Systematic review: third-line susceptibility-guided treatment for *Helicobacter pylori* infection

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Abstract

Background: Susceptibility-guided therapies (SGTs) have been proposed as preferable to empirical rescue treatments after two treatment failures. The aim of this study was to perform a systematic review and meta-analysis evaluating the effectiveness and efficacy of SGT as third-line therapy.

Methods: A systematic search was performed in multiple databases. Studies reporting cure rates of *Helicobacter pylori* with SGT in third-line therapy were selected. A qualitative analysis describing the current evidence and a pooled mean analysis summarizing the cure rates of SGT in third-line therapy was performed.

Results: No randomized controlled trials or comparative studies were found. Four observational studies reported cure rates with SGT in third-line treatment, and three studies which mixed patients with second- and third-line treatment also reported cure rates with SGT. The majority of the studies included the patients when culture had been already obtained, and so the effectiveness of SGT and empirical therapy has never been compared. A pooled mean analysis including four observational studies (283 patients) showed intention-to-treat and per-protocol eradication rates with SGT of 72% (95% confidence interval 56–87%; I^2: 92%) and 80% (95% confidence interval 71–90%; I^2: 80%), respectively.

Conclusions: SGT may be an acceptable option as rescue treatment. However, cure rates are, at best, moderate and this approach has never been compared with a well-devised empirical therapy. The evidence in favor of SGT as rescue therapy is currently insufficient to recommend its use.

Keywords: *Helicobacter pylori*, tailored treatment, susceptibility-guided treatment, third-line treatment

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Introduction

Helicobacter pylori infection is the major cause of chronic gastritis, peptic ulcers, gastric mucosa-associated lymphoid tissue lymphoma (MALT) and gastric cancer. Its worldwide prevalence approaches 50% [McColl, 2010].

The standard triple therapy combining amoxicillin (1 g b.d.) and clarithromycin (500 mg b.d.) or metronidazole (500 mg b.d.) has long been the recommended first-line therapy for *H. pylori* infection [Malfertheiner *et al.* 2012]. However, this combination seems to have lost efficacy over the last decade [Malfertheiner *et al.* 2012], mostly

due to clarithromycin resistance [Neri *et al.* 2003]. The overall clarithromycin resistance rate in Europe increased from 9% in 1998 to 17.6% in 2008 [Malfertheiner *et al.* 2012], and primary resistances are above 20% in many European countries [Megraud *et al.* 2013].

The Maastricht IV/Florence consensus [Malfertheiner *et al.* 2012] suggests that culture and standard susceptibility testing should be considered in all regions before second-line treatment if endoscopy is carried out for another reason (evidence level: 5; grade of recommendation: D). However, challenging this expert opinion, a recent

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meta-analysis of randomized controlled trials (RCTs) [Lopez-Gongora *et al.* 2015] did not observe significant differences between susceptibility-guided therapies (SGT) and empirical therapy as second-line treatment in the few comparative studies performed. Furthermore, the studies included in the meta-analyses did not compare SGT *versus* the currently recommended quadruple therapies, and allocated patients once the endoscopy and culture was performed. Whether the need for invasive testing reduces the acceptability and the effectiveness of SGT in clinical practice remains uncertain. Finally, the evidence regarding SGT in third-line treatment has not been systematically revised to date.

The aim of this study was to perform a systematic review and meta-analysis evaluating the evidence of the efficacy of SGT in the treatment of *H. pylori* after two failed attempts.

Material and methods
The study was performed in accordance with the MOOSE Statement [Stroup *et al.* 2000], because the systematic review and meta-analysis included observational studies. The MOOSE checklist is shown in the online Supplementary Table 1. A flow-chart of the studies included in the meta-analyses is shown in Figure 1.

Search strategy
A systematic computerized literature search was conducted in PubMed, Scopus, the Cochrane Library and the ISI Web of Knowledge including full-text published articles and abstracts from the of DDW, UEGW, EHSG and other relevant meetings from 1984 to May 2015. Combinations of different terms combinations are shown in the online Supplementary Table 2. In addition, references of articles retrieved and significant reviews were also examined, searching for articles to include in the present study. Finally, the personal databases of the authors were also reviewed for eligible publications.

Inclusion criteria
We included published full-text articles that fulfilled the following criteria: (a) they reported clinical trials, observational series or comparative cohort studies that evaluated the *H. pylori* eradication rates in adult patients who received SGT; (b) SGT was used as rescue treatment after two

failed attempts; (c) pretreatment diagnostic tests for *H. pylori* detection comprised one or more of the common validated tests [urea breath test, histology, rapid urease test, stool antigen, polymerase chain reaction (PCR) or culture]; (d) all of these tests (except culture) were considered adequate as control tests; (e) control diagnostic tests were performed at least 4 weeks after the end of the eradication schedule in the absence of proton pump inhibitor (PPI) use. No language limitations were imposed.

Data extraction
Data were extracted independently by four reviewers (SL, VG, MB and IP) and revised by a fifth investigator (AV). Discordances were resolved by consensus with an additional investigator (XC). Data extraction was standardized using a data extraction table. Variables compiled were: (a) number of patients; (b) type of study (RCT, quasi-RCT, comparative non-RCT, noncomparative); (c) method used to determine antibiotic susceptibility; (d) percentage of successful cultures; (e) time of randomization (before or after endoscopy); (f) intention to treat (ITT) and (g) per-protocol (PP) cure rates and their 95% confidence intervals (CIs) for the susceptibility-guided treatment group; and for the control empiric treatment group when available (h) adherence to treatment and (i) number and severity of side effects.

Risk of bias
Risk of bias was assessed independently by three reviewers (IP, SLG and MB). Discrepancies in the interpretation were resolved with a third reviewer (XC). Observational studies were assessed following the suggestions of *Methods Guide for Effectiveness and Comparative Effectiveness Reviews* [Agency for Healthcare Research and Quality, 2014]. We recorded whether the study was labeled as prospective, the completeness of outcome data, the presence of selective reporting and the validity of pre- and post-treatment eradication tests.

Statistical analysis
The primary endpoint for the different meta-analyses was the ITT efficacy. Secondary endpoints were PP efficacy, adherence and adverse events.

As the number of comparative studies (either randomized or observational) available in third-line treatments was insufficient, the mean eradication

Table 1. Characteristics of the studies reporting cure rates of SGT after two failed attempts.

Study and country	N	Study design	Method for determining antibiotic susceptibility	Successful culture	Empiric treatment cure rates				Susceptibility-guided treatment cure rates			
					ITT (%)	95% CI	PP (%)	95% CI	ITT (%)	95% CI	PP (%)	95% CI
Second-line treatment												
Cammarota et al. [2004], Italy	94	NC	E-test	94/98	–	–	–	–	85/94 (90)	81–94	85/93 (91)	82–95
Gasbarrini et al. [2000], Italy	58	NC	E-test	39/49	–	–	–	–	30/58 (52)	37–64	30/39 (77)	60–88
Liou et al. [2013], Taiwan	135	NC	PCR and agar dilution	–	–	–	–	–	109/135 (81)	73–87	109/132 (83)	75–88
Vicente et al. [2002], Spain	40	NC	E-test	41/42	–	–	–	–	24/40 (60)	43–75	24/38 (63)	46–78
Mixed second and third-line treatment												
Fiorini et al. [2012], Italy	254	NC	E-test	236/254	–	–	–	–	211/254 (83)	77–87	212/236 (90)	84–93
Tay et al. [2012], Australia	310	NC	E-test	306/310	–	–	–	–	291/310 (94)	90–96	291/310 (94)	90–96
Yahav et al. [2006], Israel	98	CNR	E-test	49/49	31/49 (63)	47–75	31/49 (63)	47–75	42/49 (86)	72–93	42/49 (86)	72–93

CI, confidence interval; CNR, controlled nonrandomized study; E-test, Epsilometer test; ITT, intention-to-treat; NC, noncontrolled study; PP, per-protocol; SGT, susceptibility-guided therapy.
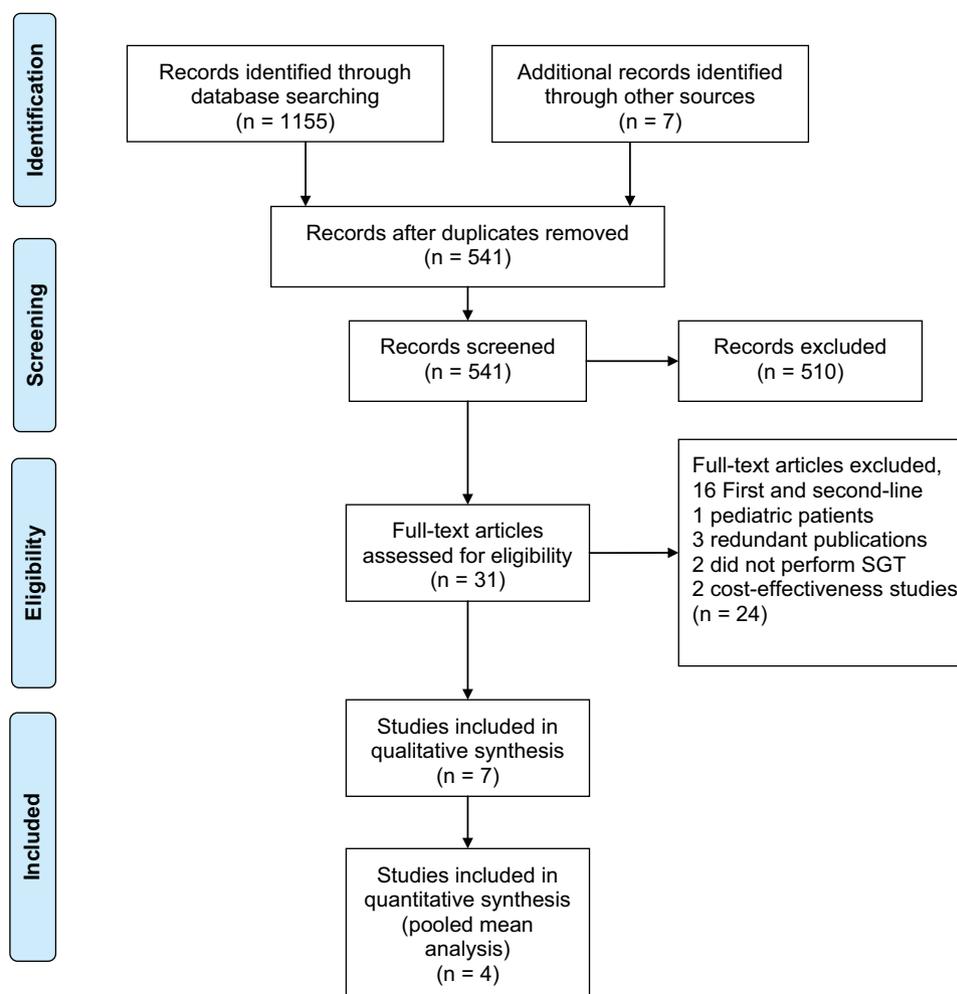
Figure 1. Flow of information through the various phases of the selection of the studies.

rate of SGT was calculated by combining data from observational noncomparative studies and using the generic inverse variance method, which involves a weighted average of the estimated effect from the individual studies. The weight for each study is taken to be the inverse of the variance (one divided by the square of the standard error) of the estimated effect. If studies differed in terms of treatments administered, a random effect model was used. The I^2 statistic was used to assess the heterogeneity of the studies following the recommendation of the *Cochrane Collaboration's Handbook for Systematic Reviews of Interventions* [Higgins and Green, 2011], as follows: 0–40%, negligible heterogeneity; 40–75%, moderate heterogeneity; 75–100% considerable heterogeneity. Analyses were performed using the freeware program Review Manager (RevMan) version 5.1 [The Cochrane Collaboration, 2012].

Results

The original searches retrieved more than 1000 articles. Following a review of the abstracts, 34 articles were assessed for eligibility. No unpublished abstracts were found. After careful evaluation of the full texts, seven were included in the qualitative analysis and four in the pooled mean analysis (Figure 1).

Studies excluded

A total of 24 studies were finally excluded, for the following reasons: (a) 16 included patients receiving only first- or second-line therapy [Neri *et al.* 2003; Furuta *et al.* 2007; Avidan *et al.* 2001; Biscontri *et al.* 2001; Cosme *et al.* 2013; Kawai *et al.* 2008; Lamouliatte *et al.* 2003; Lee *et al.* 2013; Marzio *et al.* 2006; Miwa *et al.* 2003; Molina-Infante *et al.* 2012; Romano *et al.* 2003;

Table 2. Antimicrobial therapies used in third-line therapy studies.

Study	First-line treatment	Second-line treatment	Drug resistance after the second treatment failure	Susceptibility-guided third-line treatment	N	Treatment duration
Cammarota *et al.* [2004]	PPI b.d., A 1 g b.d., M 250 mg q.d. or TI 500 mg b.d. for 7 days (n = 37)	PPI (double dose per day), A 1 g, C 250 or 500 mg b.d. for 7 days (n = 27) PPI (double dose per day), A 1 g, L 500 mg b.d. for 7 days (n = 7) O 20 mg b.d., B 120 mg q.d.s., A 1 g b.d., TE 500 mg q.d.s. for 7 days (n = 3)	M: 100% C: 95% L: 31% TE: 5% A: 0%	A and TE sensitive: B 120 mg q.d,s., O 20 mg b.d., A 1 g b.d., D 100 mg b.d.	89	7 days
	PPI, A 1 g, C 250 or 500 mg b.d. for 7 days (n = 34)	PPI (double dose per day), B 120 mg q.d.s., M 250 mg q.d.s or TI 500 mg q.d.s., TE 500 mg q.d.s. for 7 days (n = 28) PPI (double dose per day), A 1 g, L 500 mg b.d. for 7 days (n = 6)		TE, M and C resistant: O 20 mg b.d., A 1 g b.d., L 500 mg b.d.	4	7 days
	PPI b.d., A 1 g, L 500 mg b.d. for 7 days (n = 23)	PPI (double dose per day), A 1 g, C 250 or 500 mg b.d. for 7 days (n = 11) O 20 mg b.d., B 120 mg q.d.s., A 1 g b.d., TE 500 mg q.d.s. for 7 days (n = 2) PPI (double dose per day), B 120 mg q.d.s., M 250 mg q.d.s or TI 500 mg b.d., TE (500 mg q.d.s for 7 days (n = 10)		TE, M and L resistant: O 20 mg, A 1 g, C 500 mg b.d.	1	7 days
Gasbarrini *et al.* [2000]	PPI, TI, C 7 days (n = 58)	RBC, A C 7 days (n = 58)	AZ: 68% ER: 62% C: 56% M and TI 56% TE 12% A 0%	PPI, B, A, TE PPI, B, A, M PPI, B, TE, M	26 9 4	Not reported
Liou *et al.* [2013]	100% (131/131) received C and 79.1% (102/129) L in first or in second line		23S rRNA mutation (tissue) 78.9% (101/128) 23S rRNA mutation (strain) 85.7% (84/98) Gyrase A mutation (tissue) 41.3% (52/126) Gyrase A mutation (strain) 42.9% (42/98) L resistance 46.9% (45/96) A resistance 9.4% (9/96) M resistance 58.3% (56/96) TE resistance 3.2% (3/93) C resistance 86.5% (83/96)	23S rRNA wild-type: ES 40 mg, A 1 g, M 500 mg, C 500 mg b.d.	19	ES, A 7 days followed by M, C 7 days
				23S rRNA mutant-type and *gyrA* wild-type: ES 40 mg, A 1 g, M 500 mg, L 250 mg b.d.	51	ES, A 7 days followed by M, LE 7 days
				23S rRNA mutant-type and *gyrA* mutant-type: ES 40 mg, A 1 g, M 500 mg, TE 500 mg b.d.	65	ES, A 7 days followed by M, TE 7 days

(Continued)

Table 2. (Continued)

Study	First-line treatment	Second-line treatment	Drug resistance after the second treatment failure	Susceptibility-guided third-line treatment	N	Treatment duration
Vicente *et al.* [2002]	Not reported	Not reported	M resistance 44% (18/41) C resistance 52% (21/41) Both C and M resistance 19% (5/41) A 0% TE 0%	Susceptible to C and M: O 20 mg b.d., B 120 mg q.d., TE 500 mg q.d., C 500 mg b.d.	9	14 days
				Resistant to C: O 20 mg b.d., B 120 mg q.d., TE 500 mg q.d., A 1 g b.d.	13	14 days
				Resistant to M: O 20 mg b.d., B 120 mg q.d., TE 500 mg q.d., C 500 mg b.d./ A 1 g b.d.	10	14 days
				M and C resistant and penicillin allergy: O 20 mg b.d., B 120 mg q.d., TE 500 mg q.d., Cl 500 mg b.d.	8	14 days

PPI, proton pump inhibitor; A, amoxicillin; C, clarithromycin; L, levofloxacin; M, metronidazole; TI, tinidazole; b.d., twice a day; q.d.s., four times a day; TE, tetracycline; B, bismuth; O, omeprazole; D, doxycycline; RBC, ranitidine bismuth citrate; AZ, azithromycin; ER, erythromycin; ES, esomeprazole; SGT, susceptibility-guided therapy.

Sugimoto *et al.* 2014; Toracchio *et al.* 2000; Wang *et al.* 2008; Zhou *et al.* 2010]; (b) one included pediatric patients [Lopes *et al.* 2005]; (b) three reported data on patients already included in another study [Gomollón *et al.* 2000; Furuta *et al.* 2005; Romano *et al.* 2000]; (c) two did not perform susceptibility-guided treatment [Seppala *et al.* 2000; Graham *et al.* 2000]; (d) two were cost-effectiveness studies [Breuer and Graham, 1999; Zullo *et al.* 2003b].

Studies included reporting cure rates of SGT in third-line treatment

No RCTs or studies comparing SGT *versus* empirical treatment after two *H. pylori* eradication attempts were found. Only four observational noncomparative studies including 283 patients reported the mean cure rates of SGT in third-line therapy [Cammarota *et al.* 2004; Gasbarrini *et al.* 2000; Liou *et al.* 2013; Vicente *et al.* 2002]. In addition, two noncontrolled studies [Fiorini *et al.* 2013; Tay *et al.* 2012] and one controlled nonrandomized study [Yahav *et al.* 2006] reported cure rates of SGT mixing patients with second and

third-line treatment. All of the studies except one [Gasbarrini *et al.* 2000] included patients once endoscopy had been performed; furthermore, in many of them, patients were included only when a positive culture had been obtained. So, the effectiveness and applicability of SGT in clinical practice could not be evaluated.

Table 1 shows the main characteristics of the studies and cure rates of SGT. Previous antibiotic treatment and antibiotic strategy according to susceptibility tests varied widely (Tables 2 and 3). The risk of bias for observational studies is summarized in Figure 2. All of the studies were non-RCTs, but were described as prospective, included consecutive patients, used a valid diagnostic and control test and reported complete data.

Cammarota and colleagues conducted a prospective study in Italy including 94 consecutive patients who underwent endoscopy with antral biopsies and in whom successful susceptibility analyses were obtained using the Epsilometer test (E-test) [Cammarota *et al.* 2004]. In the ITT analysis they did not include 14 patients who had shown poor

Table 3. Antimicrobial therapies used in mixed second- and third-line therapy studies.

Study	Previous treatments	Drug resistances before SGT	Empiric therapy	N	Treatment duration	SGT	N	Treatment duration
Fiorini *et al.* [2013]	Not reported	L + C + M 33% [79/236] L + C 3% [8/236] L + M 3% [7/236] C + M 25% [60/236] C 20% [48/236] M 4% [10/10]	Not administered	–	–	Susceptible to L: ES 40 mg, A 1g, L 250 mg b.d. Resistant to L: ES 40 mg, A 1 g b.d., RFB 150 mg o.d.	131 105	10 days 12 days
Tay *et al.* [2012]	First line: O, A, C 7-day Second line: O, A, C/M 7–14 days	C 94% [288/306] M 68% [207/306] C and M 65% [199/306] CIP 5.6% RIF 2% A 0% TE 0%	Not administered	–	–	Susceptible to RFB and CIP, no penicillin allergy: RA 20 mg t.d.s., A 1 g t.d.s., RFB 150 mg b.d., CIP 500 mg b.d. Susceptible to RIF and CIP, penicillin allergy: RA 20 mg t.d.s., B 240 q.d, RFB 150 mg b.d., CIP 500 mg b.d. Resistant to RIF or CIP or failure of RIF and CIP-based therapy: PPI, B, FU, A or RFB PPI, A, M PPI, B, TE, FU or A	210 69 26	RA and A 10 days. RFB and CIP 5 days 10 days 10 days
Yahav *et al.* [2006]	First: O, C, A or O, M, A 7-day Second: O,C, M, A or O, B, M, TE 7-day	M 47% [23/49] C 59% [29/49] Both C and M 29% [14/49]	O 20 mg, A 1 g, C 500 mg b.d. O 20 mg, A 1 g, M 500 mg b.d. O 20 mg b.d., B 120 q.d., M 500 mg b.d., TE 500 mg q.d.	11 6 11	7 days 7 days 7 days	Susceptible to C: O 20 mg, A 1 g, C 500 mg b.d. Resistant to C and susceptible to M: O 20 mg, A 1 g, M 500 mg b.d. Allergic to penicillin: O 20 mg b.d., B 120 q.d.s., TE 500 mg q.d., M 500 mg b.d. Resistant to C and M: O 20 mg b.d., B 120 mg q.d., TE 500 mg q.d., A 1 g b.d.	34 15	7 days 7 days

A, amoxicillin; b.d., twice a day; B, bismuth; C, clarithromycin; CIP, ciprofloxacin; FU, furazolidone; L, levofloxacin; M, metronidazole; O, omeprazole; o.d., once a day; RA, rabeprazole; RFB, rifabutin; RIF, rifampicin; t.d.s., three times a day; TE, tetracycline; SGT, susceptibility-guided therapy.

Figure 2. Risk of bias of the studies included in the qualitative analysis.

compliance with the previously prescribed regimens. Four eligible patients were also excluded from the ITT analysis because *H. pylori* could not be cultured. First-line treatments consisted of a 7-day triple therapy based on a PPI, amoxicillin and clarithromycin, levofloxacin or an imidazole. In second-line treatment, a 7-day bismuth quadruple therapy or a different 7-day triple therapy was administered. In third-line treatment, *H. pylori* harbored a high prevalence of resistant strains (metronidazole 100%, clarithromycin 95%, levofloxacin 31%, tetracycline 5%, amoxicillin 0%). A 7-day bismuth quadruple therapy containing amoxicillin, tetracycline and omeprazole was used in 89 patients. The five patients harboring tetracycline-resistant strains were treated with a 7-day triple therapy with levofloxacin or clarithromycin. Following this strategy, and taking into account the restrictive inclusion criteria (patients with good previous compliance, positive culture, etc.) and the many exclusions, they obtained cure rates of 90% (ITT) and 91% (PP).

In Italy, Gasbarrini and colleagues evaluated the efficacy of a multistep strategy for *H. pylori* eradication [Gasbarrini *et al.* 2000]. A total of 2606 *H.*

pylori-positive patients were administered tinidazole, clarithromycin and a PPI for 1 week. A total of 350 patients with continuing infection were then given a second 1-week course of amoxicillin, clarithromycin and ranitidine bismuth citrate. A total of 58 patients still infected after the second course were invited to undergo an upper gastrointestinal endoscopy with *H. pylori* culture, but only 49 accepted. Finally, *H. pylori* culture was obtained in 39 out of these 49 patients. *In vitro* antibiotic susceptibility analysis by using the E-test showed a high overall prevalence of poly-antibiotic-resistant *H. pylori* strains (azithromycin 68%, erythromycin 62%, clarithromycin 56%, metronidazole and tinidazole 56%, tetracycline 12% and amoxicillin 0%). They received a 1-week quadruple PPI-bismuth-based scheme established on *H. pylori* antibiotic sensitivity, and achieved a mean eradication rate of 77% by PP analysis (30 out of 39) or 51.7% by ITT (30 out of 58).

In Taiwan, Liou and colleagues conducted a study in 135 patients who underwent an upper endoscopy and biopsies [Liou *et al.* 2013]. The study did not state the number of patients who declined endoscopy. Genotypic and phenotypic resistances were determined by PCR with direct sequencing (23S rRNA and *gyrA*) and agar dilution test, respectively. The authors found an excellent correlation between clarithromycin and levofloxacin phenotypic and genotypic resistance. The patients were re-treated with a sequential therapy containing esomeprazole and amoxicillin for the first 7 days, followed by esomeprazole and metronidazole plus clarithromycin, levofloxacin or tetracycline for another 7 days according to the genotypic resistance determined using gastric biopsy specimens. The overall eradication rate was 80.7% (109/135) in the ITT analysis and 82.6% (109/132) in the PP analysis.

Vicente and colleagues included 42 patients in a Spanish multicenter observational study who failed both a 7-day triple therapy and a second-line empirical attempt [Vicente *et al.* 2002]. These authors found a prevalence of resistance to clarithromycin and metronidazole around 50% by using the E-test and administered a 14-day bismuth-containing quadruple therapy. Four patients were not included in the PP analyses (one patient with culture failure, one with multidrug allergy, one who did not accept the treatment and one more who did not attend the follow-up visit). *H. pylori* was cured in only 60% of infected patients despite good compliance

(>90% in all the patients included). Surprisingly, cure was not achieved in six out of nine patients infected by a *H. pylori* strain susceptible to all antibiotics. The authors suggest that, in addition to antibiotic resistances, unknown factors (for instance, host immunogenicity or differences in the adherence of *H. pylori* to the mucosa) may also influence treatment efficacy.

In Italy, Fiorini and colleagues included 254 patients who failed at least one standard therapy for *H. pylori* eradication and underwent an upper endoscopy [Fiorini *et al*. 2013]. A total of 44% had failed at least two treatment attempts. Culture was obtained in 236 patients (93%) and a susceptibility analysis using the E-test identified 131 patients with levofloxacin-susceptible and 105 with levofloxacin-resistant strains. In patients with levofloxacin-susceptible *H. pylori* strains, 50% were resistant to clarithromycin and metronidazole, 43% to clarithromycin alone and 8% to metronidazole alone. In the levofloxacin-resistant *H. pylori* strains, 85% were also resistant to clarithromycin and metronidazole and 8% to both clarithromycin and metronidazole alone. Patients received either 10 days of levofloxacin or 12 days of rifabutin (in the case of patients resistant to levofloxacin) in combination with amoxicillin and esomeprazole. All patients took at least 90% of the prescribed drugs. *H. pylori* infection was cured in 90% of the patients who received levofloxacin triple therapy and 89% of the patients who received rifabutin triple therapy. Unfortunately, the results for second- and third-line treatments were not reported separately.

In Australia, Tay and colleagues successfully cultured 306 *H. pylori* strains from 310 consecutive patients who had completed and failed at least one course of the standard 7-day triple therapy containing PPI and combinations of amoxicillin, clarithromycin or metronidazole [Tay *et al*. 2012]. The proportions resistant to clarithromycin and metronidazole using the E-test were 94.1% and 67.6%, respectively, with 65% presenting resistance to both. Resistance to ciprofloxacin and rifampicin was 6% and 2%, respectively. In patients harboring rifampicin-susceptible strains, 10-day treatment with rabeprazole and amoxicillin plus rifabutin and ciprofloxacin from day 6, for 5 days, was prescribed. This schedule obtained a 95% cure rate. Again, the results for second- and third-line treatments were not reported separately.

Finally, in Israel Yahav and colleagues enrolled 49 patients who also failed at least one treatment course and in whom an upper endoscopy and culture had been performed [Yahav *et al*. 2006]. These authors compared the efficacy of SGT with a cohort of patients who received empirical rescue treatment. Resistance rates to clarithromycin and metronidazole by using the E-test were 59% and 47%, respectively (29% to both antibiotics). The empirical treatment consisted in a 7-day triple therapy containing clarithromycin or metronidazole or a 7-day bismuth-containing quadruple therapy. In the experimental group, the same therapies were administered but depending on the susceptibility of the *H. pylori* strain. SGT was significantly more efficacious than empirical therapy (86% *versus* 63%, $p = 0.02$).

Pooled mean analysis of cure rates of SGT as third-line treatment

As no RCTs or comparative observational studies reporting efficacy of SGT *versus* empirical therapy were found, a meta-analysis of cure rates of SGT of noncomparative studies was performed. ITT and PP analyses including four studies (283 patients) showed mean eradication rates of 72% (95% CI 56–87%; I^2: 92%) and 80% (95% CI 71–90%; I^2: 80%), respectively. In view of the lack of comparative trials and the high heterogeneity between the studies, this pooled mean cure rate should be interpreted with caution. The poorest results according to ITT were shown in the only study that reported rates of patients' acceptance of upper endoscopy and the rates of positive culture. As these two issues were not considered in the ITT analysis of the remaining studies, the effectiveness in clinical practice may be even lower.

Safety and side effects

Percentages of patients with adverse events were generally not reported. Cammarota and colleagues reported poor tolerance of quadruple bismuth therapy, and 1 out of 94 patients abandoned treatment due to severe side effects [Cammarota *et al*. 2004]; Liou and colleagues reported a dropout rate of 1% due to side effects [Liou *et al*. 2013]; and Vicente and colleagues reported no severe adverse events or dropouts [Vicente *et al*. 2002]. Fiorini and colleagues reported mild-to-moderate side effects in 15% and 20% of patients in each treatment arm; however, all patients complied with at least 90% of the treatment [Fiorini *et al*. 2013]. Gasbarrini and colleagues, Tay and coworkers and Yahav's group did not mention the number of patients with adverse events,

though the latter author reported a treatment compliance of 97% [Gasbarrini *et al.* 2000; Tay *et al.* 2012; Yahav *et al.* 2006].

Discussion

The present study highlights the lack of strong evidence regarding SGT in third-line therapy, as no RCTs or comparative studies with empirical treatment were found. The pooled mean analysis of cure rates including four observational noncomparative studies showed ITT and PP mean cure rates of 72% and 80%, respectively. However, the heterogeneity of the studies was high and this pooled mean cure rate should be interpreted carefully. Although the PP cure rate after two failed attempts was acceptable, ITT cure rates were not as good as desired. Furthermore, as three out of the four studies included patients once the endoscopy and the culture was obtained, the acceptance of an invasive procedure and feasibility of a successful culture was not considered. So, the effectiveness in clinical practice may be even lower than 70%. However, if the patient accepts an invasive test and a culture is feasible, SGT may be an acceptable strategy in third-line treatment.

This study agrees with the Maastricht consensus which suggested that treatment could be guided by antimicrobial susceptibility testing, whenever possible, after failure of second-line therapy (evidence level: 4; grade of recommendation: A). On the other hand, empirical treatment after two failed attempts has shown heterogeneous results: 65–85% with bismuth-containing quadruple therapies [Hsu *et al.* 2008; Gisbert *et al.* 2014], 60–90% with levofloxacin-based therapy [Sereni *et al.* 2012; Tursi *et al.* 2012; Gisbert *et al.* 2006; Zullo *et al.* 2003a] and 62–95% with a rifabutin-based therapy associated with PPIs and amoxicillin [Lim *et al.* 2014; González *et al.* 2007]. The studies differ in terms of previously administered therapies, study populations and drug schedules. So, until comparative studies are available, SGT also seems to be an acceptable treatment option in third-line treatment.

The major strong points of the study are the updated systematic review, the qualitative analysis and the pooled mean analyses of cure rates for summarizing the current evidence. The quality of the observational studies was acceptable. However, major limitations are the lack of comparative analyses, the high heterogeneity between the studies and the evaluation of acceptance of an invasive test and

the feasibility of *H. pylori* culture. All of the studies tend to use four drugs or long therapies after antibiotic sensitivity testing. Therefore, it is difficult to ascertain whether the improved cure rates are due to the determination of antibiotic resistances or to the use of far more effective therapies. This aspect requires future investigation.

In conclusion, SGT may be an acceptable option as rescue treatment. However, cure rates are, at best, moderate and this approach has never been compared with a well-devised empirical therapy, particularly with the highly effective quadruple therapies. The evidence in favor of SGT as rescue therapy is currently insufficient to recommend its use in clinical practice.

References

Agency for Healthcare Research and Quality (2014) *Methods Guide for Effectiveness and Comparative Effectiveness Reviews*. Rockville, MD: Agency for Healthcare Research and Quality (US).

Avidan, B., Melzer, E., Keller, N. and Bar-Meir, S. (2001) The effect of culture results for Helicobacter pylori on the choice of treatment following failure of initial eradication. *Isr Med Assoc J* 3: 163–165.

Biscontri, M., Lisi, L., Pellegrini, M. and Bayeli, P. (2001) Helicobacter pylori infection: Culture and antibiotic essay. *Argomenti Gastroenterol Clin* 14: 81–88.

Breuer, T. and Graham, D. (1999) Costs of diagnosis and treatment of Helicobacter pylori infection: When does choosing the treatment regimen based on susceptibility testing become cost effective? *Am J Gastroenterol* 94: 725–729.

Cammarota, G., Martino, A., Pirozzi, G., Cianci, R., Branca, G., Nista, E. *et al.* (2004) High efficacy of 1-week doxycycline- and amoxicillin-based quadruple regimen in a culture-guided, third-line treatment approach for Helicobacter pylori infection. *Aliment Pharmacol Ther* 19: 789–795.

Cosme, A., Montes, M., Martos, M., Gil, I., Mendarte, U., Salicio, Y. *et al.* (2013) Usefulness of antimicrobial susceptibility in the eradication of Helicobacter pylori. *Clin Microbiol Infect* 19: 379–383.

Fiorini, G., Vakil, N., Zullo, A., Saracino, I., Castelli, V., Ricci, C. *et al.* (2013) Culture-based selection therapy for patients who did not respond to previous treatment for Helicobacter pylori infection. *Clin Gastroenterol Hepatol* 11: 507–510.

Furuta, T., Shirai, N., Kodaira, M., Sugimoto, M., Nogaki, A., Kuriyama, S. *et al.* (2007) Pharmacogenomics-based tailored versus standard therapeutic regimen for eradication of H. pylori. *Clin Pharmacol Ther* 81: 521–528.

Furuta, T., Sugimoto, M., Nakamura, A. and Shirai, N. (2005) Susceptibility to antibiotics and drug metabolism in patients with H. pylori infection refractory to the initial treatment - therapeutic strategy based on susceptibility to CAM and CYP2C19 polymorphism. *Nippon Rinsho* 63(Suppl. 11): 426–433.

Gasbarrini, A., Ojetti, V., Armuzzi, A., Branca, G., Canducci, F., Torre, E. *et al.* (2000) Efficacy of a multistep strategy for Helicobacter pylori eradication. *Aliment Pharmacol Ther* 14: 79–83.

Gisbert, J., Castro-Fernandez, M., Bermejo, F., Perez-Aisa, A., Ducons, J., Fernandez-Bermejo, M. *et al.* (2006) Third-line rescue therapy with levofloxacin after two H-Pylori treatment failures. *Am J Gastroenterol* 101: 243–247.

Gisbert, J., Perez-Aisa, A., Rodrigo, L., Molina-Infante, J., Modolell, I., Bermejo, F. *et al.* (2014) Third-line rescue therapy with bismuth-containing quadruple regimen after failure of two treatments (with clarithromycin and levofloxacin) for H. pylori infection. *Dig Dis Sci* 59: 383–389.

Gomollón, F., Sicilia, B., Ducóns, J., Sierra, E., Revillo, M. and Ferrero, M. (2000) Third line treatment for Helicobacter pylori: A prospective, culture-guided study in peptic ulcer patients. *Aliment Pharmacol Ther* 14: 1335–1338.

González Carro, P., Pérez Roldán, F., De Pedro Esteban, A., Legaz Huidobro, M., Soto Fernández, S., Roncero Garcia Escribano, O. *et al.* (2007) Efficacy of rifabutin-based triple therapy in Helicobacter pylori infected patients after two standard treatments. *J Gastroenterol Hepatol* 22: 60–63.

Graham, D., Osato, M., Hoffman, J., Opekun, A., Anderson, S. and El-Zimaity, H. (2000) Furazolidone combination therapies for Helicobacter pylori infection in the United States. *Aliment Pharmacol Ther* 14: 211–215.

Higgins, J. and Green, S. (2011) *Cochrane Handbook for Systematic Reviews of Interventions*, Version 5.1.0 [updated March 2011]. The Cochrane Collaboration.

Hsu, P., Wu, D., Chen, A., Peng, N., Tseng, H., Tsay, F. *et al.* (2008) Quadruple rescue therapy for Helicobacter pylori infection after two treatment failures. *Eur J Clin Invest* 38: 404–409.

Kawai, T., Yamagishi, T., Yagi, K., Kataoka, M., Kawakami, K., Sofuni, A. *et al.* (2008) Tailored eradication therapy based on fecal Helicobacter pylori clarithromycin sensitivities. *J Gastroenterol Hepatol* 23: S171-S174.

Lamouliatte, H., Mégraud, F., Delchier, J., Bretagne, J., Courillon-Mallet, A., De Korwin, J. *et al.* (2003) Second-line treatment for failure to eradicate Helicobacter pylori: A randomized trial comparing four treatment strategies. *Aliment Pharmacol Ther* 18: 791–797.

Lee, H., Kim, J., Cheung, D., Kim, T., Jun, E., Oh, J. *et al.* (2013) Eradication of Helicobacter pylori according to 23S ribosomal RNA point mutations associated with clarithromycin resistance. *J Infect Dis* 208:1123–1130.

Lim, H., Lee, Y., An, B., Lee, S., Lee, Y. and Moon, B. (2014) Rifabutin-based high-dose proton-pump inhibitor and amoxicillin triple regimen as the rescue treatment for Helicobacter pylori. *Helicobacter* 19: 455–461.

Liou, J., Chen, C., Chang, C., Chen, M., Fang, Y., Lee, J. *et al.* (2013) Efficacy of genotypic resistance-guided sequential therapy in the third-line treatment of refractory Helicobacter pylori infection: a multicentre clinical trial. *J Antimicrob Chemother* 68: 450–456.

Lopes, A., Oleastro, M., Palha, A., Fernandes, A. and Monteiro, L. (2005) Antibiotic-resistant Helicobacter pylori strains in Portuguese children. *Pediatr Infect Dis J* 24: 404–409.

Lopez-Gongora, S., Puig, I., Calvet, X., Villoria, A., Baylina, M., Munoz, N. *et al.* (2015) Systematic review and meta-analysis: susceptibility-guided versus empirical antibiotic treatment for Helicobacter pylori infection. *J Antimicrob Chemother* 70: 2447–2455.

Malfertheiner, P., Megraud, F., O'Morain, C., Atherton, J., Axon, A., Bazzoli, F. *et al.* (2012) Management

of Helicobacter pylori infection - the Maastricht IV/ Florence Consensus Report. *Gut* 61: 646–664.

Marzio, L., Coraggio, D., Capodicasa, S., Grossi, L. and Cappello, G. (2006) Role of the preliminary susceptibility testing for initial and after failed therapy of Helicobacter pylori infection with levofloxacin, amoxicillin, and esomeprazole. *Helicobacter* 11: 237–242.

McColl, K. (2010) Clinical practice. Helicobacter pylori infection. *N Engl J Med* 362: 1597–1604.

Megraud, F., Coenen, S., Versporten, A., Kist, M., Lopez-Brea, M., Hirschl, A. *et al.* (2013) Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 62: 34–42.

Miwa, H., Nacahara, A., Kurosawa, A., Ohkusa, T., Ohkura, R., Hojo, M. *et al.* (2003) Is antimicrobial susceptibility testing necessary before second-line treatment for Helicobacter pylori infection? *Aliment Pharmacol Ther* 17: 1545–1551.

Molina-Infante, J., Pazos-Pacheco, C., Vinagre-Rodriguez, G., Perez-Gallardo, B., Dueñas-Sadornil, C., Hernandez-Alonso, M. *et al.* (2012) Nonbismuth quadruple (concomitant) therapy: empirical and tailored efficacy versus standard triple therapy for clarithromycin-susceptible Helicobacter pylori and versus sequential therapy for clarithromycin-resistant strains. *Helicobacter* 17: 269–276.

Neri, M., Milano, A., Laterza, F., Di Bonaventura, G., Piccolomini, R., Caldarella, M. *et al.* (2003) Role of antibiotic sensitivity testing before first-line Helicobacter pylori eradication treatments. *Aliment Pharmacol Ther* 18: 821–827.

Romano, M., Iovene, M., Montella, F., Vitale, L., De Simone, T. and Del Vecchio Blanco, C. (2000) Pretreatment antimicrobial-susceptibility testing in the eradication of H. pylori infection. *Am J Gastroenterol* 95: 3317–3318.

Romano, M., Marmo, R., Cuomo, A., De Simone, T., Mucherino, C., Iovene, M. *et al.* (2003) Pretreatment antimicrobial susceptibility testing is cost saving in the eradication of Helicobacter pylori. *Clin Gastroenterol Hepatol* 1: 273–278.

Seppala, K., Kosunen, T., Nuutinen, H., Sipponen, P., Rautelin, H., Sarna, S. *et al.* (2000) Cure of Helicobacter pylori infection after failed primary treatment: One-center results from 120 patients. *Scand J Gastroenterol* 35: 929–934.

Sereni, G., Azzolini, F., Camellini, L., Formisano, D., Decembrino, F., Iori, V. *et al.* (2012) Efficacy of a therapeutic strategy for eradication of Helicobacter pylori infection. *World J Gastroenterol* 18: 4542–4548.

Stroup, D., Berlin, J., Morton, S., Olkin, I., Williamson, G., Rennie, D. *et al.* (2000) Meta-analysis

of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 283: 2008–2012.

Sugimoto, M., Uotani, T., Sahara, S., Ichikawa, H., Yamade, M., Sugimoto, K. *et al.* (2014) Efficacy of tailored Helicobacter pylori eradication treatment based on clarithromycin susceptibility and maintenance of acid secretion. *Helicobacter* 19: 312–318.

Tay, C., Windsor, H., Thirriot, F., Lu, W., Conway, C., Perkins, T. *et al.* (2012) Helicobacter pylori eradication in Western Australia using novel quadruple therapy combinations. *Aliment Pharmacol Ther* 36: 1076–1083.

The Cochrane Collaboration (2012) *Review Manager (RevMan)*, Version 5.2. Copenhagen: The Nordic Cochrane Centre.

Toracchio, S., Cellini, L., Di Campli, E., Cappello, G., Malatesta, M., Ferri, A. *et al.* (2000) Role of antimicrobial susceptibility testing on efficacy of triple therapy in Helicobacter pylori eradication. *Aliment Pharmacol Ther* 14: 1639–1643.

Tursi, A., Picchio, M. and Elisei, W. (2012) Efficacy and tolerability of a third-line, levofloxacin-based, 10-day sequential therapy in curing resistant Helicobacter pylori infection. *J Gastrointest Liver Dis* 21: 133–138.

Vicente, R., Sicilia, B., Gallego, S., Revillo, M., Ducóns, J. and Gomollón, F. (2002) Helicobacter pylori eradication in patients with peptic ulcer after two treatments failure: A prospective culture-guided study. *Gastroenterol Hepatol* 25: 438–442.

Wang, G., Zhao, Q. and Li, S. (2008) Study of drug sensitivity test in Helicobacter pylori eradication therapy. *J Clin Intern Med* 25: 474–477.

Yahav, J., Samra, Z., Niv, Y., Evans, C., Passaro, D., Dinari, G. *et al.* (2006) Susceptibility-guided vs. empiric retreatment of Helicobacter pylori infection after treatment failure. *Dig Dis Sci* 51: 2316–2321.

Zhou, J., Huo, H., Wu, M. and Jiang, X. (2010) Role of drug sensitivity test in the triple therapy for eradication of Helicobacter pylori. *Chin J Gastroenterol* 15: 358–360.

Zullo, A., Hassan, C., Lorenzetti, R., Winn, S. and Morini, S. (2003a) A clinical practice viewpoint: to culture or not to culture Helicobacter pylori? *Dig Liver Dis* 35: 357–361.

Zullo, A., Hassan, C., De Francesco, V., Lorenzetti, R., Marignani, M., Angeletti, S. *et al.* (2003b) A third-line levofloxacin-based rescue therapy for Helicobacter pylori eradication. *Dig Liver Dis* 35: 232–236.