Prospective multicentre study on antimicrobial resistance of *Helicobacter pylori* in Germany

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Objectives: Antimicrobial resistance of *Helicobacter pylori* endangers the successful eradication of the bacteria. The aim of this prospective surveillance study (ResiNet) is to continuously keep antimicrobial resistance of *H. pylori* in Germany under surveillance and to identify risk factors for its development.

Methods: From July 2001 until December 2012, we tested the antimicrobial susceptibility of *H. pylori* strains isolated from 1651 prospectively enrolled patients. We analysed clinical and epidemiological data and identified risk factors for the development of resistance.

Results: Average primary resistances were 29.4% for metronidazole, 6.7% for clarithromycin and 3.1% for both antimicrobials. Prior unsuccessful eradication treatments, female sex and country or continent of origin were identified as independent risk factors for development of resistance.

Conclusions: *H. pylori*-positive patients without prior eradication therapy can be treated empirically; antimicrobial susceptibility testing is recommended in previously unsuccessfully treated patients and in patients who have received antimicrobial chemotherapies due to unrelated bacterial infections.

Keywords: surveillance, antimicrobial susceptibility, risk factors, follow-up, eradication

Introduction

One-quarter of German adults are colonized with *Helicobacter pylori* but only one-fifth of these present with symptoms and may develop complications including peptic ulcer disease, gastric adenocarcinoma or mucosa-associated lymphoid tissue lymphoma.1,2 German and European guidelines recommend to eradicate the bacteria by administration of an empirical antimicrobial chemotherapy that consists of two antimicrobials in combination with a proton pump inhibitor (PPI).1,3

Successful eradication of *H. pylori*, however, is substantially impaired by increasing antimicrobial resistance. Hence, knowledge of risk factors leading to antimicrobial resistance is critical to avoid administration of ineffective antimicrobials.1,3–5

A recent European multicentre study reported primary resistances of 6.9% for clarithromycin and 14.9% for levofoxacin in Germany.4 These figures, however, were based on a low number of samples collected over a short period of time in a single microbiological centre (MC) only.4

To continuously keep the resistance development of *H. pylori* under surveillance, the German National Reference Centre (NRC) for *H. pylori* established a network of 17 microbiological laboratories and launched the nationwide multicentre study ResiNet in 2001. The aims of ResiNet are as follows: (i) to collect prospective and systematic data on the prevalence of antimicrobial resistance in *H. pylori* depending on age, sex, country of origin, underlying diseases and previous unsuccessful eradication attempts; and (ii) to identify risk factors for the development of resistance. To ensure comparable and reliable results, antimicrobial susceptibility testing was standardized by using identical culture media lots and identical standard operating procedures at the participating study centres.

Methods

Study design, inclusion criteria and patients

Seventeen MCs (the location of the centres is shown in Figure 1), divided into two study subgroups with the same responsibilities and tasks,
participated in the study and collaborated with two to seven local gastroenterologists. Six times a year over a fortnight’s period, MC groups microbiologically examined gastric tissue samples of up to five patients consecutively enrolled by their associated gastroenterologists, regardless of whether the patients had undergone any prior eradication therapy or not. These tissue samples were taken from patients who were subjected to gastroduodenoscopy if medically indicated only and then sent to the associated MC. Inclusion criteria were a completed and signed informed consent form, age ≥18 years and a positive Helicobacter urease test. A total of 2762 patients met the inclusion criteria and were enrolled. After consenting to the study, pseudonyms were created for all patients. The study was conducted in line with ethical standards.

A questionnaire was used to collect patients’ epidemiological and socioeconomic data, earlier diseases of the gastrointestinal tract, prior

**Figure 1.** Map of Germany with locations of study centres. ©GeoBasis-DE/BKG 2014 (data changed), http://www.bkg.bund.de, used with permission.
eradication therapies and previously taken medicines such as painkillers or antibiotics. The attending gastroenterologists documented endoscopic findings and any prior *H. pylori* eradication therapies.

### *H. pylori* culture and antimicrobial susceptibility testing

Antrum and corpus gastric tissue samples were transported in Portagerm pylori (bioMérieux, France) to the affiliated MC. Gastric tissue samples were homogenized and cultured and the grown bacteria were identified as *H. pylori* as described previously. Study isolates were preserved and frozen at $-80^\circ$C.

MICs of metronidazole, clarithromycin, quinolones, tetracycline and amoxicillin were determined using the Etest method (bioMérieux) as described previously. The following breakpoints were applied: metronidazole, 8 mg/L; clarithromycin, 1 mg/L; ciprofloxacin (tested until 2007) and levofloxacin (tested as of 2008), 1 mg/L; amoxicillin, 2 mg/L; and tetracycline, 1 mg/L. In order to obtain comparable and reproducible results, all MCs used identical culture media lots and Etest lots and carried out antimicrobial susceptibility testing in line with standard operating procedures provided by the NRC. Furthermore, the MICs for the three reference strains CCUG 38770, CCUG 38771 and CCUG 38772 (Culture Collection, University of Gothenburg, Sweden) were determined in each centre at the beginning and at the end of a fortnight’s study period for quality control. Technicians of the participating centres were trained in these methods at the NRC during a 3 day course.

### Follow-up of the study patients

Therapy outcome of the study patients was assessed by a follow-up study. At least 3 months after endoscopy, gastroenterologists were sent a questionnaire and data were gathered on the treatment regimen administered, patient's symptoms, whether the eradication therapy was successful and how this was tested.

### Data analysis

A univariate/multivariate analysis was carried out with logistic regression using SAS 9.3. A P value $\leq 0.050$ was considered significant. CIs for ORs were calculated with the usual approach of Wald via logistic regression. Data were analysed in line with the STROBE guidelines for reporting observational studies.

### Ethics

The local ethics review committee approved this study (#33/2001). The study was conducted in line with the Declaration of Helsinki and informed consent was obtained from all study participants. All data were analysed anonymously.

### Results

#### Baseline characteristics and demographics of patients

Some 1651 patients (59.8%) with a positive *H. pylori* culture and complete antimicrobial susceptibility testing were analysed (the data are summarized in Table 1).

Female patients had more often undergone unsuccessful eradication therapies than male patients (44.1% versus 30.7%) and they more frequently revealed a macroscopically normal gastric mucosa (31.4% versus 21.3%) or gastritis (48.0% versus 44.0%). By contrast, male patients more frequently suffered peptic ulcer disease (26.6% versus 11.5%; data not shown).

#### Antimicrobial resistance of *H. pylori* clinical isolates

Antimicrobial resistance was dependent on prior eradication therapies as untreated patients ($n = 902$) showed significantly fewer resistant *H. pylori* isolates than pre-treated patients ($n = 621$). In patients who were pre-treated once, the proportion of resistant isolates significantly increased; further unsuccessful eradication treatments resulted in another significant rise of resistance (Figure 2).

Overall, only nine strains (0.5%) were resistant to tetracycline. All isolates tested were susceptible to amoxicillin. From 2001/02 to 2011/12, primary resistances to metronidazole (OR 1.06, 95% CI 1.03–1.1) and to clarithromycin (OR 1.16, 95% CI 1.12–1.20) significantly increased. Resistance to quinolones slightly increased and peaked in 2007/08 but then decreased to 11.9% in 2011/12 (not significant; Figure 3).

#### Risk factors for harbouring resistant *H. pylori* strains

Multivariate analysis identified prior unsuccessful eradication therapies and sex as the most relevant significant independent risk factors for carrying antibiotic-resistant *H. pylori*. Patients from either Africa or Eastern Europe had a significantly higher chance

### Table 1. Demographic data of ResiNet study patients

<table>
<thead>
<tr>
<th></th>
<th>All study patients ($n = 2762$)</th>
<th>Patients analysed ($n = 1651$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>1570</td>
<td>948</td>
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<tr>
<td>male</td>
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<td>662</td>
</tr>
<tr>
<td>unknown</td>
<td>61</td>
<td>21</td>
</tr>
<tr>
<td><strong>Endoscopic findings</strong></td>
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<td></td>
</tr>
<tr>
<td>gastritis</td>
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<td>755</td>
</tr>
<tr>
<td>normal mucosa</td>
<td>829</td>
<td>445</td>
</tr>
<tr>
<td>duodenal ulcer</td>
<td>309</td>
<td>213</td>
</tr>
<tr>
<td>unknown</td>
<td>186</td>
<td>126</td>
</tr>
<tr>
<td>stomach ulcer</td>
<td>122</td>
<td>73</td>
</tr>
<tr>
<td>tumour</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>other</td>
<td>106</td>
<td>32</td>
</tr>
<tr>
<td><strong>Region of origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western/Central Europe</td>
<td>1925</td>
<td>1148</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>346</td>
<td>206</td>
</tr>
<tr>
<td>Asia</td>
<td>264</td>
<td>158</td>
</tr>
<tr>
<td>Southern Europe</td>
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<td>28</td>
</tr>
<tr>
<td>Africa</td>
<td>40</td>
<td>26</td>
</tr>
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<td>8</td>
</tr>
<tr>
<td>North America</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>unknown</td>
<td>129</td>
<td>77</td>
</tr>
</tbody>
</table>

**Table 1**. Demographic data of ResiNet study patients.
**Figure 2.** Antimicrobial resistance of *H. pylori* depends on the number of prior eradication therapies and is significantly higher in patients who have undergone one or more prior eradication attempts. Asterisks indicate statistical significance. CIP, ciprofloxacin; CLR, clarithromycin; LVX, levofloxacin; MTZ, metronidazole.

**Figure 3.** Primary resistance of *H. pylori* strains to metronidazole and clarithromycin significantly increased from 2001/02 to 2011/12. CIP, ciprofloxacin; CLR, clarithromycin; LVX, levofloxacin; MTZ, metronidazole.
than patients from Western/Central Europe to harbour metronidazole-resistant strains. Patients with duodenal ulcers less often carried clarithromycin-resistant strains when compared with patients suffering from stomach ulcers (the data are summarized in Table 2).

**Follow-up of study patients**

All collaborating gastroenterologists were sent questionnaires to evaluate data on any eradication therapies and therapy outcomes. The questionnaires of a total of 606 patients (39.1% (n = 369) pre-treated patients) were analysed.

Of the as yet untreated patients, 216 out of 237 (91.1%) received therapy. The combination of amoxicillin, clarithromycin and a PPI was the predominantly prescribed first-line therapy (197 out of 216; 91.2%). Only 64.4% of these patients (139 out of 216) again presented to their physicians. In this group, eradication was successful in 82.0% (114 out of 139) and failed in 10.8% (15 out of 139) as confirmed by urea breath test, stool antigen test, histopathology or subjective improvement of symptoms. In 10 patients, eradication outcome was not tested.

Of the patients with prior unsuccessful therapies, 92.4% (341 out of 369) were treated again. Of these pre-treated patients, 285 (83.6%) again presented to their attending physicians. Therapy was successful in 195 patients (68.4%) and failed in 80 patients (28.1%) as tested by urea breath test, stool antigen test, histopathology or improvement of symptoms; 10 patients were not tested for *H. pylori* (the second-line therapies administered and the respective percentage success rates are shown in Figure 4).

Patients suffering from peptic ulcer disease (duodenal ulcer and stomach ulcer) showed significantly higher eradication rates (88.7% versus 70.1%, P < 0.010) and carried a lower proportion of clarithromycin-resistant isolates (11.3% versus 69.3%, P < 0.001) when compared with patients in whom a macroscopically normal gastric mucosa was diagnosed. The comparison of eradication success in other *H. pylori*-related diseases did not reveal any difference.

The majority of successfully treated patients were either less symptomatic (101 out of 309; 32.7%) or asymptomatic (176 out of 305; 57%). In the cohort of patients in whom therapy failed, 25.3% (24 out of 95) were less symptomatic and 58.9% (n = 56) did not feel any improvement; 11.6% (n = 11) were asymptomatic.

**Discussion**

Our data support recently published figures of a European multicentre study and a German monocentre study and show that the average primary clarithromycin resistance of *H. pylori* in Germany is substantially lower when compared with the European average.4–8 Resistance to metronidazole and quinolones, however, reached levels similar to those in other European countries.4 Although resistance to clarithromycin has steadily increased, it is still below the critical level of 15%–20%.4 Resistance to tetracycline is rare and amoxicillin resistance virtually does not exist in Germany.

Only 60% of the gastric tissue samples examined were *H. pylori* culture positive, most likely due to delayed transport of the samples to the laboratory and the intake of antibiotics or PPIs ahead of the gastroduodenoscopy.

Prior unsuccessful eradication attempts proved to be the most important risk factor for the development of antimicrobial resistance in *H. pylori*.5,9–15 As antimicrobial chemotherapies due to unrelated bacterial infections have a substantial impact on resistance development in *H. pylori*,5,12–15 physicians should take a thorough antibiotic history before prescribing any eradication treatment. They should consider either prescribing alternative empirical regimens16,17 or asking for susceptibility testing first.13,15

Women had an independently higher risk of carrying antibiotic-resistant *H. pylori* than male patients.18 Possible explanations for this may be (i) more antimicrobial therapies because of unrelated infections, e.g. infections of the urogenital tract, or (ii) a higher tendency towards less inflammatory *H. pylori* diseases (e.g. macroscopically unchanged gastric mucosa), which may impair successful eradication.

In patients with peptic ulcer disease, eradication was more successful than in patients with normal mucosa. These findings support already published data indicating that the inflammatory

<table>
<thead>
<tr>
<th>Resistance to</th>
<th>Risk factor</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLR</td>
<td>prior eradication therapies: yes versus no</td>
<td>21.41</td>
<td>15.42–29.74</td>
</tr>
<tr>
<td>MTZ</td>
<td>prior eradication therapies: yes versus no</td>
<td>3.63</td>
<td>2.85–4.62</td>
</tr>
<tr>
<td>MTZ/CLR</td>
<td>prior eradication therapies: yes versus no</td>
<td>21.73</td>
<td>14.27–33.07</td>
</tr>
<tr>
<td>MTZ/CLR/LVX</td>
<td>prior eradication therapies: yes versus no</td>
<td>12.85</td>
<td>5.71–28.95</td>
</tr>
<tr>
<td>LVX</td>
<td>sex: male versus female</td>
<td>1.40</td>
<td>1.04–1.89</td>
</tr>
<tr>
<td>CLR</td>
<td>sex: male versus female</td>
<td>0.51</td>
<td>0.37–0.70</td>
</tr>
<tr>
<td>MTZ</td>
<td>sex: male versus female</td>
<td>0.54</td>
<td>0.43–0.68</td>
</tr>
<tr>
<td>MTZ/CLR</td>
<td>sex: male versus female</td>
<td>0.62</td>
<td>0.44–0.87</td>
</tr>
<tr>
<td>MTZ/CLR/LVX</td>
<td>sex: male versus female</td>
<td>0.50</td>
<td>0.28–0.92</td>
</tr>
<tr>
<td>LVX</td>
<td>sex: male versus female</td>
<td>0.66</td>
<td>0.49–0.90</td>
</tr>
<tr>
<td>CLR</td>
<td>duodenal ulcer versus stomach ulcer</td>
<td>0.29</td>
<td>0.10–0.85</td>
</tr>
<tr>
<td>MTZ</td>
<td>Africa versus Western/Central Europe</td>
<td>2.51</td>
<td>1.06–6.00</td>
</tr>
<tr>
<td>MTZ</td>
<td>Eastern Europe versus Western/Central Europe</td>
<td>1.54</td>
<td>1.12–2.19</td>
</tr>
</tbody>
</table>

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

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Table 2. Risk factors for carrying resistant *H. pylori* (multivariate analysis)
response to *H. pylori* may play a critical role and enhances successful eradication.\textsuperscript{19–21} Whether patients with normal mucosa or only slight inflammation should therefore be administered different or longer eradication therapies than patients with peptic ulcer disease remains debatable.\textsuperscript{22}

Even though primary clarithromycin resistance was low, clarithromycin-based first-line therapies failed in 20% of patients, likely due to poor compliance to therapy. Remarkably, 25% of patients again received a clarithromycin-based first-line therapy after the first treatment failure.

Nearly 90% of the successfully treated patients, most of whom suffered from functional dyspepsia, reported that their symptoms improved. This is in contrast to publications that reported a long-term improvement of \textasciitilde10\% only in such patients.\textsuperscript{23} Further follow-up may show whether and to what degree our study patients indeed benefitted from eradication.

Continuous surveillance studies are needed to keep monitoring the antimicrobial resistance of *H. pylori* and are essential to regularly update therapeutic recommendations for empirical first- and second-line treatments. Antimicrobial susceptibility testing is highly recommended, at the latest after a second empirical therapy has failed.

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Transparency declarations
None to declare.

References
1. Fischbach W, Malfertheiner P, Hoffmann JC et al. S3-guideline ‘Helicobacter pylori and gastroduodenal ulcer disease’ of the German Society for Digestive and Metabolic Diseases (DGVS) in cooperation with the German Society for Hygiene and Microbiology, Society for Pediatric Gastroenterology and Nutrition e. V., German Society for Rheumatology, AWMF-registration-no. 021/001. Z Gastroenterol 2009; 47: 1230–63.