Empirical Second-Line Therapy in 5,000 Patients of the European Registry on Helicobacter pylori Management (Hp-EuReg)

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Short title: Empirical second-line *H. pylori* therapy

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Conflict of interest statements

Dr. Gisbert has served as speaker, consultant, and advisory member for or has received research funding from Mayoly, Allergan, Diasorin, Gebro Pharma, and Richen. Dr. Nyssen has received research funding from Mayoly and Allergan. Dr. Pérez-Aisa has received compensation from Allergan and Mylan for formative actions. Dr. Jonaitis has served as speaker for KRKA. Dr. Lanas has served as consultant to Bayer A.G. The remaining authors have declared no conflict of interest.

Data Transparency Statement

Raw data were generated at AEG-REDCap. Derived data supporting the findings of this study are available from the first and senior-corresponding author (OPN and JPG) upon request.

Data Sharing Statement

The data that support the findings of this study are not publicly available given that containing information could compromise the privacy of research participants. However, previous published data on the Hp-EuReg study, or de-identified raw data referring to current study, as well as further information on the methods used to explore the data could be shared, with no particular time constraint. Individual participant data will not be shared**.**

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Abbreviations: A, amoxicillin; AEG, Asociación Española de Gastroenterología; B, bismuth salts; C, clarithromycin; CI, confidence interval; eCRF, electronic case report form; *H. pylori*, *Helicobacter pylori;* Hp-EuReg, European Registry on *H. pylori* Management; ITT, intention-to-treat; L, levofloxacin; M, metronidazole; mITT, modified intention-to-treat; Mx, moxifloxacin; OR, odds ratio; PP, per-protocol; PPI, proton pump inhibitor; R, rifabutin; REDCap, Research Electronic Data Capture; SD, standard deviation; T, tinidazole; Tc, tetracycline.

Author contributions

Olga P. Nyssen, Hp-EuReg Scientific Director, designed the protocol and planned the study, performed the data extraction, the monitoring and the quality check, analyzed and synthesized the data, wrote the manuscript draft, and approved the submitted manuscript.

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Colm O'Morain, Francis Mégraud, Olga P. Nyssen and Ignasi Puig are Members of the Hp-EuReg Scientific Committee; they assisted with data interpretation, critically reviewed the manuscript's drafts, and approved the submitted manuscript.

Javier P. Gisbert, Principal investigator, directed the project, obtained funding, designed the protocol and planned the study, analyzed and interpreted the data,

recruited patients, critically reviewed the manuscript drafts, and approved the final submitted manuscript.

Writing Assistance

Catherine Rees of Springer Healthcare Communications provided assistance for the English editing of the manuscript prior to submission.

Abstract

BACKGROUND & AIMS: After a first *Helicobacter pylori* eradication attempt, approximately 20% of patients will remain infected. The aim of current study was to assess the effectiveness and safety of second-line empirical treatment in Europe.

METHODS: This international, multicenter, prospective non-interventional Registry aimed to evaluate the decisions and outcomes of *H. pylori* management by European gastroenterologists. All infected adult cases with a previous eradication treatment attempt were registered at AEG-REDCap up to February 2021. Patients allergic to penicillin and those having received susceptibility-guided therapy were excluded. Data monitoring was performed to ensure data quality.

RESULTS: Overall, 5,055 patients received empirical second-line treatment. Triple-therapy with amoxicillin and levofloxacin was most commonly prescribed (33%). The overall effectiveness was 82% by modified intention-to-treat analysis and 83% in the per-protocol population. After failure of first-line clarithromycin-containing treatment, optimal eradication (>90%) was obtained with moxifloxacin-containing triple-therapy or levofloxacin-containing quadruple-therapy (with bismuth). In patients receiving triple-therapy containing levofloxacin or moxifloxacin, and levofloxacin-bismuth quadruple-treatment, cure rates were optimized with 14-day regimens using high doses of proton pump inhibitors. However, three-in-one single capsule or levofloxacin-bismuth

quadruple-therapy produced reliable eradication rates regardless of proton pump inhibitor dose, duration of therapy, or previous first-line treatment. The overall incidence of adverse events was 28%, and most (85%) were mild. Three patients developed serious adverse events (0.3%) requiring hospitalization.

CONCLUSION: Empirical second-line regimens including 14-day quinolone triple-therapies, 14-day levofloxacin-bismuth quadruple-therapy, 14-day tetracycline-bismuth classical quadruple therapy, and 10-day bismuth quadruple-therapy (as single capsule) provided optimal effectiveness. However, many other second-line treatments evaluated reported low eradication rates; ClincialTrials.gov number, NCT02328131.

Keywords: bismuth, *Helicobacter pylori*, clarithromycin, levofloxacin, rescue.

INTRODUCTION

Helicobacter pylori infection affects over 50% of the population worldwide and represents a significant health burden. This infection is the leading cause of gastritis, peptic ulcer disease, and gastric cancer. However, although the bacterium was discovered in 1982, the optimal eradication treatment remains undefined.[1]

The most commonly used first-line therapy contains a proton pump inhibitor (PPI) plus two antibiotics (usually amoxicillin and clarithromycin or metronidazole), but this regimen fails to eradicate the bacteria in at least 20–30% of cases.[2] Alternative regimens, such as bismuth-containing quadruple-therapies (PPI, bismuth, tetracycline, and metronidazole) or non-bismuth quadruple regimens (PPI, clarithromycin, amoxicillin, and metronidazole administered either sequentially or concomitantly) are more effective,[3, 4] and generally recommended as first-line therapies when resistance to clarithromycin is over 15%, which is currently the case in most European countries.[5] However, even after these quadruple regimens, a considerable number of patients will have persistent *H. pylori* infection.

A major reason for treatment failure is acquired antibiotic resistance, and the rate of resistance to clarithromycin or quinolones has been gradually increasing in many parts of the world.[5] Bacterial strains surviving an eradication attempt become less susceptible to subsequent therapies either through the selection of resistant bacteria or the acquisition of *de novo* resistance.[6] As a result, the choice of a correct rescue treatment depends largely on the previous exposure

to antibiotics, especially those used in previous *H. pylori* eradications attempts.[2]

Ideally, the choice of second-line treatment would be guided by the results of antimicrobial susceptibility testing, but culture is generally unavailable in routine clinical practice.[7] Moreover, access to the optimal eradication strategy based on culture and susceptibility testing may also be hampered by the need for endoscopy, higher costs, or the time required for testing and culture.[8] Thus, there is a need to optimize empirical treatment.[9]

Currently, there is no optimal strategy to cure *H. pylori* infection in clinical practice, and available data, mainly for rescue therapies, often come from small studies with a limited number of patients in specific geographic locations. To address these gaps, the European Registry on *Helicobacter pylori* Management (Hp-EuReg) was designed to collect information on the real-world clinical practice among 30 European countries.[10] The philosophy of the project was to audit the patients' outcomes, compare current treatments with those recommended in current guidelines, detect the room for improvement, and subsequently change the routine clinical practice. Thus, the registry represents a valuable overview of current *H. pylori* management allowing continuous assessment for improvement through observation of treatment evolution.

The present study was a sub-analysis of this large-scale international multicenter prospective registry that aimed to assess the prescription patterns, the effectiveness, and the safety of empirical second-line rescue therapies used in the management of *H. pylori* in Europe.

METHODS

The Hp-EuReg is an international multicenter prospective non-interventional registry recording information of *H. pylori* infection management since 2013. Detailed information on the data collection, data management, effectiveness, safety and compliance analyses are reported in the published protocol,[10] and is summarized in Supplementary file 2.

The principal effectiveness analysis taken into account in current study was a modified intention-to-treat (mITT) aiming to reflect the closest results of the clinical practice. The mITT included all patients who had completed follow-up (i.e. confirmatory test –success or failure– available after treatment), regardless of compliance.

All authors had access to the study data and reviewed and approved the final manuscript (further information in the Authors' contribution section).

RESULTS

Baseline characteristics

Overall, 41,562 patients were registered until February 2021. Of these, 5,932 had received a second-line rescue therapy, and 5,055 cases (12%) from 27 countries (Supplementary Table 1) were treated empirically and included in present analysis (Figure 1). Further information is presented in Supplementary file 3.

Most frequent prescriptions in second-line therapy

In total, 87 second-line treatments were registered (Supplementary Table 2); however, only the most frequent ones were analyzed: PPI+A+levofloxacin (33%), PPI+bismuth+M+T as single capsule (17%), and PPI+A+levofloxacin+bismuth (13%) (Table 1). These therapies were mostly prescribed (i.e., in 78% of cases) after failure of a clarithromycin-containing first-line regimen. The other usual antibiotics used in first-line treatment, such as amoxicillin or metronidazole, were used in 79% and 24% of the rescue therapy cases, respectively.

Evolution of second-line treatment during the study period

A decrease in the use of triple regimens was observed in the 2013-2020 period: PPI+A+levofloxacin decreased from 57% to 21%; PPI+A+moxifloxacin was prescribed mainly between 2013 and 2016, but not used in the last 4 years.

Also, the PPI+C+A standard triple-therapy decreased from 12% to 9%. On the other hand, the PPI+bismuth+M+T in the standard form decreased from 9% to 6%, whereas the single capsule therapy version increased from 0% in 2013 to 51% in 2018 and decreased again to 37% in 2020. Similarly, PPI+A+levofloxacin+bismuth increased from 0.6% in 2013 to 20% in the 2015/2016 period, but decreased to 14% in 2017 and increased again up to 26% in 2020 (Figure 2).

A progressive increase in the duration of treatments was also noted from a mean (SD) of 10.8 (2.2) days in 2013, to 12.2 (2.3) days in 2020. Also, the use of longer treatment durations (14 days) increased from 29% in 2013 to 55% in 2020. Likewise, the highest potency of acid inhibition varied over time from an omeprazole mean (SD) dose equivalent of 35 mg (21) in 2013 to 41 mg (21.3) in 2020; and the use of high-dose PPIs increased from 29% to 43%.

Effectiveness of second-line treatment

Overall effectiveness of empirical second-line therapy was reported as 84% (95% CI 82%-84%) by mITT. Optimal effectiveness was reached with PPI+A+moxifloxacin (91%) and with PPI+bismuth+M+T as single capsule (90%). PPI+A+levofloxacin+bismuth and PPI+C+A+bismuth also achieved cure rates (88% and 87%, respectively) near the desired optimal threshold of 90% (Table 2).

Additionally, the analysis of the evolution of the effectiveness showed that cure rates with PPI+A+moxifloxacin constantly remained above 90%. The same was true for PPI+bismuth+M+T except in 2015, when the eradication rate was

reported as 80% (only 20 patients treated) (Figure 2).

Effectiveness after failure of a clarithromycin-containing regimen

After a clarithromycin-containing first-line treatment attempt, optimal rates of eradication were reported with PPI+A+moxifloxacin (91%), PPI+A+levofloxacin+bismuth (89%), and with 10-day PPI+bismuth+M+T as the single capsule (89%) (Table 3).

In the same scenario, further post-hoc analyses were performed to compare the overall effectiveness in regimens with and without bismuth in the two following groups: 1) PPI+C+A vs. PPI+C+A+bismuth and 2) PPI+A+levofloxacin vs. PPI+A+levofloxacin+bismuth. Significant differences were reported between both of the treatment schemes for each comparison; obtaining in both cases, higher mITT effectiveness when bismuth was added: 1) 24% vs. 87%, $p<0.001$; and 2) 80% vs. 89%, $p<0.001$; respectively.

Suboptimal effectiveness (<90%) was observed with all 7-day regimens (triple or quadruple) and most of the 10-day triple regimens; the exception was 10-day PPI+A+moxifloxacin, which achieved a cure rate of 100%. Therapy with 14-day PPI+A+levofloxacin also reported optimal cure rates (91%). When bismuth was added to this same 14-day combination, the effectiveness remained optimal, but no increase was reported (90%) (Table 4 and Supplementary Table 3).

Almost all second-line treatments studied (i.e., with available data) were more effective when high-dose PPIs were used, ranging in overall effectiveness from 89% to 100% (Table 4). Additionally, treatment with PPI+A+moxifloxacin,

PPI+C+A+bismuth, and PPI+bismuth+M+T (in the standard form) reported optimal cure rates with standard-dose PPIs (100%, 100%, and 90%, respectively). Treatment effectiveness with PPI+bismuth+M+T (single capsule) was always optimal independently of the PPI dose or the regimen (triple or quadruple) used previously (Supplementary Table 4).

Additionally, the effectiveness of PPI+A+levofloxacin, PPI+A+levofloxacin+bismuth, and PPI+C+A+M was higher (>90%) when prescribed for 14 days and with high-dose PPIs (Supplementary Table 5).

Effectiveness after failure of a bismuth-containing regimen

After a first-line bismuth-containing quadruple-therapy (PPI+bismuth+M+T) attempt, re-treatment with 10-day PPI+bismuth+M+T (single capsule) or with 10-day PPI+C+A+bismuth both achieved 94% eradication (Table 3, Table 4). The reported effectiveness of 14-day PPI+A+levofloxacin+bismuth was also high (87%).

Optimal eradication rates were obtained with both 10-day PPI+bismuth+M+T (single capsule), regardless of the PPI dose, and with PPI+A+C+M when prescribed with high-dose PPIs, reporting cure rates of nearly 90% (Table 4). Additionally, 10-day PPI+C+A+bismuth (with low-dose PPIs) and 14-day PPI+A+levofloxacin+bismuth (with either low- or high-dose PPIs) both reached optimal effectiveness (Supplementary Table 6); no data were available for these regimens using standard-dose PPIs.

Multivariate analysis

Compliance was the independent factor most closely associated with higher mITT eradication rate (OR, 3.01; 95% CI, 1.78–5.08). A significant association towards a higher effectiveness was also obtained in patients with peptic ulcer disease (compared with patients who had uninvestigated or functional dyspepsia) (OR, 1.28; 95% CI, 1.01–1.61; $P<0.05$); in patients receiving 14-day regimens (OR, 2.84; 95% CI, 1.94–4.08; $P<0.001$); and in those receiving high-dose PPIs (OR, 2.21; 95% CI, 1.77–2.75; $P<0.001$) (Table 5).

Also, prescribing either triple-therapy with quinolones (levofloxacin or moxifloxacin) or PPI+A+levofloxacin+bismuth quadruple-therapy was associated with higher mITT eradication rate; moreover, a higher association was found when PPI+bismuth+M+T (either in the standard form or with the single capsule) was used (OR, 6.30; 95% CI, 4.41–8.95; $P<0.001$). Additionally, we could observe that any treatment choice (from those included in the category 'other') except PPI+C+A was also preferable as second-line therapy; although the latter was associated to a lower eradication rate than the other reported categories.

Finally, the multivariate analysis showed that use of clarithromycin in the previous first-line treatment eradication attempt was associated with lower eradication rate with the second-line treatment (OR, 0.60; 95% CI, 0.48–0.75); $P<0.001$).

Safety of second-line treatment

The overall incidence of adverse events was 28% (95% CI, 27%–29%), although the majority were mild (85%) and of short duration (mean 6.6 days).

Further information on the safety of treatments is reported in Supplementary file 4 and Supplementary Table 7.

DISCUSSION

H. pylori treatment failure can occur due to diverse factors, but mainly due to primary or acquired bacterial antibiotic resistance (specifically to clarithromycin and metronidazole, and more recently also to levofloxacin).[5,6] Antibiotic resistance (which varies between countries in relation to antibiotic utilization) has become an important hurdle to overcome, particularly in rescue therapy, where 90% effectiveness is also demanded.[14, 15]

In our study, the overall effectiveness of second-line empirical treatment was below 90%. Treatment with PPI+A+levofloxacin was the most widely prescribed (33%) in Europe after a failed attempt with clarithromycin; however, its overall effectiveness was clearly suboptimal (81%), unless prescribed for 14 days, which provided acceptable cure rates (91%). Triple regimen with 10- or 14-day PPI+A+moxifloxacin (although prescribed in just 3% of cases) reported encouraging 90% effectiveness. Thus, only 14-day triple regimens with quinolones (either levofloxacin or moxifloxacin), showed acceptable cure rates (91% and 96%, respectively). In fact, several studies have shown optimal results with extended, optimized 14-day PPI+A+levofloxacin,[16, 17] and so are 14-day regimens currently recommended, unless shorter therapies are proven effective locally.[1, 5, 14]

Furthermore, effectiveness increased above 90% when high-dose PPIs was used in combination with longer treatment durations (i.e., 14 days), in accordance with previously published research.[1, 8, 9, 15, 18]

Bismuth was added to levofloxacin+A triple therapy in 13% of our patients, as

recommended in the last European Consensus guidelines,[1] and reported effectiveness was indeed significantly higher as compared to the triple therapy with levofloxacin (without bismuth), achieving 89% vs. 80% ($p<0.001$) cure rates, in line with previous studies.[9, 19-21]

After failure of a first-line regimen (triple or quadruple) with clarithromycin, another recommended rescue treatment is a bismuth-based quadruple-therapy with metronidazole and tetracycline.[1, 22] In our study, 10-day PPI+bismuth+M+T as single capsule was the second most frequently used treatment (17% of cases), and reported ~90% effectiveness, regardless of the PPI dose. A recent update on this 10-day treatment with the single capsule over 5,000 patients of the Hp-EuReg confirmed excellent cure rates, not only in first-line but also in second-line treatment, achieving 90% eradication.[23] Additionally, a previous meta-analysis showed similar results with single capsule bismuth quadruple-therapy, reporting high effectiveness in naïve patients and in subsequent rescue treatment lines (including those with bacterial resistance to clarithromycin or metronidazole, or both).[24]

The bismuth compound exhibits an antibacterial effect that prevents *H. pylori* colonization and adherence to the gastric mucosa, reducing the bacterial load.[9] This compound, therefore, has a synergistic effect with antibiotics, with no resistance described.[25] Adding bismuth to either triple- or quadruple-therapy may further enhance effectiveness and overcome bacterial antibiotic resistance.[19, 26, 27] Such a strategy of adding bismuth to different antibiotic combinations may explain the increase in the eradication rates of rescue treatments used in our cohort, in spite of first-line treatment failure with

clarithromycin. Such is the case of quadruple therapy with 14-day PPI+C+A+bismuth, where a cure rate of 87% was reported, significantly higher as compared to standard 14-day PPI+A+C regimen (which obtained 24% eradication rate only). This latter example, showed greater differences (with respect to other 'with vs. without' bismuth comparisons, such as PPI+A+levofloxacin vs. PPI+A+levofloxacin+bismuth) due, probably, not only to the beneficial effect of adding bismuth to the regimen but also to the repeated use of clarithromycin in second-line treatment after a failed first-line use.[2]

Also, in our study, re-treatment with 10-day PPI+bismuth+M+T (single capsule) achieved 94% eradication. It has been stated elsewhere[24] that re-treating with the single capsule is feasible given that the potential acquired bacterial resistance to tetracycline or bismuth would be minor (<3%)[28] and that resistance to metronidazole can be easily overcome.

However, after a first failed eradication attempt with PPI+bismuth+M+T, the recommended treatment is PPI+A+levofloxacin+bismuth,[1] as it is suggested not to repeat antibiotics[2] (the overall effectiveness was always <90% when repeating antibiotics[29]). In line with this, in our study, 14-day PPI+A+levofloxacin+bismuth reported ~90% effectiveness.

Additionally, prescribing clarithromycin in a quadruple regimen (with amoxicillin and bismuth) might also be an option, although there is still limited experience as rescue treatment.[9, 30] In the studied cohort, 10-day PPI+C+A+bismuth was used in a relatively small proportion of patients (5%), achieving 94% effectiveness, and confirming previous encouraging results.[30]

These results were reinforced in the multivariate analysis, where longer

treatment durations and higher PPI acid inhibition were significantly associated with higher effectiveness, as previously reported.[2, 9] Also, in our study, previous use of clarithromycin in first-line therapy was associated with a risk of second-line treatment failure; in fact, those prescribing clarithromycin after a clarithromycin failure, reported cure rates far below 90%. Indeed, repeating antibiotics resulted inadequate, as confirmed both in Europe and in the US.[2, 5, 31] Better outcomes were also confirmed with 14-day quinolone triple-therapies (also when combined with bismuth into quadruple regimens) and 10-day bismuth quadruple-therapy (either in the classical form or as single capsule).

Regarding safety, our data reported at least one .adverse event in a relatively high proportion of patients (28%). The most frequent adverse events, including diarrhea (10%), nausea (9%), or metallic taste (5%), were of mild intensity and short duration (self-limited). These results were in accordance with those recently published in the study on the safety of *H. pylori* treatments in >22,000 patients from the Hp-EuReg.[2]

In general, the tolerability of quadruple-therapies was less than that of triple-therapies, in agreement with previous research.[32, 33] Quadruple-therapies, especially PPI+C+A+bismuth but also PPI+bismuth+M+T (either in the standard version or with the single capsule), were the most poorly tolerated. Regimens containing bismuth and levofloxacin were associated with a poorer tolerance compared with triple-therapy containing levofloxacin or moxifloxacin, also in accordance with the Hp-EuReg safety study.[34]

The major limitation of our study was that the empirical regimens in the studied cohort were heterogeneous; many treatments (>50) were prescribed to

fewer than 40 patients each, and therefore, these regimens could not be used for the sub-analyses by treatment duration or PPI dosage. To some extent, this reduced the amount of information available. Nonetheless, current analysis was made on the 10 most frequently used treatments, representing >90% of the study sample. Heterogeneity was inherent to the study design of the Hp-EuReg (i.e., observational, non-interventional) and therefore difficult to avoid, as wide selection criteria were initially established in order to reflect as much as possible real clinical practice. As an example, 85% of patients came from only five countries, and the majority of patients (54%) were from a single country (Spain), and this might introduce some selection bias. Therefore, comparisons of treatments should be interpreted with caution, because allocation biases may affect effectiveness.

Another point to highlight is that we did not include patients with culture testing, and therefore information on *H. pylori* antibiotic resistance was lacking; thus, no definite conclusions could be drawn about the effect of resistance on the choice and effectiveness of second-line therapy. However, this reflects real routine gastroenterology practice in Europe, where antibiograms are not performed on a routine basis and treatments are mainly empirically prescribed.[8]

However, we believe that our study has a number of strengths, based on the invaluable information of the Hp-EuReg. To our knowledge, the present study is the largest cohort of patients treated with second-line *H. pylori* eradication treatment. The large number of patients and wide range of treatment strategies, maximize the distribution and the representativeness of the population, which may counterbalance the potential heterogeneity. Finally, a high-quality method

has been used to register, store, manage, and monitor the data by using the Online Platform for Collaborative Research AEG-REDCap, which provides robustness and coherence to the data with programmed and real-time quality controls, queries, reports and statistics.

In conclusion, the overall effectiveness of empirical second-line *H. pylori* eradication treatment was, in general, below the desired threshold. Therefore, the use of some regimens should be reconsidered and new therapeutic strategies explored by European gastroenterologists. In this respect, the empirical second-line regimens providing optimal effectiveness included 14-day quinolone triple-therapies, 14-day levofloxacin-bismuth quadruple-therapy, 14-day tetracycline-bismuth classical quadruple therapy, and 10-day bismuth quadruple-therapy as single capsule.

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TABLES AND FIGURES

Table 1. Baseline characteristics of patients receiving *Helicobacter pylori* second-line empirical treatments.

	N=5,055
Mean (SD) age, years	50 (15)
Sex, n (%)	
Female	3,221 (64)
Indication, n (%)	
Dyspepsia	4,184 (83)
Ulcer disease	861 (17)
Unknown	10 (0.2)
Diagnostic method, n (%)	
Non-invasive	2,645 (52)
Invasive (required endoscopy)	2,410 (48)
Treatment length, n (%)	
7 days	224 (4)
10 days	2,648(53)
14 days	2,063 (41)
Unknown	120 (2)
Proton pump inhibitor dose, n (%)	
Low	1,707 (34)
Standard	1,106 (22)
High	2,106 (42)
Unknown	136 (3)

Compliance, n (%)	
No (<90% drug intake)	143 (3)
Yes (≥90% drug intake)	4,548 (90)
Unknown	364 (7)
Most frequent first-line regimens, n (%)	
Triple therapy	3,395 (67)
Conc (Non-bismuth quadruple)	637 (13)
Bismuth quadruple	367 (7.3)
Seq (Non-bismuth quadruple)	197 (3.9)
Single capsule*	162 (3.2)
Other	105 (2.1)
Dual therapy	123 (2.4)
Hybrid therapy (Non-bismuth quadruple)	23 (0.5)
Unknown	46 (0.9)
Most frequent first-line antibiotics, n (%)	
Amoxicillin	3,984 (79)
Clarithromycin	3,936 (78)
Metronidazole	1,200 (24)
Bismuth	506 (10)
Tetracycline	189 (3.7)
Levofloxacin	102 (2)
Most frequent second-line treatments, n (%)	
PPI+A+L	1,631 (33)
PPI+single capsule*	820 (17)
PPI+A+L+B	648 (13)

PPI+C+A	350 (7.2)
PPI+C+A+B	257 (5.3)
PPI+C+A+M	227 (4.6)
PPI+M+Tc+B	221 (4.5)
PPI+A+Mx	143 (2.9)
PPI+A+M	103 (2.1)
PPI+C+M	38 (0.8)
Seq-PPI+C+A+T	32 (0.7)
Quadruple-A+M+B	30 (0.6)
Other	< 30 (< 0.6)

A, amoxicillin; B, bismuth; C, clarithromycin; Conc, concomitant administration; L, levofloxacin; M, metronidazole; Mx: moxifloxacin; PPI, proton pump inhibitor; R, resistance; Seq, sequential administration; T, tinidazole; Tc, tetracycline.

*Three-in-one single capsule containing bismuth, tetracycline and metronidazole.

Low-dose PPI: 4.5–27 mg omeprazole equivalents (OE) twice daily (bid) (i.e., 20 mg OE bid), standard-dose PPI: 32–40 mg omeprazole equivalents bid (i.e., 40 mg OE bid), high-dose PPI: 54–128 mg omeprazole equivalents bid (i.e., 60 mg OE bid).

Table 2. Effectiveness, safety and compliance of common empirical second-line treatments.

	Effectiveness, N (%)						Adverse events, N (%)		Compliance ≥90%, N (%)	
	ITT		mITT		PP					
	N (%)	95% CI	N (%)	95% CI	N (%)	95% CI	N (%)	95% CI	N (%)	95% CI
Triple regimens										
PPI+A+L	1,594 (72)	70-74	1,441 (81)	79-83	1,421 (81)	79-83	1,492 (22)	20-24	1,483 (98)	97-99
PPI+C+A	332 (43)	38-48	250 (57)	51-63	244 (57)	50-63	332 (41)	36-47	332 (98)	96-100
PPI+A+Mx	141 (86)	80-92	135 (91)	86-96	135 (91)	86-96	141 (19)	12-26	140 (99)	95-100
PPI+A+M	96 (50)	39-60	87 (59)	48-69	87 (59)	48-69	94 (8.5)	2-15	93 (98)	93-100
PPI+A+Rf	29 (62)	43-81	23 (78)	56-92	23 (78)	56-92	28 (18)	6-37	28 (82)	63-94
PPI+C+L	12 (75)	43-94	10 (90)	55-99	10 (90)	55-99	12 (17)	2-12	12 (100)	74-100
Quadruple regimens										
PPI+single capsule*	781 (83)	80-86	750 (90)	88-92	738 (90)	88-92	780 (31)	28-34	780 (97)	96-98
PPI+A+L+B	606 (80)	77-83	560 (88)	86-91	543 (89)	86-91	569 (30)	26-33.5	12 (92)	62-100

PPI+M+Tc+B	217 (72)	66-78	192 (83)	77-88	185 (84)	79-90	221 (37)	30.5-44	212 (95)	92-99
PPI+C+A+B	243 (51)	44-57	154 (87)	81-93	149 (87)	81-93	244 (49)	42-55	248 (95)	92-98
Conc-PPI+C+A+M	217 (79)	74-85	213 (82)	77-87	208 (83)	77-88	222 (30)	24-36	220 (96)	94-99
Seq-PPI+C+A+T	32 (59)	41-78	29 (65.5)	46-84	29 (65.5)	46-84	32 (22)	6-38	31 (93.5)	79-99
Overall effectiveness										
All 2nd line treatments	4,856 (73)	72-74	4,322 (84)	82-84	4,241 (84)	83-85	4559 (28)	27-29	4535 (97)	(96-97.5)
Number of non-evaluable cases	199 (4)	3.4-4.5	733 (14.5)	13-15	814 (16)	15-17	496 (10)	9-11	520 (10)	(9-11)

A, amoxicillin; B, bismuth; C, clarithromycin; CI, confidence interval; Conc, concomitant administration; ITT, intention-to-treat; L, levofloxacin; M, metronidazole; mITT, modified intention-to-treat; Mx, moxifloxacin.; PP, per protocol; PPI, proton pump inhibitor; Rf, rifaximin; Seq, sequential administration; T, tinidazole; Tc, tetracycline.

*Single-capsule, three-in-one single capsule containing bismuth, tetracycline and metronidazole.

Table 3. Effectiveness of second-line therapy stratified by first-line regimen.

Second-line treatments	ITT		mITT		PP	
	N (%)	95% CI	N (%)	95% CI	N (%)	95% CI
After failure of clarithromycin-containing (triple or quadruple) first-line therapy						
Triple regimens						
PPI+A+L[1]	1,301 (73)	70-75	1,186 (80.5)	78-83	1,170 (81)	79-83
PPI+C+A[2]	160 (16)	10-22	107 (24)	16-33	105 (24)	15-32
PPI+A+Mx	60 (84.5)	75-94	66 (91)	83-98	66 (91)	83-99
PPI+A+M	69 (51)	38-63	65 (57)	44-70	65 (60)	44-70
PPI+A+Rf	21 (71)	48-89	18 (83)	58-96	18 (83)	59-97
PPI+M+L	17 (65)	38-86	15 (73)	45-92	14 (71)	42-92
PPI+C+M	15 (67)	38-88	13 (77)	46-95	13 (77)	46-95
PPI+C+L	7 (100)	59-100	7 (100)	NA	7 (100)	59-100
Quadruple regimens						

PPI+single capsule*	631 (82)	79-85	609 (89)	86-91	598 (89)	87-92
PPI+A+L+B[3]	465 (81)	77-85	432 (89)	86-92	416 (90)	86-92
PPI+M+Tc+B	116 (77)	69-85	110 (83)	75-90	106 (84)	76.5-91
PPI+C+A+B[4]	87 (72)	62-82	78 (87)	79-95	76 (88)	80-97
Conc-PPI+C+A+M	120 (81)	73-88	121 (82)	74-89	120 (82)	74-89
Seq-PPI+C+A+T	25 (64)	43-85	23 (70)	47-87	23 (70)	47-87
Overall effectiveness of second line regimens						
Overall	3,302 (74.5)	73-76	3,014 (83)	82-85	2,959 (84)	82-85
Number of non-evaluable cases	234 (7)	6-7.5	522 (15)	14-16	577 (16)	16-17.5
After failure of bismuth containing quadruple first-line therapy						
Triple regimens						
PPI+A+L	25 (60)	39-81	24 (67)	46-88	23 (65)	44-87

Quadruple regimens						
PPI+single capsule*	52 (88.5)	79-98	49 (94)	83-99	49 (93)	83-99
PPI+A+L+B	92 (77)	68-86	82 (88)	80-95	81 (89)	81-96
PPI+C+A+B	86 (31)	21-42	38 (76)	61-91	36 (75)	59-91
Conc-PPI+C+A+M	49 (80)	67-92	47 (85)	74-96	44 (89)	75-96
Overall effectiveness of second-line treatment						
Overall	349 (64)	59-69	275 (84)	79-88	267 (84)	80-88
Number of non-evaluable cases	30 (8)	5-11	104 (27)	23-32	112 (30)	25-34

A, amoxicillin; B, bismuth; C, clarithromycin; CI, confidence interval; Conc, concomitant administration; ITT, intention-to-treat; L, levofloxacin; M, metronidazole; mITT, modified intention-to-treat; Mx, moxifloxacin ; N, total number of patients receiving a treatment; PP, per protocol; PPI, proton pump inhibitor; Rf, rifaximin; Seq, sequential administration; T, tinidazole; Tc, tetracycline.

*Single-capsule, three-in-one single capsule containing bismuth, tetracycline and metronidazole.

Statistical significant differences ($p<0.001$) were obtained in the Chi^2 test when comparing following schemes with and without bismuth, [2] (PPI+C+A) vs [4] (PPI+C+A+bismuth) and [1] (PPI+A+levofloxacin) vs [3] (PPI+A+levofloxacin+bismuth).

Table 4. Effectiveness of second-line therapy according to the duration and dose of the proton pump inhibitor; stratified by first-line therapy.

Duration of proton pump inhibitor													
		First-line: clarithromycin-containing triple or quadruple therapy						**First-line: bismuth quadruple therapy**					
Second-line treatment	**Length, days**	**ITT, N (%)**	**95% CI**	**mITT, N (%)**	**95% CI**	**PP, N (%)**	**95% CI**	**ITT, N (%)**	**95% CI**	**mITT, N (%)**	**95% CI**	**PP, N (%)**	**95% CI**
Triple regimens													
PPI+A+L	7	32 (50)	31-69	24 (71)	49-87	24 (71)	49-87	NA	NA	NA	NA	NA	NA
	10	799 (69)	66-72.5	737 (76)	72-79	728 (76)	73-79	15 (67)	38-88	14 (71.5)	42-92	13 (69)	39-91
	14	461 (81)	77-84	416 (91)	88-93	409 (91)	88-94	NA	NA	NA	NA	NA	NA
PPI+C+A	7	23 (30)	13-53	15 (47)	21-73	15 (47)	21-73	NA	NA	NA	NA	NA	NA
	10	95 (15)	7-22	61 (23)	12-34	59 (22)	11-33	NA	NA	NA	NA	NA	NA
	14	39 (13)	4.3-27	31 (16)	5.4-34	31 (16)	5.4-34	NA	NA	NA	NA	NA	NA
PPI+A+Mx	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10	23 (96)	78-100	22 (100)	85-100	22 (100)	85-100	NA	NA	NA	NA	NA	NA
	14	48 (79)	67-92	44 (87)	76-98	44 (86)	75-98	NA	NA	NA	NA	NA	NA
PPI+A+M	7	26 (35)	14-55	28 (32)	13-51	28 (32)	13-51	NA	NA	NA	NA	NA	NA

	10	34 (65)	47-82	30 (77)	60-93	30 (77)	60-93	NA	NA	NA	NA	NA	NA
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPI+A+Rf	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPI+M+L	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10	10 (70)	35-93	10 (70)	35-93	10 (70)	35-93	NA	NA	NA	NA	NA	NA
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPI+C+M	7	10 (60)	26-88	9 (67)	30-92	10 (70)	30-92	NA	NA	NA	NA	NA	NA
	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPI+C+L	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Quadruple regimens													
PPI+single	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
capsule*	10	614 (83)	79.5-86	593 (84)	87-92	584 (90)	87-92	52 (88.5)	79-98	49 (94)	83-99	49 (94)	83-99
	14	11 (82)	48-98	11 (82)	48-98	11 (82)	48-98	NA	NA	NA	NA	NA	NA

PPI+A+L+B	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10	23 (57)	34-79	18 (78)	52-94	18 (78)	52-94	NA	NA	NA	NA	NA	NA
	14	442 (82)	79-96	414 (90)	86-92	398 (90)	87-93	88 (76)	67-86	78 (87)	79-95	77 (88)	80.5-96
PPI+M+Tc+B	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10	48 (71)	57-85	47 (72)	58.5-86	45 (76)	62-89	NA	NA	NA	NA	NA	NA
	14	61 (84)	73.5-94	57 (93)	83-98	55 (93)	82-98	NA	NA	NA	NA	NA	NA
PPI+C+A+B	7	NA	NA	NA	NA	NA	NA	9 (11)	0.3-48	5 (20)	0.5-72	5 (20)	0.5-72
	10	41 (78)	64-92	37 (86)	71-95.5	37 (86)	71-95.5	33 (51.5)	33-71	18 (94)	73-100	17 (94)	71-100
	14	45 (69)	54-83.5	41 (88)	74-96	39 (90)	76-97	42 (21)	8-35	14 (79)	49-95	13 (77)	46-95
Conc-PPI+C+A+M	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10	38 (76)	61-91	13 (54)	25-81	36 (78)	63-93	NA	NA	NA	NA	NA	NA
	14	77 (84.5)	76-93	79 (85)	76-93	79 (85)	76-93	44 (77)	64-91	42 (83)	71-96	39 (87)	73-96
Seq-PPI+C+A+T	7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	10	25 (64)	43-85	23 (70)	47-87	23 (70)	47-87	NA	NA	NA	NA	NA	NA
	14	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Dose of the proton pump inhibitor

PPI dose	**First-line: clarithromycin-containing triple or quadruple therapy**						**First-line: bismuth quadruple therapy**					
	ITT, N (%)	**95% CI**	**mITT, N (%)**	**95% CI**	**PP, N (%)**	**95% CI**	**ITT, N (%)**	**95% CI**	**mITT, N (%)**	**95% CI**	**PP, N (%)**	**95% CI**

Triple therapy combinations													
PPI+A+L	Low	437 (67)	62-71	401 (73)	68-77	395 (74)	69-78	14 (50)	23-77	13 (54)	25-81	13 (54)	25-81
	Standard	307 (71)	66-76	289 (76)	71-81	284 (77)	72-82	NA	NA	NA	NA	NA	NA
	High	551 (78)	75-82	491 (89)	86-92	486 (89)	86-92	NA	NA	NA	NA	NA	NA
PPI+C+A	Low	91 (21)	12-30	67 (28)	17-40	66 (29)	17-40	NA	NA	NA	NA	NA	NA
	Standard	50 (8)	2-19	28 (14)	4-33	27 (11)	2.3-29	NA	NA	NA	NA	NA	NA
	High	16 (19)	4-46	12 (25)	5.5-57	12 (25)	5.5-57	NA	NA	NA	NA	NA	NA
PPI+A+Mx	Low	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Standard	18 (94)	73-100	17 (100)	80-100	17 (100)	80-100	NA	NA	NA	NA	NA	NA
	High	51 (80)	68-92	47 (87)	77-98	47 (87)	77-98	NA	NA	NA	NA	NA	NA
PPI+A+M	Low	49 (47.5)	31-64	41 (51)	35-68	41 (51)	35-68	NA	NA	NA	NA	NA	NA
	Standard	12 (50)	21-79	9 (67)	30-92	9 (67)	30-92	NA	NA	NA	NA	NA	NA
	High	17 (59)	33-81	15 (67)	38-88	15 (67)	38-88	NA	NA	NA	NA	NA	NA
PPI+A+Rf	Low	9 (78)	40-97	9 (78)	40-97	9 (78)	40-97	NA	NA	NA	NA	NA	NA
	Standard	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	High	12 (67)	35-90	9 (89)	52-100	9 (89)	52-100	NA	NA	NA	NA	NA	NA
PPI+M+L	Low	8 (62.5)	24-91	7 (71)	29-96	7 (71)	29-96	NA	NA	NA	NA	NA	NA
	Standard	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

	High	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPI+C+M	Low	11 (64)	31-89	10 (70)	35-93	10 (70)	35-93	NA	NA	NA	NA	NA	NA
	Standard	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	High	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PPI+C+L	Low	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Standard	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	High	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Quadruple therapy combinations													
PPI+single capsule*	Low	306 (80)	75-85	291 (86)	82-90	286 (86)	82-90	9 (89)	52-100	8 (100)	63-100	8 (100)	63-100
	Standard	101 (79)	71-88	92 (90)	84-97	914 (90)	83-97	19 (89.5)	67-99	17 (100)	80.5-100	17 (100)	80-100
	High	222 (86.5)	82-91	224 (92)	88-96	219 (92)	88-96	24 (87.5)	67-97	24 (87.5)	67-97	24 (87.5)	67-97
PPI+A+L+B	Low	44 (61)	46-77	39 (72)	56-87	39 (72)	56-87	16 (68)	41-89	12 (92)	61.5-100	12 (92)	61-100
	Standard	42 (69)	54-84	36 (83)	67-94	35 (83)	66-93	NA	NA	NA	NA	NA	NA
	High	378 (85)	81-89	356 (92)	88-94	341 (92)	89-95	73 (79.5)	69.5-89	67 (86)	78-96	66 (88)	79-97
PPI+M+Tc+B	Low	44 (68)	53-83	39 (77)	62-91	38 (79)	65-93	NA	NA	NA	NA	NA	NA
	Standard	48 (73)	59-86	45 (78)	64-91	42 (79)	65-92	NA	NA	NA	NA	NA	NA
	High	23 (100)	85-100	25 (100)	86-100	25 (100)	86-100	NA	NA	NA	NA	NA	NA
PPI+C+A+B	Low	14 (50)	23-77	11 (64)	31-89	11 (64)	31-89	29 (38)	19-57	14 (79)	49-95	13 (77)	46-95

	Standard	50 (82)	70-94	47 (96)	85-99	47 (96)	85-99	20 (35)	15-53	11 (64)	31-89	11 (64)	31-90
	High	22 (64)	41-86	19 (79)	54-94	17 (82)	56-96	33 (27)	11-44	12 (92)	62-100	11 (91)	59-100
Conc-PPI+C+A+M	Low	39 (69)	53-85	39 (69)	53-85	38 (68)	52-84	18 (89)	65-99	19 (84)	60-97	18 (89)	65-99
	Standard	25 (80)	59-93	25 (80)	59-93	25 (80)	59-93	9 (56)	21-86	6 (83)	36-99	5 (100)	48-100
	High	56 (89)	80-98	57 (91)	81-97	57 (91)	81-97	22 (82)	60-95	22 (86)	66-98	21 (86)	64-97
Seq-PPI+C+A+T	Low	16 (56)	30-80	15 (60)	32-84	15 (60)	32-84	NA	NA	NA	NA	NA	NA
	Standard	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	High	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

A, amoxicillin; B, bismuth; C, clarithromycin; CI, confidence interval; Conc, concomitant administration; ITT, intention-to-treat; L, levofloxacin; M, metronidazole; mITT, modified intention-to-treat; Mx, moxifloxacin; NA, not available; PP, per protocol; PPI, proton pump inhibitor; Rf, rifaximin; Seq, sequential administration; T, tinidazole; Tc, tetracycline.

*Single-capsule, three-in-one single capsule containing bismuth, tetracycline and metronidazole.

Low-dose PPI: 4.5-27 mg omeprazole equivalents (OE) twice daily (bid) (i.e., 20 mg OE bid), standard-dose PPI: 32-40 mg omeprazole equivalents bid (i.e., 40 mg OE bid), high-dose PPI: 54-128 mg omeprazole equivalents bid (i.e., 60 mg OE bid).

Table 5. Multivariate analysis in empirical second-line treatment.

	OR (95%CI)	*P*-value
Indication [ref. dyspepsia]	1.280 (1.014–1.616)	0.038
Treatment length [ref. 7 days]		
10 days	2.089 (1.476–2.957)	0.000
14 days	2.814 (1.942–4.079)	0.000
PPI dose [ref. low dose]		
Standard	1.507 (1.215–1.869)	0.000
High	2.208 (1.774–2.748)	0.000
Use of clarithromycin first-line	0.600 (0.479–0.751)	0.000
Second-line treatment [ref. PPI+C+A]		
PPI+A+L or PPI+A+Mx	3.112 (2.276–4.255)	0.000
PPI+A+L+B	3.638 (2.395–5.525)	0.000
Bismuth Quadruple*	6.284 (4.411–8.951)	0.000
Other (remaining therapies)	2.944 (2.130–4.069)	0.000
Compliance [ref. No, <90% drug intake]	3.013 (1.788–5.077)	0.000

A, amoxicillin; B, bismuth; C, clarithromycin; CI, confidence interval; L, levofloxacin; Mx, moxlifloxacin; OR, odds ratio; PPI, proton pump inhibitor; ref, reference category.

*accounting for PPI + metronidazole + tetracycline + bismuth and single

capsule.

Low-dose PPI: 4.5–27 mg omeprazole equivalents (OE) twice daily (bid) (i.e., 20 mg OE bid), standard-dose PPI: 32–40 mg omeprazole equivalents bid (i.e., 40 mg OE bid), high-dose PPI: 54 to 128 mg omeprazole equivalents bid (i.e., 60 mg OE bid).

Figure 1. Study flow chart

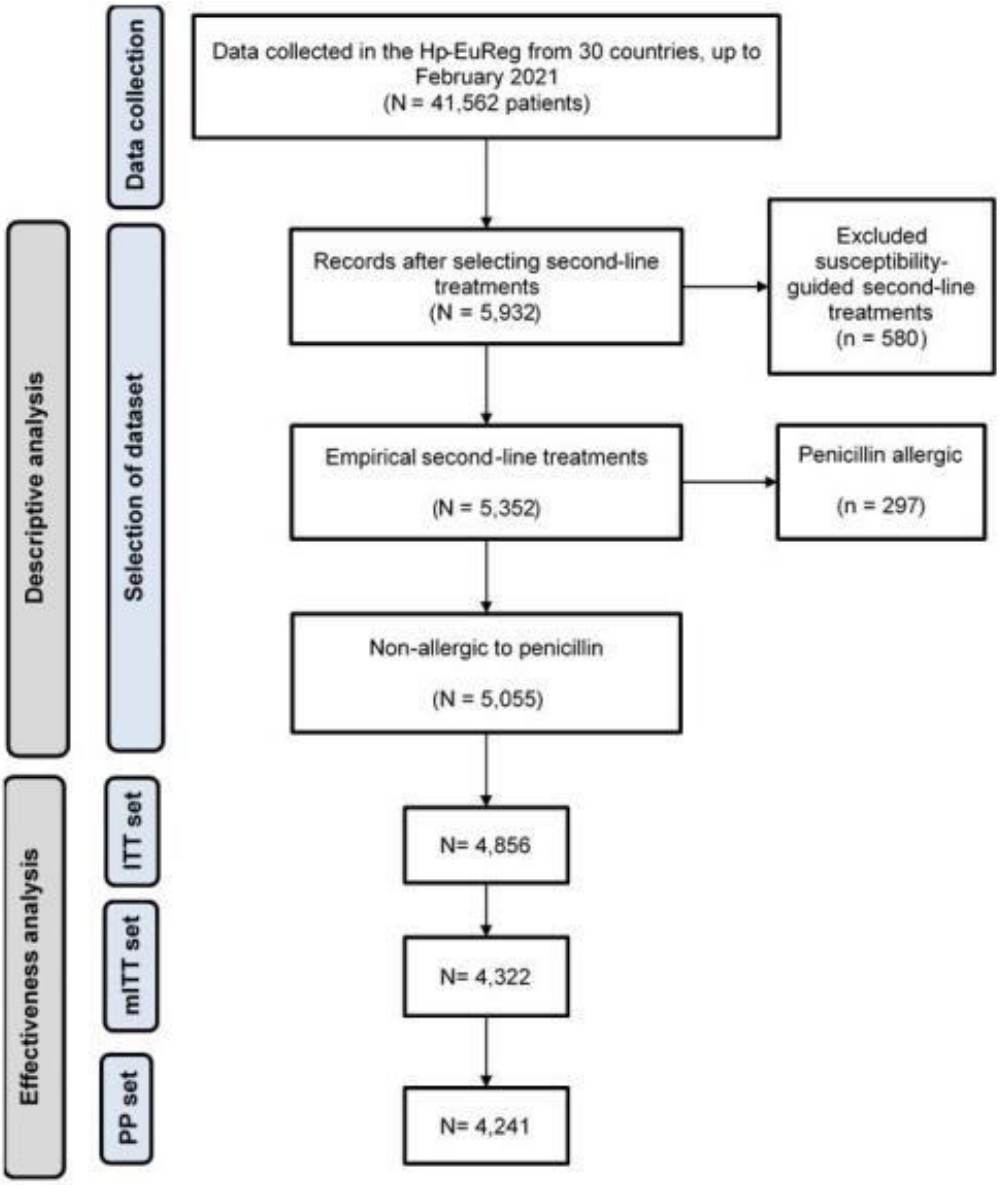

Figure 2. Evolution (A) in prescriptions and (B) effectiveness (mITT) of most common second-line treatments from 2013 to 2020. A, amoxicillin; B, bismuth; C, clarithromycin; L, levofloxacin; M, metronidazole; mITT, modified intention-to-treat; Mx, moxifloxacin; Tc, tetracycline