Outcome of second- and third-line *Helicobacter pylori* eradication therapies based on antimicrobial susceptibility testing

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Objectives: The objective of this study was to assess the outcome of antimicrobial susceptibility-guided therapies in *Helicobacter pylori*-infected individuals who had undergone unsuccessful prior eradication treatments.

Methods: From October 2004 to December 2013, 481 *H. pylori*-positive patients with prior unsuccessful eradication treatments were administered susceptibility-guided salvage eradication treatments. Six months on, treatment outcome was assessed by urea breath test, stool antigen ELISA, *Helicobacter* urease test or microbiology and/or histopathology.

Results: Resistance to metronidazole and clarithromycin was high in patients with prior unsuccessful eradication treatments and was dependent on the number of treatment failures. Susceptibility-guided salvage eradication treatments achieved eradication rates of nearly 70% in these patients. No particular regimen was significantly better than another.

Conclusions: Antimicrobial susceptibility testing prevents prescription of inefficient antimicrobials and enables individualized and promising salvage treatments in patients with prior unsuccessful eradication treatments.

Introduction

Infections with *Helicobacter pylori* cause gastritis and may result in complications such as peptic ulcer disease, gastric adenocarcinoma or mucosa-associated lymphoid tissue (MALT) lymphoma. To eradicate the bacteria and prevent these complications, patients are prescribed empirical first-line antimicrobial therapies with clarithromycin and metronidazole or amoxicillin in combination with a proton pump inhibitor (PPI). These treatments, however, may fail due to a lack of compliance and increasing antibiotic resistance.

Several factors drive the development of antimicrobial resistance with the number of prior treatment failures being the most important one. We recently showed that after just one unsuccessful therapy, resistance to clarithromycin rose to 60%; further vain treatment attempts resulted in resistance as high as 80%. In parallel, double resistance to clarithromycin and metronidazole soared to 65% and triple resistance to clarithromycin, metronidazole and quinolones increased to 15%. The German National Reference Centre (NRC) for *H. pylori* receives and microbiologically examines gastric tissue samples primarily from patients who had undergone unsuccessful eradication treatments in the past. Such patients frequently carry *H. pylori* resistant to two or more antibiotics, making treatment particularly challenging. To improve patients’ prospects of successful eradication of *H. pylori*, the NRC supports gastroenterologists and general practitioners by testing antimicrobial susceptibility of *H. pylori* and giving guideline-based treatment recommendations.

In this retrospective study, we assessed the outcome of such susceptibility-based eradication treatments in *H. pylori*-positive patients and whether clinical symptoms improved. We show that susceptibility testing-guided eradication therapies achieve eradication rates as high as 70% in those patients.

Methods

Patients, gastric tissue samples and microbiological examination

A total of 481 *H. pylori*-positive patients from across Germany were retrospectively evaluated and included in the study. Patients were routinely endoscoped between 2004 and 2013 by their attending gastroenterologists. Gastric tissue samples were taken and sent to the NRC for *H. pylori* using a special transport medium (Portagerm pylori, bioMérieux, Germany) within 48 h. Data on prior eradication therapies and diagnosed gastric diseases were routinely collected from endoscopy reports.

On receipt, gastric tissue samples were crushed in 1.8 mL reaction tubes (Eppendorf, Germany) and streaked onto Columbia agar-based culture medium containing 10 vol% washed human erythrocytes and 10 vol% heat-inactivated horse serum. Culture media were then incubated under microaerobic conditions at 37°C for 2–10 days. Growth...
bacteria were identified as \textit{H. pylori} by typical morphology, biochemical reactions and Gram staining.

Phenotypic susceptibility to amoxicillin, metronidazole, clarithromycin, ciprofloxacin/levofloxacin and tetracycline was tested on either Iso-Sensitest (Oxoid, Germany) or Mueller–Hinton agar plates (with 10% horse blood; Oxoid, Germany) by using the Etest\textsuperscript{6} method (bioMérieux) as described previously.\textsuperscript{6} As there is no rifabutin Etest\textsuperscript{6} available in Europe, rifampin was used to screen for rifabutin resistance. Isolates were classified as susceptible or resistant by applying the following breakpoints: metronidazole, 8 mg/L; clarithromycin, 1 mg/L; ciprofloxacin/levofloxacin, 1 mg/L; amoxicillin, 1 mg/L; tetracycline, 1 mg/L; and rifampin, 4 mg/L.\textsuperscript{6}

**Susceptibility-guided therapy recommendations and outcome**

Treatment recommendations based on susceptibility testing were given on request in line with the German S3 guideline\textsuperscript{6} and the Maastricht IV consensus.\textsuperscript{1} Ahead of any recommendation, attending gastroenterologists and/or general practitioners were asked to provide information on any prior antimicrobial treatments of \textit{H. pylori} and unrelated bacterial infections.

In the case of resistance to metronidazole and clarithromycin, the NRC recommended regimens consisting of: (i) PPI (twice the standard dose, three-times daily) and amoxicillin (1000 mg, three-times daily) for \(\geq 14\) days; (ii) PPI (standard dose, twice daily), amoxicillin (1000 mg, twice daily) and levofloxacin (500 mg, once daily) for 10 days; (iii) PPI (standard dose, twice daily), amoxicillin (1000 mg, twice daily) and rifabutin (150 mg, twice daily) for 10 days; (iv) PPI (standard dose, twice daily), levofloxacin (500 mg, once daily) and rifabutin (150 mg, twice daily) for 10 days; and (v) PPI (standard dose, twice daily), metronidazole (400 mg, three-times daily), tetracycline (500 mg, four-times daily) and bismuth subcitrate (120 mg, four-times daily) for 10 days.

To eradicate triple-resistant \textit{H. pylori} (resistant to metronidazole, clarithromycin and quinolones), suggested therapies included: (i) PPI (twice the standard dose, three-times daily) and amoxicillin (1000 mg, three-times daily) for \(\geq 14\) days; (ii) PPI (standard dose, twice daily), amoxicillin (1000 mg, twice daily) and rifabutin (150 mg, twice daily) for 10 days; and (iii) PPI (standard dose, twice daily), metronidazole (400 mg, three-times daily), tetracycline (500 mg, four-times daily) and bismuth subcitrate (120 mg, four-times daily) for 10 days.

Six months on, gastroenterologists and/or general practitioners were sent questionnaires to collect information on the treatment prescribed, patients' symptoms and therapy success as tested by the urea breath test, Helicobacter urease test, stool antigen ELISA or microbiology and/or histopathology. In line with national and international guidelines,\textsuperscript{6,7} treatment controls were carried out by the attending physicians \(\geq 4\) weeks after eradication treatment (test methods and the respective results are summarized in Table S1, available as Supplementary data at JAC Online). Improvement of symptoms as reported by patients was evaluated and documented by the attending physicians without using a particular scoring sheet or scale.

Even though not reported, poor compliance and any side effects cannot be ruled out.

**Ethics**

The local ethics review committee confirmed this study was surveillance and hence did not require approval. All data were analysed anonymously.

**Statistical analysis**

Independence of criteria was tested by \(\chi^2\) or Fisher's exact tests applying a significance level of 0.05.

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**Results**

**Baseline characteristics of patients**

Four-hundred-and-eighty-one patients with positive \textit{H. pylori} culture and phenotypic antimicrobial susceptibility testing were evaluated (data on patients are summarized in Table 1). As all patients had undergone prior unsuccessful eradication treatments, the proportions of \textit{H. pylori} resistant to clarithromycin, metronidazole and levofloxacin were markedly high and associated with the number of treatment failures (Table 2); only a few patients (\(n = 23\)) carried rifabutin-resistant isolates and none of the strains tested was resistant to amoxicillin or tetracycline. The resistance of \textit{H. pylori} from patients with peptic ulcer disease was not significantly different from the resistance of \textit{H. pylori} from patients with non-ulcer diseases (resistance data are summarized in Table S2).

Patients with one prior treatment had primarily been administered amoxicillin and clarithromycin (109 out of 149; 73.2\%) and patients with two unsuccessful treatments in the past had most often received amoxicillin, clarithromycin and metronidazole (122 out of 215; 56.7\%). Nearly half of the patients (50 out of 107; Table 1. Baseline characteristics of patients included in the study (\(n = 481\))

<table>
<thead>
<tr>
<th></th>
<th>(n)</th>
<th>%</th>
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<td>69</td>
</tr>
<tr>
<td>male</td>
<td>149</td>
<td>31</td>
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<tr>
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<tr>
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<th>(n = 10)</th>
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<tr>
<td>Resistant to</td>
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<tr>
<td>CLR</td>
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<td>25.5</td>
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<td>MTZ/CLR/LVX</td>
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<td>16.8</td>
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</table>

| CLR, clarithromycin; LVX, levofloxacin; MTZ, metronidazole. |

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46.7%) with three or more prior treatments had taken at least four different antibiotics (antimicrobials used in prior treatments are listed in Table S3).

**Administered therapies and outcome**

Patients carrying *H. pylori* resistant to clarithromycin and metronidazole (n = 205) had undergone one (n = 53) prior unsuccessful treatment, two (n = 101) prior unsuccessful treatments or three or more (n = 44) prior unsuccessful treatments (for 7 patients the number of prior eradication therapies was unknown). In patients with *H. pylori* resistant to metronidazole and clarithromycin, treatment with levofloxacin, rifabutin and PPI proved to be most successful (success rate = 80.1%; Figure 1a); there was, however, no significant statistical difference when compared with other regimens. Patients with *H. pylori* resistant to clarithromycin, metronidazole and quinolones (n = 121) had one (n = 25) unsuccessful eradication treatment, two (n = 54) unsuccessful eradication treatments or three or more (n = 40) unsuccessful eradication treatments in the past (for 2 patients the number of prior eradication therapies was unknown). Even though treatments with amoxicillin and PPI (success rate = 79.6%) proved to be most successful in this group of patients (Figure 1b), none of the various therapies administered was significantly better than the others.

Treatments and outcome in patients with either entirely susceptible *H. pylori* (n = 14) or *H. pylori* resistant to solely clarithromycin (n = 63); solely metronidazole (n = 40); solely levofloxacin (n = 7); clarithromycin and levofloxacin (n = 20); and metronidazole and levofloxacin (n = 11) are summarized in Table S4.

Six months on, the majority of successfully treated patients were either less symptomatic or asymptomatic (279 out of 336; 83%). Thirty-nine patients (11.6%) did not report any clinical improvement; for the remaining patients, no information was available.

In patients in whom therapy failed, 73.1% (106 out of 145) did not feel any improvement; 20% (n = 29) were asymptomatic and for 10 patients no information on symptoms was available.

Neither underlying diseases such as gastritis or peptic ulcer disease (P > 0.99) nor patients’ sex (P = 0.82) had any impact on treatment outcome.

**Discussion**

In an estimated 20% of *H. pylori*-positive patients, empirical first-line therapies, mostly including clarithromycin, fail primarily due to a lack of compliance and antimicrobial resistance of the bacteria.7–10 Thus, physicians are prompted to prescribe second-line therapies to eradicate *H. pylori*. In line with a national guideline, German gastroenterologists are requested to have antimicrobial susceptibility-guided treatments succeed in 336 (69.9%) patients. This eradication rate was below treatment success rates reported by others,11 which might be due to the patient cohort we investigated; more than one-third had two or even more unsuccessful treatments in the past. Other factors such as the grade of gastric inflammation, gastric acid secretion and patients’ genetics such as polymorphisms in cytochrome P450 2C19 or IL-1β may also have an impact on successful eradication and might explain why susceptibility-guided treatments fail in 30% of patients.6,7,12,13

Of note, most patients in our study were female. We and others have shown that women have a higher risk than men to harbour metronidazole- and clarithromycin- and quinolone-resistant *H. pylori*.6,14 Hence, female patients may get prescribed more eradication treatments that may result in more treatment failures.

In patients with double and triple resistant *H. pylori*, treatment with rifabutin, levofloxacin and PPI or amoxicillin and PPI achieved higher but not significantly better outcomes. A novel quadruple combination of bismuth subcitrate, metronidazole and tetracycline in one capsule with a PPI for 10 days has achieved excellent eradication rates in recent studies.15 As this combination of drugs, however, was not yet available in Germany during the study period and physicians were required to purchase bismuth subcitrate via international pharmacies abroad, only few patients were

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**Figure 1.** (a) Percentage success of eradication treatments in patients carrying *H. pylori* resistant to metronidazole and clarithromycin. Twelve patients received therapies including tetracycline, metronidazole, bismuth salt and PPI; for four patients, the therapy administered was unknown (not shown). AMX, amoxicillin; LVX, levofloxacin; RFB, rifabutin. (b) Percentage success of eradication treatments in patients carrying *H. pylori* resistant to metronidazole, clarithromycin and levofloxacin. Eight patients received unknown treatments (not shown). Quadruple therapies included tetracycline, metronidazole, bismuth salt and PPI. AMX, amoxicillin; RFB, rifabutin.
administered bismuth-based quadruple therapies. Whether the novel quadruple therapy is as successful in patients with multiple prior treatment failures needs to be demonstrated in further studies.

More than 80% of the successfully treated patients reported that their symptoms improved. As this percentage is far higher than reported by others, further follow-up is needed to show whether the patients indeed benefitted from eradication treatment. In case avoided the development of resistance, it at least spares patients the reduction of inefficient antibiotics and reduces costs.17,18 In case of resistance to clarithromycin and/or levofloxacin with high specificity and sensitivity.19–22 As resistance to tetracycline and rifabutin is rare and resistance to amoxicillin has not yet been described in Germany, knowledge of resistance to clarithromycin and levofloxacin may be sufficient for an adequate therapy to be selected. If susceptibility testing is not available, physicians may refrain from administering clarithromycin in previously unsuccessfully treated patients. Instead, they may prescribe regimens that are quick and reliable and several commercial test kits based on real-time PCR methods and reverse hybridization are available. These methods work straight from gastric tissue samples and predict resistance to clarithromycin and/or levofloxacin with high specificity and sensitivity.21,22

In summary, we suggest having antimicrobial susceptibility tested not later than after the second therapy failure. Susceptibility testing is cost-effective, prevents the administration of inefficient antibiotics and enables tailoring of an individual treatment that is successful in ~70% of patients.

References
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Transparency declaration
None to declare.

Supplementary data
Tables S1 to S4 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).
Antimicrobial resistance of *Helicobacter pylori* in Germany


