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ORIGINAL RESEARCH—CLINICAL

Two-dimension Tailor-made Therapy: A New Salvage Therapy After Multiple Eradication Failures for *Helicobacter pylori* Infection

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BACKGROUND AND AIMS: Vonoprazan-based eradication therapies have a higher eradication rate than usual proton pump inhibitor (PPI)–based therapies in treating *Helicobacter pylori* infection. Should we use vonoprazan to treat patients who failed multiple eradication therapies? Because the drug is not available in most countries, we propose 2-dimension tailor-made therapy (2dTMT) without using vonoprazan. **METHODS:** Patients who failed twice or more PPI-based triple therapies were recruited. Patients underwent CYP2C19 genotype and antibiotic susceptibility tests (ASTs). PPI doses per day were decided as per the CYP2C19 genotype: twice for poor and 4 times for extensive metabolizers (dimension 1). Two antibiotics were selected as per the results of the AST in each patient (dimension 2). Regimens of 2dTMT included 2 susceptible antibiotics and a PPI. For those who could not have enough information with the AST, tailor-made PPI dosing was indicated with empirically selected 2 antibiotics (one-dimension tailor-made therapy [1dTMT]). **RESULTS:** Of 51 candidates with multiple eradication failures, 37 patients underwent the genotype test and AST, and 24 succeeded to obtain sufficient information to select 2 susceptible antibiotics. Of them, 22 patients accepted to receive 14-day 2dTMT. Of the residual patients, 12 accepted to receive 14-day 1dTMT. The mean eradication rate of 2dTMT was 86.4% (95% confidence interval [CI]: 65.1%–98.8%) in intention-to-treat and 90.5% (95% CI: 69.6%–98.8%) in per-protocol analyses, whereas that of 1dTMT was 75.0% (95% CI: 42.8%–94.5%) in intention-to-treat and 90.0% (95% CI: 55.5%–99.7%) in per-protocol analyses. **CONCLUSION:** Without vonoprazan, 14-day 2dTMT could be one of the salvage therapies for patients with multiple eradication failures. In cases of insufficient information with the AST, 14-day 1dTMT could be an alternative therapy. Clinical Trials Registry number, UMIN000022154 (https://www.umin.ac.jp/icdr/index.html).

Keywords: Vonoprazan; Proton Pump Inhibitor; CYP2C19 Genotype; Antibiotics Susceptibility Test; Third-line Therapy

Introduction

Helicobacter pylori infection causes chronic gastritis and is related to the pathogenesis of peptic ulcer, gastric cancer, mucosa-associated lymphoid tissue lymphoma, idiopathic thrombocytopenic purpura, hyperplastic gastric polyp, and so on.[1,2] Guidelines from both Western and Eastern countries recommend eradication therapy to cure or prevent the diseases, and eradication therapies are proposed as per the eradication rate and the prevalence of antibiotic-resistant strains.[1,2] In most countries, combination therapies with the proton pump inhibitor (PPI) and 2 or 3 antibiotics are recommended. For example, in Japan, 7-day therapies with amoxicillin and clarithromycin or metronidazole with the PPI are approved as the first- or second-line eradication therapies, respectively.[1] Although the overall eradication rate with the 2 regimens is estimated almost 98%–99%, the rest 1%–2% still need effective third-line or salvage therapies.[1]

Dore et al[3] reported that PPI-based quadruple therapy with omeprazole, tetracycline, metronidazole, and bismuth subcitrate twice a day for 14 days showed a 97% eradication rate in per-protocol (PP) analysis for patients who failed 2 or more treatment courses. However, Gisbert et al[4] reported that the quadruple therapy with the same drugs showed a 67% eradication rate in PP analysis, indicating that the quadruple therapy was not always the best regimen. In cases of eradication failure with PPI-based triple therapy, fluoroquinolone-containing triple or quadruple therapies are recommended.[2] However, for the third-line therapy after twice failures with PPI-based triple therapies, a multicenter randomized controlled study

Abbreviations used in the paper: **1dTMT, one-dimension tailor-made therapy; 2dTMT, two-dimension tailor-made therapy; CI, confidence interval; EUCAST, European Committee on Antimicrobial Susceptibility Testing; ITT, intention to treat; LAL, lansoprazole, amoxicillin, levofloxacin; LAS, lansoprazole amoxicillin, sitafloxacin; MIC, minimum inhibitory concentration; PP, per protocol; PPI, proton pump inhibitor; SNP, single-nucleotide polymorphism; UBT value, delta ^{13}C-CO_2.**

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revealed that an eradication rate with lansoprazole, amoxicillin, and levofloxacin twice a day for 7 days (lansoprazole + amoxicillin + levofloxacin [LAL] therapy) was only 43.7% in PP analysis.[5] Because they did not concern susceptibilities of the bacteria to the antibiotics, the eradication rates may have been lower than those with antibiotics which are selected based on the results of the antibiotic susceptibility test. On the other hand, susceptibility-guided therapies had an 80% eradication rate in PP analysis in a meta-analysis.[6]

Recently, a potassium-competitive acid blocker, vonoprazan, has been available in some countries.[1] Vonoprazan is stronger than usual PPIs in suppressing acid secretion, and the effect was little affected by the CYP2C19 genotype.[7] Therefore, vonoprazan-based eradication therapies have a higher eradication rate than usual PPI-based therapies.[8,9] Vonoprazan is promised in the future salvage therapy; however, the drug is not available in most countries until now. Should we wait and use vonoprazan to treat patients who failed multiple eradication courses?

The PPI has different effects on the patients as per their CYP2C19 genotypes.[10] For example, extensive metabolizers with homozygous or heterozygous CYP2C19 genotypes showed lower effects on the suppression of gastric acid secretion than poor metabolizers. However, if the PPI was administered 4 times a day in extensive metabolizers, gastric acid suppression showed the same as in poor metabolizers who took the PPI twice a day. If the eradication success depends on the acid suppression, the eradication rate may be elevated in extensive metabolizers if they were administered the PPI 4 times a day.[11]

In addition, antibiotics have different pharmacokinetic and pharmacodynamic characteristics.[12] For example, the antibacterial effect of amoxicillin depends on the time above minimum inhibitory concentration (MIC). However, the metabolism of amoxicillin is rapid, so that the effect is limited if the drug was administered twice a day. If the drug was administered 4 times a day, the time above MIC becomes twice longer than that with twice a day, and the antibacterial effects become greater.[11]

To make the eradication rate greater, we took 4 improvements in the salvage therapy as in the following. First, to make PPI effects greater in extensive metabolizers, we measured CYP2C19 genotypes of the patients and increased the frequency of PPI doses per day as per the genotype, namely, tailor-made PPI dosing. Second, to choose effective antibiotics, we performed the antibiotic susceptibility test. Third, to make antibacterial effects greater, we used antibiotics as per their pharmacokinetic and pharmacodynamic profiles. Fourth, we prolonged the duration of eradication therapy from 1 to 2 weeks because longer therapy was reported to be more effective.[2] Combining these 4 improvements, we designed 2-dimension tailor-made therapy (2dTMT) for 14 days without using vonoprazan. To clarify the effect of 14-day 2dTMT as the salvage therapy after twice or more eradication failures, we conducted the present study.

Materials and Methods

Patients

Patients were recruited from those who visited the Outpatient Departments of Gastroenterology or General Medicine, JCHO Shiga Hospital, from June 2004 until April 2016 to receive salvage therapy to eradicate *H pylori* after twice failures with PPI-based first and second eradication therapies. Those who had received more than twice therapies were also included in the study if *H pylori* was still positive after the last therapy. After enough information about the following tests and eradication therapy, patients gave written consent to enter the program of our salvage therapies. Those who had severe liver or renal diseases or other conditions which were thought to be inadequate were excluded. Those who discontinued previous eradication therapies because of adverse events were also excluded.

Tests for the Serum H pylori Antibody and CYP2C19 Genotype

Blood sample was obtained from each patient after informed consent for measuring serum *H pylori* antibody and single-nucleotide polymorphism (SNP) in the CYP2C19 gene. The antibody was measured with E-plate Eiken *H pylori* IgG antibody II (Eiken Chemical Co, Ltd, Tokyo, Japan). SNPs in the CYP2C19 gene were measured in the laboratory of BioMedical Laboratories, Inc (Tokyo, Japan). Briefly, DNA was isolated from peripheral blood, and mutations from guanine to adenine substitution at the position 681 of exon 5 (CYP2C19*2) and at the position 636 of exon 4 (CYP2C19*3) were identified with Invader assay.[13–16] The oligonucleotides used are listed in the supplementary table (Table A2). As per the results of the SNPs, patients were classified into 6 combinations of alleles (genotype) and 3 CYP2C19 phenotypes: homozygous and heterozygous extensive metabolizers and poor metabolizers as in Table 1.[17]

Antibiotic Susceptibility Test

Patients underwent esophagogastroduodenoscopy to take 2 biopsy specimens for *H pylori* culture from the greater curvature of the antrum and the middle corpus of the stomach. Each biopsy specimen was placed in a microaerophilic transportation medium for *H pylori* (Seed Tube, Eiken Chemical Co, Ltd) and sent to the laboratory of SRL Inc (Tokyo, Japan), where *H pylori* was cultured and then MICs to amoxicillin, clarithromycin, and metronidazole were measured with the agar dilution method. MICs to levofloxacin and minocycline were added since 2012. The MIC was measured separately with 2 biopsy specimens from antrum and corpus, and the larger MIC was adopted for determining susceptibility. Breakpoints were adopted from clinical breakpoints and dosing of antibiotics of the European Committee on Antimicrobial Susceptibility Testing (EUCAST Clinical Breakpoint Tables v. 11.0, valid from 2021-01-01: https://www.eucast.org/clinical_breakpoints/) (Table 2). The breakpoint of minocycline was not described in EUCAST's list, so we adopted that of tetracycline because minocycline was classified in tetracycline-family antibiotics.

Table 1. CYP2C19 Genotypes and Phenotypes as Per the Combinations of Single-nucleotide Polymorphisms

Exon	Position	Pair		Pair		Pair		Pair		Pair		Pair	
5	681	G	G	G	A	G	G	A	G	A	A	G	G
4	636	G	G	G	G	G	A	G	A	G	G	A	A
Genotype		*1/*1		*1		*1		*2		*2		*3	
Phenotype		HomoEM		HeteroEM						PM			

Genotype *1: wild type; *2: G681A; *3: G636A.
EM, extensive metabolizer; hetero, heterozygous; homo, homozygous; PM, poor metabolizer.

Two-dimension Tailor-made Therapy

Our policy is that the dose of the PPI should be determined based on the patient's CYP2C19 genotype (dimension 1) and that the antibiotics should be selected based on the susceptibility of the bacteria (dimension 2). That is why we call the therapy 2dTMT.

For dimension 1, the frequency of PPI doses was twice a day for poor metabolizers and 4 times a day for extensive metabolizers as per the CYP2C19 genotype. One of the PPIs was selected from the available 4 PPIs: lansoprazole 30 mg, omeprazole 20 mg, esomeprazole 20 mg, and rabeprazole 10 mg. Lansoprazole was preferably selected in the early period of the study because it was the only PPI approved for *H pylori* therapy at that time, and then, esomeprazole or rabeprazole was selected as they were approved for eradication therapy. Although esomeprazole and rabeprazole (second-generation PPIs) are reported to be more effective in eradicating *H pylori* than omeprazole and lansoprazole (first-generation PPIs),[18] it is also reported that acid suppression was greater in poor metabolizers than that in extensive metabolizers in all PPIs.[19] In fact, eradication was failed in some cases in which they had used esomeprazole or rabeprazole in the preceding therapies as shown later. Therefore, we did not concern about which PPI was selected, and all the PPIs were prescribed as per the CYP2C19 genotype as described previously.

For dimension 2, we selected 2 antibiotics from the results of the antibiotic susceptibility test. If the MIC was below the breakpoint, the antibiotic was included in the candidates for 2dTMT. The priority order to choose antibiotics was amoxicillin, clarithromycin, metronidazole, levofloxacin, and minocycline in accordance with the history of the proposal of the Japanese Society for Helicobacter Research.[1,20] In patients for whom minocycline was selected because of multiple antibiotic-resistant strain, 0.5 g bismuth subnitrate was added 4 times a day (2 g per day) because PPI-based quadruple therapies usually contain the bismuth compound with tetracycline.[3] Because bismuth subcitrate was not available in Japan, we used bismuth subnitrate which was approved for diarrhea. If we could not find 2 susceptible antibiotics, we treated with one-dimension tailor-made therapy (1dTMT) as described later.

Doses and frequencies of the antibiotics were decided as per the pharmacokinetic/pharmacodynamic properties.[21] Briefly, amoxicillin: 250 mg or 500 mg 4 times a day; clarithromycin: 200 mg twice a day; metronidazole: 250 mg twice a day; levofloxacin: 500 mg once a day; and minocycline: 100 mg twice a day were administered.

The duration of the therapy was 14 days. To expect antidiarrhea effect, 4 tablets per day of antibiotic-resistant *Streptococcus faecalis* (Biofermin-R, Biofermin Co, Kobe, Japan) were added in most patients.

Table 2. Breakpoints of the Antibiotics

Antibiotics	Abbreviation	S/R	Breakpoint (μg/mL)
Amoxicillin	AMPC	S R	≤0.125 >0.125
Clarithromycin	CAM	S R	≤0.25 >0.5
Metronidazole	MNZ	S R	≤8 >8
Levofloxacin	LVFX	S R	≤1 >1
Minocycline	MINO	S R	≤1 >1

Breakpoints were adopted from EUCAST 2021: clinical breakpoints and dosing of antibiotics.
R, resistant; S, susceptible.

One-dimension Tailor-made Therapy

During the study period, we could not always obtain enough information on the MICs because of the failure of bacterial culture, the patient's refusal, avoidance of biopsy during antithrombotic therapy, or the lack of examined antibiotics in the early period of the study. For those who did not have enough information on the antibiotics' susceptibility, amoxicillin and levofloxacin were selected empirically for the salvage therapy. For those who had resistant bacteria to multiple antibiotics, we empirically selected amoxicillin, minocycline, and bismuth subnitrate with a PPI as in 2dTMT. The frequency of PPI administration per day was determined as per the CYP2C19 genotype as in 2dTMT. After all, the regimens included 2 antibiotics and a PPI, but at least one of the antibiotics was selected empirically, and the PPI was used with tailor-made dosing, so the therapies were called 1dTMTs. Duration of the therapy was 2 weeks, and 4 Biofermin R tablets were also administered.

Evaluation of Eradication Therapy

Eradication was evaluated mainly with the ^{13}C-urea breath test. The urea breath test was performed 2 months or later after finishing the therapy. Briefly, 100 mg ^{13}C-urea (UBIT tablet, Otsuka Pharmaceutical Co, Tokyo, Japan) was ingested by the patient, and expiration gas was taken before and 20 minutes after ingestion, and then, delta $^{13}C\text{-}CO_2$ (UBT value) was measured with an isotope-selective infrared spectral analyzer (POC one, Otsuka Electronics Co, Osaka, Japan). To

make the UBT value accurate as possible, we took patients' breath at the end of expiratory phase to collect high-concentration carbon dioxide gas to reduce false results.[22] In patients whose UBT value was near the cutoff value, a single test with urea breath test was not adequate to evaluate eradication, so a stool antigen test or other *H pylori* tests were added. For the stool antigen test, we used Meridian HpSA ELISA II (Fujirebio Inc, Tokyo, Japan). The serum antibody test was performed before and approximately a year after the therapy. If the antibody titer declined more than half of that before the therapy, eradication success was suggested. Endoscopic examination was performed approximately a year after the therapy, and 3 biopsy specimens were taken from the greater curvature of the antrum and middle corpus and incisura angularis for histology and 2 biopsy specimens from the greater curvature of the antrum and middle corpus for bacterial culture. Specimens for culture were proceeded as mentioned previously. Specimens for histology were stained with hematoxylin and eosin and Giemsa staining. No *H pylori* and no neutrophil infiltration in all specimens were the findings for negative infection in pathology. Finally, we evaluated eradication success with the combination of the abovementioned tests.

The eradication rate was calculated with intention-to-treat (ITT) analysis and PP analysis. The mean eradication rate and 95% confidence interval (95% CI) were calculated. To demonstrate and evaluate the impact of the eradication rate in each regimen, we calculated the *P* value with the Clopper-Pearson binomial sample test in comparison with the virtual therapy which had a 50% eradication rate using IBM SPSS statistics 25. PP analyses were adopted in ad hoc analyses.

Ethics

Patients were informed of the tests and the following therapies and then accepted to undergo the abovementioned tests and the salvage therapies. The tests and eradication therapies were approved by the Ethics Committee of JCHO Shiga Hospital. This retrospective study was also approved by the Institutional Review Board of JCHO Shiga Hospital. Candidates of the study were given a chance to opt out as per the Ethical Guidelines for Medical and Health Research Involving Human Subjects issued by Japan Ministry of Health, Labour and Welfare in 2015. The study was enrolled as UMIN000022154. All authors had access to the study data and reviewed and approved the final article.

Results

Forty-one patients with persistent *H pylori* infection after twice eradication therapies and 10 cases after 3 or more eradication therapies were enrolled in the study. No patients reported insufficient ingestion of drugs during the previous therapies, indicating they had good adherence. Among the total 51 patients, 6 refused the salvage therapy and one revealed that the *H pylori* was already eradicated without any salvage therapies. Thus, 44 patients were recommended to undergo the CYP2C19 genotype test, but 7 refused. Then, 37 patients underwent endoscopy, and 24 patients succeeded to obtain sufficient information from the antibiotic susceptibility test to select 2 susceptible antibiotics. Among them, 2 refused to take medication for 14 days. Thus, 22 patients received 14-day 2dTMT (Figure, right arm). Among 13 patients who did not have sufficient information from the antibiotic susceptibility test, 1 refused 14-day therapy. Thus, 12 patients received 14-day 1dTMT (Figure, left arm).

The sex and age distribution of the 34 patients who underwent 14-day 2dTMT or 1dTMT were analyzed. The female-to-male ratio was 22:12, and the mean age was 56.1 years old (range: 33–79, standard deviation: 13.4, Table A3). Upper gastrointestinal tract diseases of the patients were peptic ulcer diseases, gastric polyp, and chronic gastritis in 14 (41.2%) subjects, 1 (2.9%) subject, and 19 (55.9%) subjects, respectively (Table A4). Twenty-eight patients (82.4%) had failed twice therapies, and 6 patients (17.6%) had failed 3 or more therapies before the tailor-made therapies (Table A5).

Details of the cases in the study are shown in Table 3. Three patients withdrew, but the other patients completed the therapy. The infection in a patient (ID 10) had previously been eradicated, but he had revealed reinfection, and only the data of reinfection are listed here. As shown in the top 11 cases of 2dTMT in Table 3, 4 cases had both amoxicillin- and clarithromycin-susceptible and 7 cases had both amoxicillin- and metronidazole-susceptible strains even after 7-day amoxicillin and clarithromycin and then 7-day amoxicillin and metronidazole PPI-based triple therapies. The CYP2C19 genotype was an extensive metabolizer in all 11 cases. The former 4 cases were successfully eradicated with 2dTMT with amoxicillin and clarithromycin, and 6 of 7 latter cases were successfully eradicated with 2dTMT with amoxicillin and metronidazole. A patient (ID 23), who was an extensive metabolizer, had failed 5 courses of therapies including prolonged-duration therapies and finally succeeded with 2dTMT with 4 times a day of the PPI and amoxicillin. The next 5 cases in Table 3 had 2-antibiotic (clarithromycin and metronidazole)–resistant but levofloxacin-susceptible strains and were prescribed with 2dTMT with amoxicillin and levofloxacin. Four of the 5 cases were evaluated and revealed eradication success. The bottom 6 cases of 2dTMT in Table 3 had 3-antibiotic–resistant but minocycline-susceptible strains and were prescribed with 2dTMT using amoxicillin, minocycline, and bismuth subnitrate. Eradication was achieved in 5 of 6 cases. ID 509 was a case previously reported elsewhere.[23] The evaluation of amoxicillin susceptibility in the present study is different from that in the previous report because the breakpoint of MIC was different at that time in Japan from EUCAST 2021.

As shown in the top 5 cases of 1dTMT in Table 3, we could not have any information about antibiotic susceptibility and indicated 1dTMT with empirically selected amoxicillin and levofloxacin. A case withdrew, and a case revealed eradication failure, but 3 cases were successfully eradicated. The ID 50 patient, who had a failure with 1dTMT, wanted to have another therapy and was listed in the following as a different case (ID 51). The next 3 cases in Table 3 had 2-antibiotic–resistant strains, but we could not

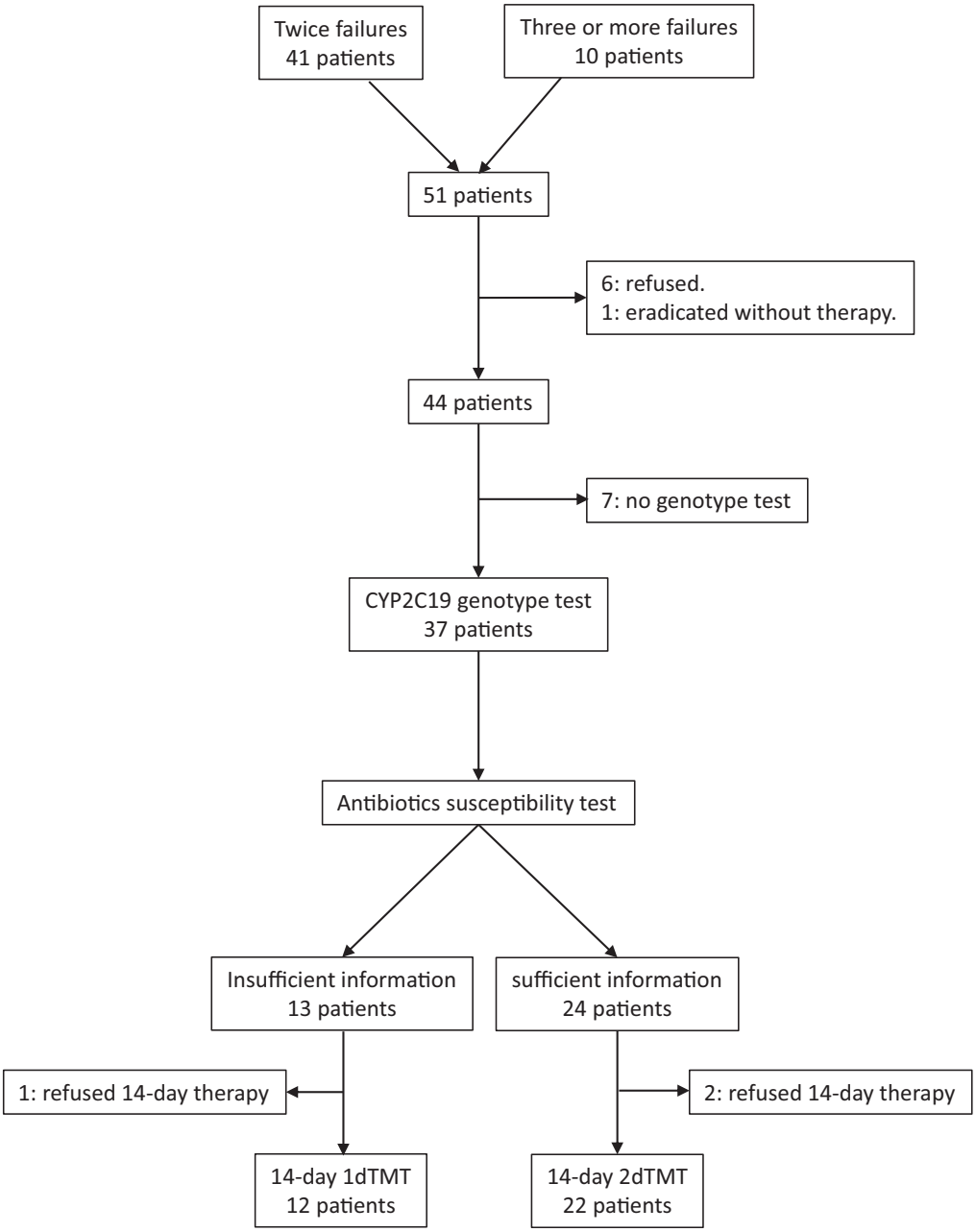

Figure. The flowchart of the patients.

have information about susceptibility to the other antibiotics at that time. Therefore, we indicated 1dTMT with empirically selected levofloxacin, but a case withdrew, and the rest 2 cases had eradication success. A case of ID 7 had a 3-antibiotic–resistant but minocycline-susceptible strain. We could select 2dTMT but performed 1dTMT with an increased dose of amoxicillin with levofloxacin because the doctor hesitated to use minocycline at that time, although eradication was a success. The bottom 3 cases of 1dTMT in Table 3 had 4-antibiotic–resistant strains. ID 51 was the case transferred from the previous 1dTMT as described before. We could not select 2 susceptible antibiotics and decided to do 1dTMT with an increased dose of amoxicillin with minocycline and bismuth subnitrate. The 3 cases had eradication success. A patient (ID 24) failed 4 courses of previous therapies, one of which included vonoprazan, but succeeded with 1dTMT without vonoprazan.

In 2dTMT, lansoprazole, rabeprazole, or esomeprazole was used in 10, 7, or 5 cases, respectively, whereas in 1dTMT, 10 or 2 cases used lansoprazole or rabeprazole, respectively. All cases using amoxicillin and levofloxacin used lansoprazole in either 2dTMT or 1dTMT. Four patients had adverse events, but no serious adverse events were reported in either of the groups (Table 3).

Summary of the antibiotic susceptibility, selected regimens, and CYP2C19 genotype of the patients are shown in Table 4. The numbers of patients who were administered amoxicillin with clarithromycin, metronidazole, levofloxacin, or minocycline were 4, 7, 5, or 6, respectively, in 2dTMT, and those with levofloxacin or minocycline were 9 or 3,

Table 3. Details of the Cases With 2dTMT and 1dTMT

2dTMT

ID	Age	Sex	Disease	First	Second	Third or more therapies	MIC	CYP2C19	2dTMT	Biofermin R	Result	Memo
10	48	M	DU	LAC	LAM		AMPC0.06S, CAM<0.03S, MNZ 0.12S	HeteroEM (G681G/G, G636G/A)	L30x4, AMPC500x4, CAM200x2, 14d	-	Success	Previously eradicated patient. This is for the reinfection. LAC and LAM therapies had been tried but failed for the reinfection.
19	42	M	GU	LAC	LAM		AMPC<0.03S, CAM<0.03S, MNZ2S, LVFX0.25S, MINO0.12S	HeteroEM (G681A G/A, G636A G/G)	R10x4, AMPC250x4, CAM200x2, 14d	4 tablets/d	Success	
30	68	F	CG	OAC	OAM		AMPC<0.03S, CAM<0.03S, MNZ32R, LVFX0.12S, MINO<0.03S	HomoEM (G681A G/G, G636A G/G)	R10x4, AMPC250x4, CAM200x2, 14d	4 tablets/d	Success	
44	38	F	CG	LAC	LAM		AMPC<0.03S, CAM<0.03S, MNZ4S, LVFX0.25S, MINO0.12S	HomoEM (G681A G/G, G636A G/G)	R10x4, AMPC500x4, CAM200x2, 14d	4 tablets/d	Success	
12	44	F	CG	LAC	LAM		AMPC<0.03S, CAM8R, MNZ2S, LVFX0.25S, MINO0.06S	HeteroEM (G681A G/A, G636A G/G)	R10x4, AMPC250x4, MNZ250x2, 14d	4 tablets/d	Success	
14	43	F	CG	LAC	RAM		AMPC<0.03S, CAM1R, MNZ1S, LVFX0.5S, MINO0.12S	HeteroEM (G681A G/A, G636A G/G)	E20x4, AMPC250x4, MNZ250x2, 14d	-	Success	Tongue pain at the end of the therapy.
15	35	M	CG	LAC	LAM		AMPC<0.03S, CAM1R, MNZ1S	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, MNZ250x2, 14d	4 tablets/d	Success	
23	37	M	CG	LAC	LAM	L30x2, AMPC750x2, MNZ250x2, 14d; R10x2, MNZ250x2, LVFX300x2, 7d; R10x2, AMPC500x4, LVFX500x1, 14d	AMPC<0.03S, CAM16R, MNZ4S	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, MNZ250x2, 14d	-	Success	

Table 3. Continued

2dTMT

ID	Age	Sex	Disease	First	Second	Third or more therapies	MIC	CYP2C19	2dTMT	Biofermin R	Result	Memo
26	71	M	CG	LAC	LAM		AMPC<0.03S, CAM16R, MNZ4S, LVFX4R, MINO0.06S	HomoEM (G681A G/G, G636A G/G)	E20x4, AMPC250x4, MNZ250x2, 14d	4 tablets/d	Success	He had slightly abnormal liver function test before the 2dTMT, but the data did not change after the therapy.
29	69	M	GU	PAC	PAM		AMPC<0.03S, CAM16R, MNZ4S, LVFX0.5S, MINO0.12S	HeteroEM (G681A G/G, G636A G/A)	E20x4, AMPC250x4, MNZ250x2, 14d	4 tablets/d	Failure	
34	46	F	CG	LAC	PAM		AMPC<0.03S, CAM4R, MNZ2S, LVFX0.12S, MINO<0.03S	HomoEM (G681A G/G, G636A G/G)	E20x4, AMPC500x4, MNZ250x2, 14d	4 tablets/d	Success	
31	53	M	CG	LAC	LAM		AMPC<0.03S, CAM8R, MNZ64R, LVFX<0.03S, MINO0.06S	HeteroEM (G681A G/G, G636A G/A)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
35	60	M	DU	LAC	LAM		AMPC<0.03S, CAM16R, MNZ64R, LVFX0.25S, MINO0.12S	HeteroEM (G681A G/A, G636A G/G)	L30x4. AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
57	33	F	CG	LAC	LAM		AMPC<0.03S, CAM16R, MNZ32R, LVFX0.12S, MINO<0.03S	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
46	46	F	CG	LAC	PAM		AMPC<0.03S, CAM16R, MNZ 128R, LVFX0.25S, MINO<0.03S	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
18	53	F	CG	EAC	LAM		AMPC<0.03S, CAM4R, MNZ16R, LVFX0.12S, MINO0.06S	HeteroEM (G681A G/A, G636A G/G)	L30x4, AMPC250x4, LVFX500x1, 14d	4 tablets/d	Withdrawal	She did not come to evaluate.
9	53	M	CG	RAC	RAM		AMPC0.06S, CAM16R, MNZ64R, LVFX4R, MINO<0.03S	HomoEM (G681A G/G, G636A G/G)	R10x4, AMPC500x4, MINO100x2, Bis0.5gx4, 14d	4 tablets/d	Success	
13	73	F	GU	OAC	EAM		AMPC<0.03S, CAM32R, MNZ128R, LVFX4R, MINO0.12S	PM (G681A A/A, G636A G/G)	R10x2, AMPC500x4, MINO100x2, Bis0.5gx4, 14d	-	Success	
17	51	F	GP	LAC	LAM		AMPC<0.03S, CAM8R, MNZ16R, LVFX8R, MINO0.06S	PM (G681A A/A, G636A G/G)	E20x2, AMPC250x4, MINO100x2, Bis0.5gx4, 14d	4 tablets/d	Success	Fever at the tenth day, but not discontinued.

Table 3. Continued

2dTMT

ID	Age	Sex	Disease	First	Second	Third or more therapies	MIC	CYP2C19	2dTMT	Biofermin R	Result	Memo
39	76	F	CG	RAC	RAM		AMPC<0.03S, CAM16R, MNZ64R, LVFX8R, MINO0.12S	HeteroEM (G681A G/A, G636A G/G)	R10x4, AMPC500x4, MINO100x2, Bis0.5gx4, 14d	4 tablets/d	Success	
54	60	M	GU	LAC	EAM		AMPC0.12S, CAM16R, MNZ64R, LVFX4R, MINO0.06S	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC250x4, MINO100x2, Bis0.5gx4, 14d	-	Failure	
509	46	F	CG	LAC	RAM	L30x2, AMPC750x2, CAM800x2, 7d, sequentially followed by L30x2, AMPC750x2, MNZ250x2, 7d	AMPC0.12S, CAM8R, MNZ32R, LVFX32R, MINO<0.03S	HeteroEM (G681 G/A, G636 G/G)	L30x4, AMPC500x4, MINO100x2, Bis0.5gx4, 14d	-	Success	

1dTMT

ID	Age	Sex	Disease	First	Second	Third or more therapies	MIC	CYP2C19	1dTMT	Biofermin R	Result	Memo
2	71	F	CG	OAC	RAM		Culture failed	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
25	50	F	DU	PAC	PAM		Culture refused	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
45	67	F	GU + DU	PAC	LAM		Culture failed	PM (G681A G/A, G636A G/A)	L30x2, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
48	66	M	CG	PAC	PAM		Culture failed	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Withdrawal	He did not take medicine.
50	71	F	GU	LAC	LAM		Culture failed	HeteroEM (G681A G/G, G636A G/A)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Failure	Another 1dTMT was performed (ID: 51).
6	79	F	CG	LAC	RAM		AMPC0.12S, CAM32R, MNZ128R	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	Soft stool at day 5–7.
8	39	F	DU	LAC	PAM		AMPC<0.03S, CAM 64R, MNZ32R	HeteroEM (G681A G/A, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
59	51	M	GU	OAC	LAM	L30x2, CAM400x2, MNZ250x2, 7d	AMPC<0.03S, CAM8R, MNZ64R	HomoEM (G681S G/G, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Withdrawal	He did not come to evaluate.

Table 3. Continued

1dTMT

ID	Age	Sex	Disease	First	Second	Third or more therapies	MIC	CYP2C19	1dTMT	Biofermin R	Result	Memo
7	60	F	GU	LAC	LAM		AMPC<0.03S, CAM8R, MNZ128R, LVFX4R, MINO<0.03S	HomoEM (G681A G/G, G636A G/G)	L30x4, AMPC500x4, LVFX500x1, 14d	4 tablets/d	Success	
24	68	F	CG	OAC	OAM	R20x2, MNZ250x2, LVFX100x3, 7d; Vonoprazan20x2, MNZ250x2, LVFX500x1, 7d	AMPC1R, CAM4R, MNZ128R, LVFX16R, MINO0.12S	HeteroEM (G681A G/A, G636A G/G)	R10x4, AMPC500x4, Mino100x2, Bis0.5gx4, 14d	4 tablets/d	Success	
49	71	F	GU	OAC	OAM	O20x2, AMPC750x2, CAM800x2, 14d	AMPC2R, CAM16R, MNZ8R, LVFX4R, MINO0.25S	HeteroEM (G681A G/A, G636A G/G)	L30x4, AMPC500x4, Mino100x2, Bis0.5gx4, 14d	4 tablets/d	Success	
51	71	F	GU	LAC	LAM	L30x4, AMPC500x4, LVFX500x1, 14d	AMPC0.25R, CAM2R, MNZ16R, LVFX32R, MINO0.12S	HeteroEM (G681A G/G, G636A G/A)	R10x4, AMPC500x4, Mino100x2, Bis0.5gx4, 14d	4 tablets/d	Success	Re-entered from ID 50. Soft stool at day 4–14.

In the Disease column, DU, duodenal ulcer; GU, gastric ulcer; GP, gastric polyp; CG, chronic gastritis only.
In First and Second therapies columns, the first letter means the proton pump inhibitor; E, esomeprazole 20 mg; L, lansoprazole 30 mg; O, omeprazole 20 mg; R, rabeprazole 10 mg; P, unknown proton pump inhibitor, and the second and third letters mean antibiotics used; A, amoxicillin 750 mg; C, clarithromycin 200 or 400 mg; M, metronidazole 250 mg, each twice per day for 7 days as approved by medical insurance in Japan.
In Third or more therapies and 2dTMT columns, the first letter means the proton pump inhibitor as described previously, and the following abbreviations mean antibiotics; AMPC, amoxicillin; CAM, clarithromycin; MNZ, metronidazole; LVFX, levofloxacin; MINO, minocycline; Bis, bismuth subnitrate, and the values and numbers mean doses (mg each ingestion except for bismuth) and times per day and the duration of the therapy; d, days.
In the MIC column, the abbreviations of antibiotics are the same as the abovementioned abbreviations, and the values mean minimum inhibitory concentration (MIC) (μg/mL), followed by susceptibility as per EUCAST 2021; R, resistant; S, susceptible.
ID 509 was the same case as previously reported elsewhere (Ref. 23). The evaluation of amoxicillin susceptibility is different from that in the previous report because the breakpoint was different at that time in Japan from EUCAST2021.
ID, identification number for this research only.

Table 4. Summary of the Results of AST, Antibiotics Used, and CYP2C19 Genotype

Results of AST						CYP2C19 genotype		
AMPC	CAM	MNZ	LVFX	MINO	Antibiotics used	EM	PM	Total
2dTMT								
S	S	WE	WE	WE	AMPC + CAM	4	0	4
S	R	S	WE	WE	AMPC + MNZ	7	0	7
S	R	R	S	WE	AMPC + LVFX	5	0	5
S	R	R	R	S	AMPC + MINO + Bis	4	2	6
						20	2	22
1dTMT								
U	U	U	U	U	AMPC + LVFX	4	1	5
S	R	R	U/R	U		4	0	4
R	R	R	R	S	AMPC + MINO + Bis	3	0	3
						11	1	12

AMPC, amoxicillin; Bis, bismuth subnitrate; CAM, clarithromycin; EM, extensive metabolizer; LVFX, levofloxacin; MINO: minocycline; MNZ, metronidazole; PM, poor metabolizer; R, resistant; S, susceptible; U, unknown (no results); WE, whatever it is.

respectively, in 1dTMT. The numbers of patients whose genotype was an extensive metabolizer or poor metabolizer were 20 or 2 in 2dTMT and 11 or 1 in 1dTMT, respectively.

Summary of eradication was shown in Table 5. In 2dTMT, in which 2 susceptible antibiotics were used, the eradication rate of each regimen was not significantly higher than that of the virtual therapy with a 50% eradication rate, probably due to the small size of the number of patients. However, in total, the eradication rate of 2dTMT was significantly higher ($P < .001$) in both ITT and PP analyses (mean: 86.4%; 95% CI: 65.1–97.1 in ITT and mean: 90.5%; 95% CI: 69.6–98.8 in PP analyses) than that of the virtual therapy (Table 5, upper table). In 1dTMT, in which at least one antibiotic was empirically selected, the eradication rate of each regimen was not significantly higher than that of the virtual therapy with a 50% eradication rate. In total 1dTMT, the eradication rate was not significantly higher than that of the virtual therapy in ITT analysis (mean: 75.0%; 95% CI 42.8–94.5), but significantly higher in PP analysis (mean: 90.0%; 95% CI: 55.5–99.7, $P = .021$) (Table 5, middle table). When combining 2 tailor-made therapies, the eradication rate was significantly higher in the therapies with amoxicillin and levofloxacin or minocycline than that of the virtual therapy in PP analyses ($P < .05$). In total tailor-made therapies, the eradication rate was significantly higher ($P < .001$) in both ITT and PP analyses (mean: 82.4%; 95% CI: 65.5–93.2 in ITT; mean: 90.3%: 95% CI: 74.2–98.0 in PP analyses) than that of the virtual therapy (Table 5, lower table).

From the viewpoint of the CYP2C19 genotype, the eradication rate of poor metabolizers was 100%, but it was not significantly higher than that of the virtual therapy with a 50% eradication rate in either 2dTMT or 1dTMT because of the small numbers of the patients (Table 6). On the other hand, heterozygous, homozygous, or total extensive metabolizers had a significantly higher eradication rate in 2dTMT than that of the virtual therapy in PP analyses (Table 6, upper table). In 1dTMT, the eradication rate was not significantly higher in either heterozygous or homozygous metabolizers than that of the virtual therapy, but significantly higher in total extensive metabolizers ($P = .039$, Table 6, middle table). Combined with 2 tailor-made therapies, heterozygous, homozygous, and total extensive metabolizers had a significantly higher eradication rate than that of the virtual therapy in PP analyses (85.7%, 92.9%, and 89.3%, respectively, Table 6, lower table). Furthermore, in total patients including all genotypes, the eradication rate was significantly higher in 2dTMT, 1dTMT, or total tailor-made therapies (90.5%, $P < .001$; 90.0%, $P < .05$; 90.3%, $P < .001$) than that of the virtual therapy in PP analyses (Table 6).

Discussion

In the present study, we found that the eradication success was obtained in 86.4% (ITT) and 90.5% (PP) in patients who took 2dTMT after multiple eradication failures (Table 5, upper table). Although the present study was not a randomized prospective controlled study, the rate of eradication success in 2dTMT was significantly higher than that of the virtual therapy which had a 50% eradication rate ($P < .001$). We did not obtain a significant superiority of 2dTMT to 1dTMT because the study was not a comparative study and the patients' number was small. However, when combined with 1d and 2dTMTs, the eradication rate of total tailor-made therapies was 82.4% in ITT and 90.3% in PP analyses (Table 5, lower table), and the rates were significantly higher ($P < .001$) than that of the virtual therapy. In the viewpoint of the CYP2C19 genotype, the eradication rates of extensive metabolizers were significantly higher in 2dTMT, 1dTMT, or total tailor-made therapies than that of the virtual therapy. These results suggest that 14-day 2dTMT could be a new salvage therapy for *H pylori* infection after multiple eradication failures, and 14-day 1dTMT could be an alternative therapy in cases with insufficient information with the antibiotic susceptibility test.

Table 5. Summary of Eradication Therapies

	Numbers of patients			Eradication rate, mean		PP analysis in each regimen		
Antibiotics used	Total (ITT)	Total (PP)	Success	ITT	PP	95% CI	*P* value	Significance
2dTMT								
AMPC + CAM	4	4	4	100.0%	100.0%–	42.1–100.0	$P = .125$	NS
AMPC + MNZ	7	7	6	85.7%	85.7%–	42.1–99.6	$P = .125$	NS
AMPC + LVFX	5	4	4	80.0%	100.0%–	42.1–100.0	$P = .125$	NS
AMPC + MINO + Bis	6	6	5	83.3%	83.3%–	35.9–99.6	$P = .219$	NS
Total	22	21	19	86.4%	90.5%			
			95% CI	65.1–97.1	69.6–98.8			
			P value	$P < .001$	$P < .001$			
			Significance	***	***			
1dTMT								
AMPC + LVFX	9	7	6	66.7%	85.7%–	42.1–99.6	$P = .125$	NS
AMPC + MINO + Bis	3	3	3	100.0%	100.0%–	29.2–100.0	$P = .250$	NS
Total	12	10	9	75.0%	90.0%			
			95% CI	42.8–94.5	55.5–99.7			
			P value	$P = .146$	$P = .021$			
			Significance	NS	*			
Total TMTs								
AMPC + CAM	4	4	4	100.0%	100.0%–	42.1–100.0	$P = .125$	NS
AMPC + MNZ	7	7	6	85.7%	85.7%–	42.1–99.6	$P = .125$	NS
AMPC + LVFX	14	11	10	71.4%	90.9%–	58.7–99.8	$P = .012$	*
AMPC + MINO + Bis	9	9	8	88.9%	88.9%–	51.8–99.7	$P = .039$	*
Total	34	31	28	82.4%	90.3%			
			95% CI	65.5–93.2	74.2–98.0			
			P value	$P < .001$	$P < .001$			
			Significance	***	***			

*$P < .05$; **$P < .01$; ***$P < .001$.
AMPC, amoxicillin; Bis, bismuth subnitrate; CAM, clarithromycin; LVFX, levofloxacin; MINO, minocycline; MNZ, metronidazole; NS, not significant.

Table 6. Eradication Rates in Different CYP2C19 Genotypes

				ERadication rate, mean		PP analysis		
CYP2C19 genotype	ITT	PP	Success	ITT	PP	95% CI	*P* value	Significance
2dTMT								
PM	2	2	2	100.0%	100.0%–	15.8–100.0	$P = .500$	NS
Hetero EM	10	9	8	80.0%	88.9%–	51.8–99.7	$P = .039$	*
Homo EM	10	10	9	90.0%	90.0%–	55.5–99.7	$P = .021$	*
(EM, total)	20	19	17	85.0%	89.5%–	66.9–98.7	$P < .001$	***
Total (PM + EM)	22	21	19	86.4%	90.5%–	69.6–98.8	$P < .001$	***
1dTMT								
PM	1	1	1	100.0%	100.0%–	25.0–100.0	$P = 1.00$	NS
Hetero EM	5	5	4	80.0%	80.0%–	28.4–99.5	$P = .375$	NS
Homo EM	6	4	4	66.7%	100.0%–	39.8–100.0	$P = .125$	NS
(EM, total)	11	9	8	72.7%	88.9%–	51.8–99.7	$P = .039$	*
Total (PM + EM)	12	10	9	75.0%	90.0%–	55.5–99.7	$P = .021$	*
Total TMTs								
PM	3	3	3	100.0%	100.0%–	29.2–100.0	$P = .25$	NS
Hetero EM	15	14	12	80.0%	85.7%–	57.2–98.2	$P = .013$	*
Homo EM	16	14	13	81.3%	92.9%–	66.1–99.8	$P = .002$	**
(EM, total)	31	28	25	80.6%	89.3%–	71.8–97.7	$P < .001$	***
Total (PM + EM)	34	31	28	82.4%	90.3%–	74.2–98.0	$P < .001$	***

*$P < .05$; **$P < .01$; ***$P < .001$.
EM, extensive metabolizer; hetero, heterozygous; homo, homozygous; NS, not significant; PM, poor metabolizer.

Compared with the previous study performed by Murakami et al[5] in the same country in which the 7-day LAL therapies showed a 43.7% eradication rate in PP analysis, our success rate with the same PPI and the same antibiotics was superior (90.9% in PP analysis) because 95% CI of our study (58.7%–99.8%) was greater than that of LAL therapy (95% CI: 31.6%–55.8%) (Table 5, lower table). The differences between Murakami's LAL and our studies were the duration of the therapy (1 vs 2 weeks), the times of amoxicillin ingestion per day (twice vs 4 times), the times of PPI doses per day (twice vs 4 times) in extensive metabolizers, and effective antibiotics selection as per the antibiotic susceptibility test in 2dTMT. These results suggest that the 4 improvements may have contributed to the increase of the eradication rate.

Tan et al[24] reported that tailored therapy with the antibiotic susceptibility test revealed a low eradication rate (44.4%) in the United States in 2018. The difference between Tan's and our 2dTMT was that they did not consider the CYP2C19 genotype, so that the times of PPI ingestion per day in extensive metabolizers may be one of the factors to make the difference.

In Murakami's study, they also used sitafloxacin, which was a new broad-band quinoline-family antibiotic recently available in some countries.[5] They clearly showed the superiority of sitafloxacin-containing regimen lansoprazole + amoxicillin + sitafloxacin (LAS) to LAL therapy. However, sitafloxacin is not available in every country and is also expensive. The drug should be saved for the future multiple antibiotic-resistant bacterial infection outside the stomach. We did not use sitafloxacin in the present study, but the eradication rates of our tailor-made therapies were not inferior or might be superior to Murakami's LAS therapy in which the mean eradication rate was 72.1% with 95% CI: 61.9%–82.3%. Although the medical expense was large in our tailor-made therapies because we measured the CYP2C19 genotype and used drugs for 14 days, eradication therapy without a novel broad-band antibacterial agent must have prevented bacterial flora from acquisition of unnecessary antibacterial resistance.

Interestingly, there were 11 cases with clarithromycin- or metronidazole-susceptible strains even after twice 7-day eradiation therapies which included the same antibiotics. It suggests that the reason why preceding eradications were failed was not because they had antibiotic-resistant strains. Because they were all extensive metabolizers and eradication success was achieved in 10 of the 11 cases with 2dTMT, increasing PPI and amoxicillin doses and prolongation of the therapy may have been effective. It is noted that in case of ID 23, simple prolongation of the therapy was not effective but only increasing PPI and amoxicillin doses per day was effective.

In cases with 3- or 4-antibiotic–resistant strains, eradication was succeeded in 1 of 1 with amoxicillin and levofloxacin therapy (ID: 7) and 8 of 9 with amoxicillin, minocycline, and bismuth therapies in either 2dTMT or 1dTMT. These results suggest that multiple-antibiotic resistance including amoxicillin resistance may be overcome with these therapies and encourage us to prove the efficacy of 2d or 1dTMT for treating such patients in the future.

Although the potassium-competitive acid blocker, vonoprazan, has been promised for eradication therapies,[1] the drug is not available in most countries until now. Our present study demonstrated that CYP2C19 genotype–based tailor-made therapies could be used in countries where vonoprazan is not available. In fact, in the case of ID 24, vonoprazan was used in the previous therapy but failed, and 1dTMT successfully salvaged without using vonoprazan. This case suggests that vonoprazan is not always necessary to treat patients with multiple eradication failures.

We had 3 poor metabolizers: two and one treated with 2dTMT and 1dTMT, respectively. They all succeeded in eradication. Because the number of poor metabolizers was small, we cannot say any statistical comments about it. One of the reasons why poor metabolizers were rare is that poor metabolizers probably had a higher eradication rate than extensive metabolizers in the preceding therapies.[16] In addition, approximately 20% population are poor metabolizers in Japan,[25] so that the number of such patients was very small in this study period. However, the effectiveness of our tailor-made therapy was 100% in poor metabolizers so far. We may be able to speculate that we do not need to increase the dose of the PPI in poor metabolizers and that the susceptibility-based selection of antibiotics, 4 times per day administration of amoxicillin, and prolongation of therapy period contribute to the success. Although the results of poor metabolizers are important, those of extensive metabolizers are more meaningful as discussed previously.

Limitations of the study are discussed. This was not a prospective controlled study and performed in a single center in a country, and the number of patients was small. Because the patients with multiple eradication failures were rare in Japan, it was difficult to collect large number of such patients in a single center. We should prove the efficacy of 2dTMTs with more numbers of patients in a prospective controlled manner.

In conclusion, we propose 14-day 2dTMT as one of the salvage therapies for patients with multiple eradication failures against *H pylori* infection in countries where vonoprazan is not available. In cases of insufficient information with the antibiotic susceptibility test, 14-day 1dTMT may be an alternative therapy. Combination with a conventional PPI and non-novel antibiotics are still effective for the salvage therapy.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2021.11.006.

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Authors' Contributions:
The principal author Shigemi Nakajima designed, performed, and interpreted the study and wrote the manuscript. Hisayuki Inoue and Hiroshi Satake participated in the recruitment and referral of the patients and discussed. Other authors partially participated in recruiting patients and discussion.

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The authors do not have any conflict of interest.

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Ethical Statement:
The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
Medical doctors who referred the patients to JCHO Shiga hospital are listed in the supplementary table (Table A1). Deidentified individual participant data were shared by the authors before submission but were not made available to the others. Deidentified individual participant data are available in UMIN (https://www.umin.ac.jp/). Individual participant data will not be shared.