradication Rates in Italian Subjects Heterogeneously Managed for *Helicobacter pylori* Infection. Time to Abandon Empiric Treatments in Southern Europe

Agostino Di Ciaula[1], Giuseppe Scaccianoce[2,3], Marino Venerito[4], Angelo Zullo[5], Leonilde Bonfrate[3], Theodore Rokkas[6], Piero Portincasa[3]

1) Division of Internal Medicine, Hospital of Bisceglie, Italy;
2) Gastrointestinal Endoscopy Unit, Ospedale della Murgia F. Perinei, Altamura, Italy;
3) Clinica Medica A. Murri, Department of Biomedical Sciences & Human Oncology, University of Bari Medical School, Bari, Italy;
4) Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University Hospital, Magdeburg, Germany;
5) Gastroenterology Unit, Nuovo Regina Margherita Hospital, Rome, Italy;
6) Gastroenterology Clinic, Henry Dunant Hospital, Athens, Greece.

Address for correspondence:
Prof. Piero Portincasa
University of Bari Medical School, Clinica Medica A. Murri; Department of Biosciences and Human Oncology (DIMO); Policlinico Hospital - 70124 Bari, Italy
piero.portincasa@uniba.it

Received: 09.03.2017
Accepted: 18.04.2017

ABSTRACT

Background & Aims: *H. pylori* eradication is strongly affected by various factors, including the ongoing antibiotic resistance. We describe a "real life" scenario in patients managed for *H. pylori*-related conditions, living in a southern Italian region (Apulia), an area with clarithromycin resistance >15%.
Methods: 2,224 subjects were studied in two tertiary referral centers in Apulia. Analyses included: reason for referral, *H. pylori* infection rates (^{13}C-urea breath test – UBT or upper endoscopy), and eradication rates following distinct regimens previously prescribed or prospectively prescribed (such as the bismuth-based quadruple therapy Pylera®, recently marketed in Italy).
Results. Over 80% of the patients were referred by family physicians (60% naïve subjects). The overall infection rate was 32.5% and it was similar in asymptomatic patients (31.1%) or with *H. pylori*-related symptoms/clinical conditions (34.3%). In the 987 *H. pylori*+ve patients receiving therapy, the overall eradication rate was 80.2% (ITT). Observed eradication rate varied greatly across different regimens: 57.1% (2nd line levofloxacin), 59.6% (unconventional), 70.7% (7-day triple), 73.2% (7-day undefined), 89% (10-day sequential) and 96.9% (ITT, 10 day Pylera®, 1st to 5th line regimens given to 227 patients).
Conclusions. A heterogeneous "real life" scenario in Southern Europe shows that *H. pylori*+ve patients are put at risk of poor outcomes and points to the need of a susceptibility-based therapy according to guidelines and local microbial resistance. In the present setting (i.e. high clarithromycin resistance), despite the high observed eradication rate, sequential therapy should not be recommended (absent in guidelines, unneeded antibiotic). Bismuth-based quadruple treatment (1st, 2nd or subsequent lines) yields the highest eradication rates.

Key words: bismut – clarythromicin – chronic gastritis – levofloxacin – Pylera.

Abbreviations: ALT: Altamura; BA: Bari; EGDS: esophagogastroduodenoscopy; GERD: gastro-esophageal reflux disease; H. pylori: Helicobacter pylori; ITT: intention-to-treat; PP: per-protocol; PPI: proton pump inhibitor; UBT: urea breath test.

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is one of the commonest bacterial infections worldwide. Current diagnostic techniques include noninvasive procedures (i.e. ^{13}C-urea breath test - UBT, fecal *H. pylori* antigen) and esophagogastroduodenoscopy (EGDS) followed by rapid urease test or histology. The treatment of *H. pylori* infection is a matter of debate because of several implications arising from the prevalence of the disease, the presence of symptoms, the potential evolution toward atrophic gastritis, metaplasia, gastric cancer and mucosa-associated MALT lymphoma. Current guidelines suggest eradication of *H. pylori* in all diagnosed subjects [1], as this infectious agent is included in the list of IARC group I carcinogens [2-4].

The choice of the diagnostic test (in particular invasive vs noninvasive tests) strongly depends on several logistic variables (i.e. patients' needs, local availability, clinical context). Generally, UBT is appropriate in younger patients with uncomplicated upper gut symptoms, with recourse to endoscopy if the "test and treat" strategy fails to relieve symptoms [5].

On the other hand, the best therapeutic approach in the clinical practice is not easily identifiable. According to recent guidelines [5], current therapy includes first- and second-line regimens. Of note, clarithromycin resistance is

rapidly increasing worldwide [6] and this trend undermines the efficacy of the standard first-line triple (proton pump inhibitor - PPI, amoxicillin, clarithromycin) therapy, yielding a low eradication rate (<80%) [7-9]. Other first-line regimens included the "sequential" (PPI, amoxicillin followed by clarithromicin and tinidazole) therapy [9-11]. Alternative second-line (i.e. when first-line therapy fails) therapeutic approaches contain PPI plus amoxicillin plus levofloxacin [12]. More recently, a combination of tetracycline hydrochloride, metronidazole, and bismuth subcitrate potassium (Pylera®) has been developed as either the first- and second-line regimen [5, 8, 13]. Pylera entered the Italian market in March 2016.

Current diagnostic and therapeutic guidelines are often not completely coherent with practice in primary care, since the individual behavior [14], and the overall adherence of primary care physicians to guidelines can be very low in some contexts [15].

All these factors increase the risk of an inadequate management of *H. pylori* infection and this practical problem becomes even more challenging considering the overall costs related to consultations, diagnostics and therapies, and the risks related with the increasing antibiotic resistance.

With this background in mind, we aimed at describing a "real life" scenario about the management of a heterogeneous group of patients with *H. pylori* - related problems, and to report on the therapeutic outcomes of standard regimens and (prospectively) of the novel bismuth-based quadruple therapy (Pylera).

SUBJECTS AND METHODS

Study design

The survey was conducted in a geographical area with high clarithromycin resistance (i.e. approximately 30% in Italy [6, 13, 16], more than 15% in Apulia [7]).

A total of 2,224 subjects were enrolled at two hospitals (tertiary referral centers) in the province of Bari (Apulia region about 4M inhabitants, Southern Italy): Clinica Medica "A. Murri", Dept. of Biomedical Science and Human Oncology, University of Bari (BA), and Gastrointestinal Endoscopy Unit, district Hospital "Fabio Perinei", Altamura, Bari (ALT) due to *H. pylori*-related clinical problems and were included in an observational protocol aimed to assess the appropriateness of referral and diagnostic procedures and the efficacy of both prior and subsequent eradication regimens. The study was based on routine diagnostic and therapeutic practice.

In the first part of the study, all patients underwent a full clinical evaluation (including an analysis of symptoms and the existence/absence of prior eradication treatments) and diagnostic procedures (UBT or endoscopy) aimed to verify the *H. pylori* infection.

No attempt was made to contact the general physicians to obtain additional information regarding prior eradication regimens, apart from the information provided by the patients themselves.

In the second (prospective) part of the study, subjects with a positive test for *H. pylori* infection (n=651, 27.7%) underwent eradication treatments in the two referral centers and subsequent observation and confirmation tests (see below). In particular, a subgroup of 227 subjects underwent eradication treatment with bismuth subcitrate potassium (see below). All patients provided full informed consent to the procedures and the study protocol was approved by the local Ethics Committee of the University of Bari Medical School.

Characteristics of symptoms

Three categories of subjects were identified according to the presence of symptoms:

1. asymptomatic subjects: subjects referred by their physicians because of a family history of *H. pylori* infection, or because of a personal concern about *H. pylori* infection and potential health consequences (including gastric cancer);

2. subjects with *H. pylori*-related symptoms/clinical conditions: i.e. mainly because of uninvestigated "functional" dyspepsia without alarm symptoms [5, 17], previous history of peptic ulcer, symptoms suggestive of gastro-esophageal reflux disease (GERD), chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), and extra-gastric diseases such as unexplained iron-deficiency anemia, idiopathic thrombocytopenic purpura (ITP), vitamin B12 deficiency [5];

3. subjects with symptoms/clinical conditions unrelated to *H. pylori*: i.e. presence of non-gastroenterological symptoms/diseases: allergies, urticaria, hair loss, eye pain likely allergic, dermatological symptoms. In all these cases the indication for UBT was based on a specific physician request. This subgroup of patients was observed exclusively in the BA center.

Diagnosis of *H. pylori* infection and confirmation of eradication

The UBT was performed according to the manufacturer's instruction (in BA by Breath Quality UBT-75 mg ^{13}C urea, AB Analitica SRL, Padova, Italy; in ALT by Expirobacter UBT/100 mg ^{13}C - urea, Sofar, Milano, Italy) in fasting subjects for at least 8 hrs and free of medications able to influence results: i.e., off antibiotics in the prior month, and no proton pump inhibitors in the previous 2 weeks [5, 18]. Smoking and physical exercise were not allowed on the morning of the test. The reported sensitivity and specificity of UBT ranges from 88 to 95% and 95 to 100%, respectively [19]. Post-therapy UBT was planned not earlier than four weeks after the end of the eradication treatment [20]. UBT samples were analyzed by an infrared mass spectrometry (HeliFANplus®, FAN GmbH, Leipzig, Germany). Delta values over baseline (DOB, δ‰) were abnormal if > 4‰.

The diagnosis of *H. pylori* infection by EGDS was established by gastric biopsies and histology (two samples from the antrum and two from the body of the stomach), and a rapid urease test (Urease Liquid, LTA srl, Bussero, Milano, Italy) on antral biopsies. Confirmation of eradication was performed by UBT in all patients receiving treatment for *H. pylori* in both referral centers of BA and ALT. Prior eradication treatments had to be discontinued for at least four weeks and PPIs for at least two weeks, to reduce the chance of false-negative results [5].

Eradication treatments

The following regimens were considered in this study. In particular, the two referral centers used treatments n. 1.2, n. 2 (before Pylera was marketed) and n. 3 after March 2016, when Pylera was marketed in Italy.

1. First-line regimens:

1.1. Standard triple treatment: PPI + clarithromycin 500 mg + amoxicillin 1000 mg each administered twice daily. In all cases the treatment was protracted for 7 days [21], although extension to 10–14 days was recommended by previous guidelines [5].

1.2. Sequential treatment [9, 11]: PPI (pantoprazole 40 mg) + amoxicillin 1000 mg each administered twice daily for 5 days followed by PPI + clarithromycin 500mg + tinidazole 500 mg, each administered twice daily for the remaining 5 days.

2. Second-line regimens: levofloxacin-containing triple treatment: PPI + amoxicillin 1000 mg + levofloxacin 250 mg, each administered twice daily for 10 days [5, 22];

3. First-line, second-line or subsequent regimens: Pylera® (Allergan SpA, Rome, Italy): quadruple therapy consisting of a 10 day treatment with a combination formulation containing bismuth subcitrate potassium 140 mg + metronidazole 125 mg + tetracycline hydrochloride 125 mg as three capsules given 4 times daily (q.d.s.), and omeprazole 20 mg given twice daily [5, 20, 22].

4. Other treatments

4.1. "Unconventional" regimen: this was a subgroup of patients (N=47) who were prescribed various combinations of antibiotics but had no place in current guidelines [1].

4.2. "Undefined" regimen: this was a subgroup of patients (N= 395) who had undergone various therapies (likely triple for 7 days), which could not be properly described (i.e. when written notes were missing).

Statistical analysis

H. pylori eradication rate was calculated as the percentage of subjects with a negative diagnostic test performed at least one month after end of treatment.

Results are provided as the means ± standard error (SEM). Variables were checked for normal distribution by the Kolmogorov-Smirnov goodness of fit test. Proportions were compared by contingency tables and chi-squared or Fisher's exact tests. Comparison of continuous variables was performed with the unpaired Student's *t*-test or Mann-Whitney rank sum test, as appropriate. In the prospective part of the study, if drop-outs were recorded, both per-protocol (PP) (all subjects who completed the study without any events that could potentially bias the study outcome) and intention-to-treat (ITT) analyses were performed. Statistical analyses were performed with the NCSS statistical software (NCSS9 Statistical Software 2013. NCSS, LLC. Kaysville, Utah, USA, www.ncss.com/software/ncss) [23, 24]. The difference was considered statistically significant when the probability (P-value) was less than 0.05.

RESULTS

Diagnosis and clinical profile

Overall, 2,224 subjects entered the study: 80% of the patients were referred by their physicians and 20% by specialists on an outpatient basis. All patients lived in the same geographical area (i.e. the Province of Bari).

Table I. General characteristics of the 2,224 subjects according to referral center

	Bari (BA)	Altamura (ALT)
Patients n (%)	2023	201
Females	1,211 (50.9%)	112 (55.7%)
Males	812 (40.1%)	93 (46.3%)
	(P=0.0000)	(P=0.0000)
Age (years)		
Females	49.7±0.51	52.8±1.77
Males	51.8±0.67	53.8±2.0
	(P=0.02)	(P=0.6)
Diagnosis		
UBT, basal n (%)	2,023 (100%)	-
EGDS + histology, basal n (%)	633 (31.3%)	201 (100%)
UBT, post-eradication	2,023 (100%)	201 (100%)

Data are mean ± SE; significant difference for P<0.05; UBT: urea breath test; EGDS: esofagogastroduodenoscopy; *patients had both EGDS (in external centers, before enrollment) and UBT (at admission)

The general characteristics of the study group according to the referral center are depicted in Table I.

Females were better represented than males within each center, and in BA they were slightly younger than males. In BA all patients underwent UBT before and after eradication regimen, and 31.3% had also previously undergone EGDS at an external unit. In ALT, all patients underwent EGDS before- and UBT after treatment at the same endoscopy unit.

The clinical profile of the study group is given in Table II. Overall, naïve subjects entering the two centers to confirm a suspicion of *H. pylori* infection were 59.8% (1201+128 = 1329/2224), with females more prevalent than males in both centers. Patients undergoing post-eradication control were 40.2% (822+73 = 895/2224) and in BA females were more prevalent than males.

About one-third of the subjects were asymptomatic in BA, compared to 2% in ALT (P=0.0000), without gender difference. By contrast, females were more prevalent in the group of symptomatic subjects in both centers.

Overall, 1480 patients (66.5%) had specific *H. pylori*-related symptoms/clinical conditions. The prevalence was significantly lower in BA than in ALT (63.4% vs. 98.0%), but a gender difference (i.e. higher prevalence in females than males) was observed in both centers. Among subjects referred for UBT in BA, 6.1% had *H. pylori*-unrelated symptoms and 8.3% had a family history of *H. pylori* infection.

Prevalence of *H. pylori* infection

Overall, a total of 723 patients were positive for *H. pylori* infection (adding up both naïve patients and patients referred after one prior eradication regimen in both centers). The overall infection rate was 32.5%. The detailed data (Table III) show that the prevalence of *H. pylori* infection in the post-eradication group was lower in BA than in ALT (32.5% vs. 47.9%, P=0.0074). The rate of *H. pylori* infection in the 185 patients with a family history of *H. pylori* infection was 34.1% (63/185).

Table II. Clinical profile of the 2,224 patients referred for diagnostic test, according to the referral center

	Bari (BA)	Altamura (ALT)	P value^
Patients n (%)	2023	201	
H. pylori testing*			
Naïve	1,201 (59.4%)	128 (63.7%)	0.56
Females	711 (59.2%)	76 (59.4%)	0.89
Males	490 (40.8%)	52 (40.6%)	0.89
	(P=0.01)	(P=0.01)	
Post-eradication	822 (40.6%)	73 (36.3%)	0.56
Females	508 (61.8%)	40 (54.8%)	0.39
Males	314 (38.2%)	33 (45.2%)	0.32
	(P=0.001)	(P=ns)	
Asymptomatic	616 (30.4%)	4 (2.0%)	0.0000
Females	338 (54.9%)	2 (50%)	0.48
Males	278 (45.1%)	2 (50%)	0.48
	(P=ns)	(P=ns)	
Symptomatic	1,407 (69.6%)	197 (98.0%)	0.0000
Females	886 (63.0%)	114 (57.9%)	0.47
Males	521 (37.0%)	83 (42.1%)	0.47
	(P=0.0002)	(P=0.02)	
H. pylori - related symptoms	1,283 (63.4%)	197 (98.0%)	0.0000
Females	816 (63.6%)	115 (58.4%)	0.47
Males	467 (36.4%)	82 (41.6%)	0.39
	(P=0.0002)	(P=0.02)	
H. pylori - unrelated symptoms	124 (6.1%)	0	
Females	68 (54.8%)	0	
Males	56 (45.2%)	0	
	(P=ns)		
Family history of *H. pylori* infection	185 (8.3%)	0	
Females	88 (47.6%)	0	
Males	97 (52.4%)	0	
	(P=ns)		

*subjects entering the referral centers to confirm a suspicion of *H. pylori* infection; ^ significant difference for P <0.05

Table III. Prevalence of *H. pylori* infection according to referral center, indication for *H. pylori* testing, symptoms, and family history

BARI	Reason for referral		
	Naïve (n=1,201)	Post eradication* (n=822)	P value^
H. pylori infection n (%)	384 (32.0%)	267 (32.5%) **	0.9
Asymptomatic (n=616)	101/297 (34.0%)	88/319 (27.6%)	0.29
Symptomatic			
H. pylori -related symptoms (n=1283)	263/825 (31.9%)	172/458 (37.6%)	0.56
H. pylori -unrelated symptoms (n=124)	20/79 (25.3%)	7/45 (15.6%)	0.56
ALTAMURA	Reason for referral		
	Naïve (n=128)	Post eradication* (n=73)	P value^
H. pylori infection n (%)	37 (28.9%)	35 (47.9)**	0.005
Asymptomatic (n=4)	-	0/4 (0%)	
Symptomatic			
H. pylori -related symptoms (n=197)	37/128 (28.9%)	35/69 (50.7%)	0.001
H. pylori -unrelated symptoms (n=0)	-	-	

* at least one prior eradication regimen; **significantly different between BA and ALT (P= 0.0074); ^ significant difference for P < 0.05

Overall, the infection rate was 31.1% in asymptomatic subjects, 34.3% in patients with -, and 21.8% in patients without *H. pylori*-related symptoms.

Eradication rates according to the different therapeutic regimens and outcome with Pylera®

As shown in Table IV, the eradication therapy (any type) was performed in a total of 987 subjects (i.e. already treated before referral, N=264; treated after referral with sequential/ Pylera, N=723). In the prospective part of the study, recorded drop-outs were 3, 0, and 2 for sequential, levofloxacin, and Pylera®, respectively.

Table IV. Outcomes of different eradication regimens

	Treated (n)	Eradicated n [% (95% CI)]^
Total	987	792
		PP [80.7% (95% CI 75.1-86.5)]
		ITT [80.2% (95% CI 74.8-86.0)]
1st-line (all)	725	552 [76.1% (95% CI 72.9-79.1)]
Sequential	191	170
		PP [89.9% (95% CI 83.7-92.7)]
		ITT [89.0% (95% CI 76.1-100)]
Triple-7d	92	65 [70.7% (95% CI 60.0-78.9)]
Unconventional	47	28 [59.6% (95% CI 45.3-72.3)]
Undefined*	395	289 [73.2% (95% CI 68.5-77.3)]
2nd-line (all)	35	
Levofloxacin	35	20 [57.1% (95% CI 40.8-72.0)]
Bismuth subcitrate potassium (Pylera®, all)	227	220
		PP [97.8% (95% CI 85.3-100)]
		ITT [96.7% (95% CI 84.5-100)]
1st-line	85	85 [100% (95% CI 95.7-100)]
2nd-line	118	116
		PP [99.1% (95% CI 81.9-100)]
		ITT [98.3% (95% CI 81.2-100)]
3rd-line	13	12
		PP [100% (95% CI 51.7-100)]
		ITT [92.3% (95% CI 47.7-100)]
4th-line	9	5 [55.6% (95% CI 18.0-100)]
5th-line	2	2 [100% (95% CI 34.2-100)]

*no definitive information about exact therapeutic regimen; ^ in each therapeutic subgroup within each line regimen (see Figure 1).

The percentage of treatments was 73.5% (1st line), 3.5% (2nd line levofloxacin) and 23.0% (Pylera® 1st-5th line). A total of 792 patients were successfully eradicated, with an overall eradication rate of 80.2% (ITT population) (Table IV).

First line regimens (excluding regimens with bismuth subcitrate potassium) induced an overall eradication rate of 76.1% as ITT (95% CI 72.9-79.1). In this subgroup of patients, the highest eradication rate (89.0%, ITT) was achieved with the sequential regimen (prescribed prospectively), an intermediate eradication rate (70.7% and 73.2%) was achieved, respectively, with the triple (7-day) and undefined (likely triple 7-day) regimens, while the lowest eradication rate (59.6%) was observed with other unconventional regimens. Second line regimen with levofloxacin yielded an eradication rate of 57.1%.

Pylera® was prospectively administered in a total of 227 patients and yielded an eradication rate of 96.7% (ITT population) with no eradication in 5 cases (1 patients on 2nd- and 4 patients on 4th-line treatment), and 2 drop-outs (Fig. 1).

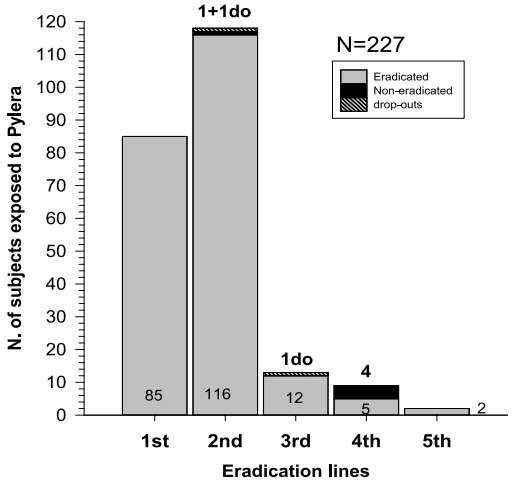

Fig. 1. Outcome of eradication treatment in the 227 *H. pylori* positive patients exposed to Pylera® (for the regimen, see Methods). Bars indicate the absolute numbers of eradicated subjects, non-eradicated subjects and drop-outs (do) at each line of treatment.

Overall, minor side effects were recorded in 5% of the patients on eradication therapies: bloating during sequential therapy, and bloating, mild abdominal pain, nausea, vaginal candidosis, transient urticaria during treatment with Pylera®. Metallic taste and/or dark stools were observed on Pylera® starting from day 2 but the finding was not classified as a side effect.

DISCUSSION

Over the years, expert panels have proposed a number of guidelines [5, 25-27], which target populations and subgroups of patients with *H. pylori* infection. However, guidelines can vary according to geographical areas, increasing clarithromycin resistance rate, economical considerations and access to health care. With respect to the management of patients with *H. pylori* infection, the results from the present study suggest that in a "real life" scenario and in a well-defined and limited geographical context, several factors play a critical role. Factors include difference in management, available diagnostic techniques, physicians and patients' preferences and available standard and novel eradication regimens.

Additionally, as in all infectious diseases, also in the case of *H. pylori* infection the best therapeutic outcomes are achieved when the antimicrobial treatment is susceptibility-based.

In our series, females were generally more represented than males irrespective of diagnostic technique or referral center, and symptomatic subjects were more represented in the female than in the male subgroup. A previous study found that females report several physical symptoms more frequently than males

[28] and, according to our results, this is also the case for *H. pylori*-related symptoms.

Asymptomatic subjects without a history of peptic ulcer disease should not be routinely tested for *H. pylori* infection [5], unless a family history or fear of gastric carcinoma occurs or in the case of patients belonging to populations where the incidence of gastric cancer is increased [29, 30]. In our series, about one third of enrolled subjects with a family history of *H. pylori* infection resulted positive to diagnostic tests. Irrespective of a family history of *H. pylori* infection, a comparable infection rate (31.1% and 34.3%, respectively) was observed between asymptomatic and symptomatic subjects. Subjects with symptoms/clinical conditions not specifically related with *H. pylori* infection showed an infection rate only slightly lower (21.8%) than that recorded in the other two subgroups of patients. Thus, the presence and the characteristics of symptoms/clinical conditions seem not to be sufficient to discriminate subjects infected with *H. pylori*. These results are in line with previous evidences documenting the lack of significant associations between *H. pylori* infection and gastrointestinal symptoms both in children with non-ulcer dyspepsia [31, 32], and in adults [33, 34]. As a consequence, as also suggested by the Working Group of the WHO IARC and considering the easy availability of noninvasive tests, it might be recommended that countries evaluate the possibility of introducing population-based *H. pylori* screening and treatment programs on the basis of a correct evaluation of local factors such as the disease burden and cost-effectiveness analysis [2]. Population *H. pylori* screening and treatment strategies may therefore become cost-effective, at least in selected populations [35-38].

In the present series, naïve subjects had, overall, a prevalence of *H. pylori* infection (31.7%) similar to that observed in patients who had undergone previous eradication therapies (33.7%), pointing to a possible inefficacy/inappropriateness of previous treatments.

Effective *H. pylori* eradication therapy can save between 750,000 USD and 1,000,000 USD per year per million inhabitants in western Europe, as compared to maintenance or episodic therapy [39]. As a consequence, inappropriate therapy for *H. pylori* infection (mainly first-line eradication treatment) could lead to increased health costs [1]. The global expenditure, furthermore, might increase due to repeated post-treatment testing to confirm eradication [40]. Thus, adherence to current guidelines improves both outcomes and the final cost of *H. pylori* management [1].

From this point of view, one of the most popular first-line eradication treatments in our region (externally to the two referral centers) was still the PPI-clarithromycin-based triple 7-day treatment. However, as expected in an area where clarithromycin resistance is quite high - approximately 30% in Italy [6, 13, 16, 41], and >15% in Apulia [7], the eradication rate was low and below what should be expected for an infectious disease [5, 42]. In this respect, some additional considerations are required: i) the rising prevalence of clarithromycin-resistant *H. pylori* in southern Europe [41, 43] and therefore in Italy [13], with regional differences [7], makes the PPI-clarithromycin-containing triple therapy unacceptable [5], at least in these geographical areas; ii) the duration of the PPI-clarithromycin-containing triple therapy was 7 days in our area, instead of 10 or 14 days, a policy aimed to further increase the success rate by another 4-5% [5] and possibly >80% [44, 45] and, iii) the majority of PPI-clarithromycin-containing triple therapy used the PPI omeprazole 20 mg twice daily, instead of the more potent second-generation PPIs, i.e. esomeprazole or rabeprazole 40 mg twice daily, which may increase the cure rates by 8-12% [5, 46].

In our series and, after enrollment, under the control of two experienced referral centers, the 10-day sequential treatment proved to be effective as first line regimen, suggesting that tinidazole resistance was uncommon in the explored geographical area. This finding confirms previous positive observations from our [9] and other groups [47, 48]. Although the sequential therapy has been considered by the 2012 Maastricht/Florence Consensus [5] and by the recent Italian Guidelines [13], the current guidelines indicate that the presence of clarithromycin resistance undermines the efficacy of this therapeutic approach [1]. However, in our series, the sequential therapy allowed a high eradication rate (89%), even though enrolled patients lived in a geographical area characterized by high clarithromycin resistance. This outcome was probably due to the presence of tinidazole-susceptible *H. pylori* strains [1, 49, 50]. Evidence suggests that the sequential therapy besides being highly effective (91.1% and 92.1% eradication rates at ITT and PP analysis) [9], can overcome the issue of clarithromycin resistance [47].

Moreover, the final success is similar to the 14-day triple regimen [10, 51]. The so-called 10-day "concomitant" therapy (non-bismuth quadruple), although representing an option as first line therapy [10, 52], has been used by our group in a previous trial [9] with eradication rates of 85.5% (ITT) and 91.6% (PP) but was not used in the present study.

Despite the high eradication rate recorded in the present series, the sequential therapy should not be recommended, since it is not considered in current guidelines and clarithromycin is a completely unneeded antibiotic, depicting this practice as an antimicrobial misuse able to promote resistance.

As far as the second line treatment is concerned, this study found that, outside of the referral centers, the PPI-clarithromycin-containing triple therapy had been used as second, third or even fourth line treatment. This approach is surprising and is not in line with the clear recommendations of Maastricht/Florence Consensus [5] and the Italian guidelines [13], which suggest the use of levofloxacin-containing triple therapy or, if available, the bismuth-containing quadruple therapy. Indeed, the first option with levofloxacin was chosen by the two referral centers involved in the study before the marketing of Pylera® (see below). Although the use of levofloxacin-containing triple therapy has been endorsed in Italy [53], we confirmed a rather low eradication rate. It should be emphasized that primary levofloxacin resistance is being described in Italy, a trend which can negatively affect the efficacy of this regimen [54]. The local unavailability of antimicrobial susceptibility testing, furthermore, did not permit selecting additional antibiotic regimens, including rifabutin (which is characterized by increased costs and additional side effects).

Bismuth subcitrate potassium (Pylera®), recently introduced in the Italian market, has been recommended by the current Maastricht Guidelines in the first-line as well as in the second line after failure of another regimen. However, this drug became available in Italy only from March 2016, and data on its efficacy in southern Italy are still scarce. Our data confirm the high eradication rates with Pylera®, which seems able to overwhelm the limits linked to antibiotic resistance in the explored geographical area, characterized by a clarithromycin resistance rate higher than 15% [6, 7, 13, 16, 41].

The eradication rate (96.9%, ITT analysis) obtained by treatment with Pylera® in the present series of patients from Southern Italy was higher than that reported by previous international studies [12, 51, 55], but similar to that reported by another recent Italian study both in patients on first treatment and in rescue therapy [56], confirming the elevated therapeutic efficacy of regimen with bismuth subcitrate potassium in this specific geographical area.

Both the European guidelines and Italian guidelines suggest this regimen as a second line option [5, 13]. The eradication rate is significantly better than the levofloxacin-based triple regimen, an accepted alternative as second line treatment. We found cases treated with 7-day PPI-clarithromycin-based triple treatment as second line, even if guidelines discourage this approach. As expected, the quadruple therapy was better than the triple therapy.

An additional observation, in our series, is that the bismuth-containing regimen was also highly effective as a first line treatment. This finding is in line with the results of previous important studies [8, 20], and is a valid alternative to standard 14-day PPI-clarithromycin-based triple treatment or 10-day sequential therapy or 10-day concomitant therapy [13].

Both sequential and quadruple therapies have similar safety and tolerability to standard therapy but provide superior eradication rates. Our data also indicate that recommendations of European and Italian guidelines allowed optimal eradication rates in our areas, and therefore the final decision of treatment depends on the best available therapeutic option and on the patient's choice [57].

CONCLUSIONS

The present study contributed to depict a "real life" scenario in Southern Europe and showed that heterogeneous and empiric managements put *H. pylori*+ve patients at risk of poor outcomes, pointing to the need of a susceptibility-based therapy based on local microbial resistance.

Although results are specific for the region where the study was performed, there is no place for empiric therapy for *H. pylori* infection, unless the choice is based on knowledge of local susceptibility patterns obtained by culture or, in alternative, by a careful monitoring of treatment success. In the described setting, indications from current guidelines were only partly observed. The choice greatly depended on specific characteristics of patients (age, gender, symptoms, familiarity for *H. pylori* infection), on local availability of diagnostic techniques (i.e. invasive or noninvasive tests), and unexplained personal choices in family medicine. The implementation of educational and collaborative programs might minimize this gap and the risk of raised health costs, poor eradication rates, and inappropriate antibiotic use. The bismuth-containing regimens (i.e. quadruple therapy) has been able to ensure the highest eradication rates. Furthermore, the observation that sequential therapy yields high eradication rates does not necessarily support the use of a regimen not reported in the current guidelines and leading to antibiotic misuse and resistance.

Conflicts of interest: None declared.

Authors' contribution: A.DC. was responsible for data collection, data analysis, writing and editing the manuscript. G.S. was responsible for endoscopic and UBT analyses and data collection. M.V. and A.Z. provided critical revision of the manuscript. T.R. provided statistical support and revision of the manuscript. L.B. edited the manuscript. P.P. was responsible for the design of the study, data collection, data analysis and interpretation, critical revision of the manuscript and drawing of the figure.

Acknowledgements: The authors are indebted to Rosa De Venuto, Paola De Benedictis, Michele Persichella, Ornella de Bari for their skillful technical assistance, and to Francesco Conte for the initial data collection, as part of his Degree Thesis at the University of Bari Medical School.

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