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Culture-based antibiotic susceptibility testing for *Helicobacter pylori* infection: a systematic review

Vincenzo De Francesco[a], Angelo Zullo[b], Raffaele Manta[c], Alissa Satriano[d], Giulia Fiorini[e], Matteo Pavoni[e], Ilaria M. Saracino[e], Fabrizio Giostra[f], Giorgio Monti[f], Dino Vaira[e]

Riuniti Hospital, Foggia; Nuovo Regina Margherita Hospital, Rome; Generale Hospital, Perugia; Villa Aurora Clinic, Foligno, Perugia; University of Bologne; Sant'Orsola Hospital, Bologna, Italy

Abstract

Background Primary antibiotic resistance in *Helicobacter pylori* (*H. pylori*) strains is increasing worldwide, affecting therapy success. The use of therapies tailored on susceptibility pre-testing at culture has been proposed, but data are still conflicting.

Method We performed a systematic review to evaluate the role of a culture-based therapeutic approach for *H. pylori* treatment, taking into account the sensitivity of culture and the success rates achieved with tailored therapies in different therapeutic steps.

Results We analyzed data from 51 studies. Overall, *H. pylori* strains were isolated in 80.7% of 7889 patients, the success rates being 78.1%, 77.5%, 86.3% and 86.6%, before first-, second-, third-line or more therapies, respectively. In comparative studies, the infection was cured in 89.9% of 2052 patients treated with tailored therapies, and in 77.6% of 2516 patients receiving empiric therapy (P<0.001). However, in the subanalysis, the tailored approach achieved optimal eradication rates (>90%) only when it was applied before first- and second-line therapies, but not before third-line or more attempts (<80%). Moreover, no significant difference emerged between the 2 approaches when data from only the most recent (last 5 years) studies were considered, as well as in those performed in Western populations.

Conclusions The attempt to achieve antibiotic susceptibility testing before treatment failed in 20% of infected patients managed in dedicated laboratories. Culture-tailored therapies administered after 2 or more therapies achieved suboptimal eradication rates. The role of bacterial culture in patients whose therapeutic management failed to eradicate *H. pylori* probably needs to be corroborated by further data.

Keywords *Helicobacter pylori*, culture, therapy, resistance

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[a]Gastroenterology Unit, "Riuniti" Hospital, Foggia (Vincenzo De Francesco); [b]Gastroenterology and Digestive Endoscopy, "Nuovo Regina Margherita" Hospital, Rome (Angelo Zullo); [c]Gastroenterology and Digestive Endoscopy, "Generale Hospital", Perugia (Raffaele Manta); [d]Clinical Nutrition Unit, "Villa Aurora" Clinic, Foligno, Perugia (Alissa Satriano); [e]Department of Medical and Surgical Sciences, University of Bologna (Giulia Fiorini, Matteo Pavoni, Ilaria M. Saracino, Dino Vaira); [f]Emergency Unit, Sant'Orsola Hospital, Bologna (Fabrizio Giostra, Giorgio Monti), Italy

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Correspondence to: Dr. Angelo Zullo, Gastroenterologia ed Endoscopia Digestiva, Ospedale Nuovo Regina Margherita, Via Emilio Morosini, 30, 00153, Rome, Italy, e-mail: angelozullo66@yahoo.it

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Introduction

Helicobacter pylori (*H. pylori*) infection plays a major role in the pathogenesis of different gastroduodenal disorders. These comprise benign diseases, such as non-ulcer dyspepsia, peptic ulcer, idiopathic thrombocytopenic purpura, and idiopathic iron deficiency anemia, as well as tumors, including gastric mucosa-associated lymphoid tissue lymphoma and adenocarcinoma [1-5]. Indeed, *H. pylori* eradication definitely changes the natural history of both peptic ulcer disease and gastric lymphoma, and significantly improves dyspeptic symptoms and platelet count. Moreover, the risk of gastric cancer development is reduced by 44% following the cure of *H. pylori* infection [5]. Unfortunately, curing this infection remains challenging for clinicians, as no available empiric therapy regimen is able to achieve bacterial eradication in all treated patients. Therefore, a definite portion of patients requires 2 or more treatments in clinical practice. To overcome the impact of antibiotic resistance, administration of therapies tailored on susceptibility pre-testing at culture has been

proposed as a more rational approach than empirical use of standard treatment regimens. According to the Kyoto consensus, *H. pylori* gastritis is fundamentally an infectious disease, and principles of antimicrobial stewardship focusing on optimization of therapy to reliably achieve high cure rates and prevent resistance to the antimicrobials should be used [6]. As for other infectious diseases, this approach is expected to achieved bacterial eradication in virtually all patients. Moreover, the diffusion of bacterial resistance toward different antibiotics in other bacterial strains might be limited by reducing the use of empiric therapies. Current Italian and European guidelines suggest that susceptibility pretesting should be performed following 2 or more consecutive therapy failures [7,8]. Some studies have even proposed the use of bacterial culture to choose the first-line therapy according to antibiotic susceptibility results [9,10]. Nevertheless, clear evidence favoring the tailored over the empiric treatment is still lacking. In addition, isolating *H. pylori* from gastric biopsies is challenging, and dedicated laboratories for culture, with skilled personnel and specific equipment, are not widespread, limiting the implementation of this approach in clinical practice. Based on these observations, we aimed to evaluate the role of a culture-based therapeutic approach for *H. pylori* treatment by considering the sensitivity of culture and the success rate achieved with tailored therapies in different therapeutic steps.

Materials and methods

Literature review

A systematic literature review was performed to find studies reporting data on: a) *H. pylori* culture sensitivity and/or b) the cure rates with successive tailored treatments. Only those studies that provided these data separately according to different therapeutic steps – from before first-line to 3 or more attempts – were considered. Studies that included pediatric patients and those in languages other than English were excluded, and case series with less than 5 patients were not considered. The search was performed in PubMed for publications from January 1, 2004, through March 5, 2021, using the following algorithm (all fields): (*Helicobacter pylori* OR *H. pylori*) AND culture. Following title and abstract evaluation, the full text of all relevant studies was retrieved, and the reference lists from the selected articles were reviewed to identify additional studies of potential interest. For each study, the variables extracted were as follows: author, year of publication, country, setting (pre- or post-treatment step), number of patients, the type of culture (E-test or agar dilution) as well as the rate of both successful culture and bacterial eradication. Authors were not contacted in case of absent/incomplete data; those studies were excluded from the analysis. Two investigators (VDF and AZ) extracted data according to a specifically designed database and conflicting data were eventually resolved by another investigator (DV).

Statistical analysis

Eradication rates and their 95% confidence intervals (CI) on intention-to-treat (ITT) analysis were computed for each subanalysis. Comparison of cure rates was performed using the chi-squared test. Differences were considered significant at a 5% probability level. Analyses were performed using Statsoft version 7.1 (StatSoft Europe GmbH, 22301 Hamburg, Germany) for Windows 10.

Results

Descriptive analysis

A total of 3015 citations were found, of which 51 met the inclusion criteria on evaluation (Fig. 1) [11-61]. Eleven studies originated from Japan, 10 from China, 7 from each of Italy and Taiwan, 5 from Spain, 4 from Korea, 2 studies from each Australia and Poland, and 1 study from each of Portugal, USA and Israel. The main characteristics of the studies are shown in Table 1.

Culture sensitivity

Data on culture sensitivity separately reported for different lines of treatment were available in 41 studies [11-51]. Overall, *H. pylori* strains were isolated in 6371 (80.7%, 95%CI 79.8-81.6) of 7889 infected patients. More specifically, culture was performed using a single antral specimen in 5053 patients and it was positive in 4052 of them (80.1%, 95%CI 79-81.2), and by antral plus gastric body mucosa specimens in 2836 patients, being positive in 2319 cases (81.7%, 95%CI 80.3-83.1), without a significant difference between groups. Bacterial growth was

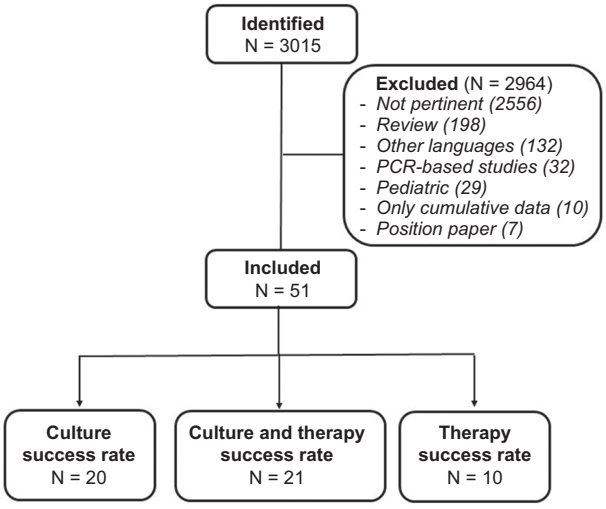

Figure 1 The PRISMA flow diagram
PCR, polymerase chain reaction

Table 1 Characteristics of studies

Ref.	Country	Patients (N)	Study design	Therapy line
31	China	70	Prospective	3
32	Italy	40	Prospective	3
33	China	90	Prospective	1
34	South Korea	219	Prospective	2
35	Spain	104	Prospective	1
36	Spain	221	Retrospective	1
37	Spain	298	Prospective	1
38	Italy	80 83	Randomized Randomized	1 2
39	South Korea	114	Randomized	2
40	China	260	Randomized	2
41	China	813	Randomized	2
42	China	233	Randomized	2
43	Italy	415 310 312	Retrospective Retrospective Retrospective	2 >3
44	China	200	Prospective	3
45	Poland	58	Prospective	3
46	USA	27	Retrospective	3
47	Italy	94	Prospective	3
48	Italy	236	Prospective	3
49	Australia	279	Prospective	3
50	Japan	22	Randomized	2
51	Japan	53	Prospective	2
52	Israel	98	Prospective	3
53	Spain	297	Prospective	1
54	Spain	88	Prospective	1
55	China	356	Randomized	3
56	China	316	Randomized	1
57	Poland	120	Prospective	1
58	China	668	Randomized	1
59	South Korea	146	Retrospective	1
60	South Korea	74	Prospective	1
61	Portugal	42	Retrospective	3

achieved in 2055 (78.1%, 95%CI 76.5-79.7) of 2538 patients before first-line therapy, in 2691 (77.5%, 95%CI 76.1-78.9) of 3470 patients before second-line, in 1313 (86.3%, 95%CI 84.6-88) of 1521 patients before third-line, and in 312 (86.6%, 95%CI 83.1-90.1) of 360 patients following more therapeutic failures. The success rates of culture performed following either 2 (86.3%) or more (86.6%) treatments were significantly higher than those achieved before (78.1%) and after (77.5%) the first-line therapy (P<0.001).

Eradication rates

A total of 31 studies reported data on therapeutic outcomes [31-61]. Of these, 19 studies compared the cure rates between culture-tailored and empiric therapies [31-42,52-58], whilst only the cure rates of tailored therapies were reported in the remaining 12 studies [43-51,59-61].

In comparative studies, the overall cure rate following tailored therapies (1842 of 2052 patients; 89.7%, 95%CI 88.4-91.2) was higher (P<0.001) than that of empiric treatments (1954 of 2516 patients; 77.6%, 95%CI 76-79.2). Specifically, the eradication rates were 91.6% (914/997) and 78.2% (1006/1285), respectively, before first-line therapy (P<0.001), 91.2% (702/769) and 79% (753/953) (P<0.001) before second-line, and 79% (226/286) and 70.1% (195/278) (P=0.03) before third-line (or more) approaches (Table 2). When subanalyses of these data were performed according to either different periods or diverse geographic areas, some differences emerged. Indeed, a comparison of the results of studies published in the last 5 years with earlier studies showed that tailored therapies administered before third-line treatments were significantly more effective than empiric therapy only in the older, but not in the more recent studies (Table 3). Considering the different populations, the overall *H. pylori* cure rates following tailored and empiric therapy were, respectively, 84.3% (2115/2491) and 79.1% (563/711) (P<0.001) in Western and 89.2% (1632/1828) and 77% (1391/1805) (P<0.001) in Asian countries. The success rate of tailored therapies in Western populations was lower than that of Asian patients (84.3% vs. 89.2%; P<0.001), whilst no difference emerged between empiric therapies (79.1% vs. 77%; P=0.25).

Data from 12 non-comparative studies showed a cumulative eradication rate of 83.1% (1885/2268), with values of 88.6% in 220 patients, 82.4% in 490, and 82.5% in 1558 cases, when the culture was performed before first-, second- and third-line (or more) therapies, respectively (Table 4). The cure rate achieved before first-line (88.6%) therapy was significantly higher than that following other steps (82.4% and 83.8%; P=0.04). By totaling all available (comparative and non-comparative) studies, the susceptibility-based approach achieved a cure rate of 91.1% (1109/1217), 87.8% (1106/1259) and 83% (1532/1844) before first-, second- or third-line (or more) therapies, respectively, with a statistically significant (P=0.03) trend in the reduction of cure rates among the 3 steps.

Susceptibility-based method

As far as the method for antibiotic susceptibility testing used in the considered studies is concerned, the E-test was utilized in 19 series with a total of 2935 patients, and the agar dilution method in 12 studies with 1259 cases. In comparative studies, a similar eradication rate was achieved by therapies tailored on agar dilution testing (811 of 905; 89.6%, 95%CI 87.6-91.6) and that based on the E-test (918 of 1021; 89.9%, 95%CI 88-91.7). In non-comparative studies, the overall cure rate achieved in the 4 studies (321 of 354 patients) with

Table 2 Cure rates at intention to treat analysis following culture-tailored and empiric therapies

Culture-tailored* [days]	Cure rate N (%)	Empiric [days]	Cure rate N (%)	P-value	Ref.
Before first-line					
E-A-B-C-F-L-M [14]	41/45 (91.1)	EBAC [14]	33/45 (73.3)	0.03	33
O-A-C-L-M [10]	47/50 (94)	OAC [10]	36/54 (66.6)	<0.001	35
O-A-B-C-M-Tet [10]	103/117 (88)	OAC [10]	51/104 (49)	<0.001	36
O-A-C-Tin [14]	87/89 (97.7)	OACT [10]	182/209 (87)	<0.001	37
R-A-C-L-Tin [10]	39/41 (95.1)	RAL [10]	36/39 (92.3)	0.6	38
O-A-C-L-M [10]	171/181 (94.4)	EACM [10]	103/116 (88.7)	0.07	53
O-A-C-M [10]	39/43 (90.6)	Pylera [10]	43/45 (95.5)	0.37	54
P-A-C-L-M [10]	64/67 (95.5)	PACM [10]	47/53 (88.6)	0.16	57
R-A-C-Tin [10]	282/318 (88.6)	RACTin or RACB [10]	271/350 (77.4)	<0.001	58
P-A-C-M [10]	41/46 (89.1)	PAC [10] or PBMTet [10] or Seq [10]	204/270 (75.5)	0.07	56
Before second-line					
O-A-C-M [10]	117/125 (93.6)	OAC [10]	107/135 (79.2)	<0.001	40
E-A-C-L-M-F [14]	281/313 (89.7)	EAC [14]	405/500 (81)	<0.001	41
E-A-B-L-F [10]	163/182 (89.5)	EBAL [10]	44/51 (86.2)	0.51	42
R-A-C-L-Tin [10]	50/51 (98)	RAL [10]	26/32 (81.2)	0.007	38
P-A-C-M [7]	54/57 (94.7)	PAC [7]	41/57 (71.9)	<0.001	39
E-A-B-M-Tet-Mox [14]	37/41 (90.2)	EBMTet or EAMox[14]	130/178 (73)	0.02	34
Before third-line					
O-A-B-C-L-M-Tet [14]	128/164 (78)	PAL [10]	142/192 (73.9)	0.36	55
E-A-C-L-M-Tet [14]	21/30 (70)	EBMTet [14]	8/10 (80)	0.53	32
O-A-B-C-M-Tet [14]	42/49 (85.7)	OBMTet [10]	31/49 (63.2)	0.01	52
E-A-T-M-L [14]	35/43 (81.4)	EAMTet [14]	14/27 (51.8)	0.008	31

Combinations of 2 or more of the cited drugs

O, omeprazole; P, pantoprazole; E, esomeprazole; R, rabeprazole; A, amoxicillin; B, bismuth; C, clarithromycin; F, furazolidone; L, levofloxacin; M, metronidazole; Mox, moxifloxacin; Tet, tetracycline; Tin, tinidazole; Seq, sequential therapy; Pylera, 3-in-1 capsules containing bismuth subcitrate potassium (140 mg), metronidazole (125 mg), and tetracycline (125 mg)

Table 3 Eradication rates following tailored and empiric therapy in different periods

Therapy step	Period <2016		P-value	Period ≥2016		P-value
	Tailored	Empiric		Tailored	Empiric	
Before first N (%)	317/342 (92.6)	338/447 (75.6)	<0.001	597/655 (91.1)	668/834 (80.1)	<0.001
Before second N (%)	502/546 (91.9)	579/724 (79.9)	<0.001	200/223 (89.6)	174/229 (75.9)	<0.001
Before third (or more) N (%)	42/49 (85.7)	31/49 (63.2)	0.01	184/237 (77.6)	164/229 (71.6)	0.13

therapies tailored on agar dilution was higher than that of 8 studies (1584 of 1914 patients) based on the E-test results (90.6% vs. 82.7%, P<0.001). By totalling the data of all studies, the success rate was significantly (P<0.001) higher when therapies were tailored on agar dilution (1132 of 1254; 89.9%, 95%CI 88.2-91.6) as compared to the E-test (2502 of 2935; 85.2%, 95%CI 84-86.5).

Discussion

Although several factors are involved, primary and secondary bacterial resistance towards the few available antibiotics are the most relevant for *H. pylori* therapy failure. More specifically, resistance to clarithromycin, metronidazole and levofloxacin is increasing worldwide and affects the success rate of therapeutic regimens containing these drugs [10]. In contrast, the prevalence of primary resistance towards amoxicillin, tetracycline and rifabutin remains distinctly low [62,63]. Therefore, tailoring eradication therapy on the antibiotic susceptibility testing is expected to be successful. We performed this study in order to assess the probability of bacterial recovery at culture and to estimate the success rate of antibiotic susceptibility-tailored therapies in different steps of the therapeutic sequence for *H. pylori* eradication.

Overall, our pooled-data analysis found that the bacterial growth failed in 20% of infected patients managed in dedicated microbiology laboratories. The data showed that the success rate of culture was significantly higher when performed following

Table 4 Overall eradication rates at intention to treat following culture-tailored therapies

Culture-tailored* [days]	Cure rate N (%)	Ref.
Before first-line		
O-A-B-C-Tet [7 or 10]	126/146 (86.3)	59
E-A-B-C-L-Tet [7]	69/74 (93.2)	60
Before second-line		
O-A-L-Rif or Seq or Pylera [10]	335/415 (80.7)	43
L-A-M-L [7]	18/22 (81.8)	50
L-A-C-M [7]	51/53 (96.2)	51
Before third-line (or more)		
O-L-A-Rif or Seq or Pylera [10 or 12]	249/310 (80.3)	43
E-A-B-C-L-M [14]	189/200 (94.5)	44
E-A-C-M [7]	32/58 (55.1)	45
E-A-B-M-Tet [10]	12/27 (44.4)	46
O-A-B-L-C-Dox [7]	85/94 (90.4)	47
O-A-L-Rif [10 or 12]	211/236 (89.4)	48
R-A-B-Cip-Rif [10]	265/279 (94.9)	49
E-A-B-L-M-Tet [14]	25/42 (59.5)	61
O-A-L-Rif or Seq or Pylera [10 or 12]	238/312 (76.2)	43

*Combinations of 2 or more of cited drugs

E, esomeprazole; O, omeprazole; R, rabeprazole; A, amoxicillin; B, bismuth; C, clarithromycin; Cip, ciprofloxacin; Dox, doxycycline; L, levofloxacin; M, metronidazole; Rif, rifabutin; Tet, tetracycline; Seq, sequential therapy; Pylera, 3-in-1 capsules containing bismuth subcitrate potassium (140 mg), metronidazole (125 mg), and tetracycline (125 mg)

2 or more therapeutic attempts, compared to that performed in naïve patients or just following first-line therapy, when the value was even lower than 80%. Therefore, the attempt to guide first-line therapy for *H. pylori* failed in 1 of every 5 infected patients. On the other hand, when the culture was attempted in unselected naïve patients who underwent upper endoscopy, the antibiotic susceptibility testing was achieved in only 247 (13.3%) of 1862 cases [59]. Based on all these observations, tailoring first-line therapy seems to be impractical in clinical practice.

By considering data from more than 4500 patients, we compared the cure rates achieved by therapies tailored on antibiotic susceptibility and those empirically administered. As expected, the culture-tailored therapies were more successful overall than empiric regimens. Moreover, another advantage of the tailored strategy is to achieve bacterial eradication, in keeping with the rules of antibiotic stewardship, whilst empiric therapies inevitably lead to the misuse of antibiotics. Nevertheless, optimal (>90%) eradication rates were achieved only when the tailored strategy was adopted before first- and second-line regimens, whilst suboptimal (<80%) cure rates were observed following 2 or more therapy failures, namely when current European guidelines suggest that culture-tailored therapies should be administered [8]. In addition, the therapeutic advantage of culture-tailored first-line therapy was mainly due to the low efficacy of the empiric therapies used as comparator in the studies we evaluated. Indeed, despite the use of first-line regimens including only those antibiotics with a proven susceptibility at culture, the eradication rate was not distinctly higher than 90%. This success rate would appear to be no different to that achieved by the more effective therapies currently available, such as sequential, concomitant and quadruple first-line regimens administered empirically [64]. In addition, the lack of any therapeutic advantage of culture-based therapies clearly emerged in more recent studies, and in Western populations. Some host factors, especially those affecting the proton pump inhibitor metabolism (e.g., CYP 2C19 polymorphisms, etc.) may be involved in the difference between Asian and Caucasian studies.

The failure of an antibiotic-based therapy may depend on several factors, such as drug doses, therapy duration, and potency of the anti-secretory drug used. Moreover, tailored therapy may fail to achieve a 100% eradication rate because of the impossibility of assembling in this phase a successful therapy regimen that includes only the still available antibiotics to which *H. pylori* is susceptible. Indeed, the synergism among different antibiotics, chosen because of the absence of bacterial resistance, could be lacking. For instance, following the administration of a 14-day bismuth–tetracycline–amoxicillin combination, 3 drugs with no or a very low (<5%) primary resistance rate, the eradication rate was as low as 43% [65]. Therefore, apart from antibiotic susceptibility, drug combinations are a matter for concern in *H. pylori*. On other hand, the possibility of a hetero-resistant status—i.e., the coexistence of susceptible and resistant *H. pylori* strains at different gastric sites in the same patient—may undermine the correct classification of antibiotic susceptibility *in vitro* [66]. The same result is expected when antibiotic susceptibility is genetically assessed using a culture-free, polymerase chain reaction-based tool, which overcomes the culture limitation of bacterial growth.

Notably, data from a recent systematic review showed that by adopting an empiric therapeutic sequence with bismuth-based quadruple therapy, levofloxacin- and rifabutin-based regimens following first-line therapy failure, *H. pylori* infection might be cured in virtually all patients, with only 1 in every 170 patients eventually remaining infected [67]. Therefore, the real advantage of resorting to bacterial susceptibility testing before third-line therapy, as suggested in the current guidelines [7,8], probably needs to be corroborated by further data. Finally, our analysis found that the tool used for antibiotic susceptibility testing seem to plays a role in predicting the success rate. Specifically, higher eradication rates were achieved when the therapy was tailored on agar dilution as compared to the E-test. Therefore, the agar dilution method should be preferred when deciding on culture-based antibiotic susceptibility testing, but this method is impractical as it is very laborious and time-consuming.

Some limitations of our study should be acknowledged, such as inclusion of case series with heterogeneous samples (from 20 to 813), the use of an arbitrary timeline, and the absence of any search for publications in other databases. However, we identified as many as 51 studies with more than 4500 patients, so it is highly improbable there are many other studies not present in PubMed able to significantly change the results of this systematic review.

In conclusion, our study found that the probability of isolating *H. pylori* in infected patients by culture in dedicated laboratories is 80%. The overall success rate of tailored

regimens was higher than that of empiric therapies, but data from more recent studies and those performed in Western population failed to find a statistically significant difference, particularly following 2 or more therapy failures. Even when only antibiotics to which the infection is susceptible were used, the eradication rate was not higher than 90%.

Summary Box

What is already known:

- *Helicobacter pylori* (*H. pylori*) eradication remains challenging for clinicians, as no available empiric therapy regimen is able to achieve bacterial eradication in all treated patients
- Primary and secondary bacterial resistance towards antibiotics is the most relevant factor for therapy failure
- Culture-based therapeutic approaches are expected to cure the infection in virtually all treated patients

What the new findings are:

- Data found that *H. pylori* growth failed in 20% of infected patients managed in dedicated microbiology laboratories, particularly when performed in naive patients
- Culture-tailored therapies were more successful than empiric regimens, but optimal (>90%) eradication rates were achieved only when the tailored strategy was adopted before first- and second-line therapies, whilst suboptimal (<80%) cure rates were observed following 2 or more therapy failures
- The lack of advantage of culture-based therapies over empiric approaches emerged in more recent studies, and in the Western populations

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