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Primary resistance of *Helicobacter pylori* is still low in Southern Austria

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ABSTRACT

Objectives: We determined primary and secondary resistance rates of *H. pylori* in different regions of Austria and potential bacterial and host factors associated with resistance.
Methods: In a prospective multicentre study *H. pylori* was cultivated from biopsies and susceptibility testing was performed according to EUCAST. Resistance to clarithromycin and levofloxacin was determined by sequencing of the resistance-determining regions of *23S rRNA* and *gyrA* genes. *cagA*, *vacA* and *babA2* genotypes were determined.
Results: A total of 1266 patients were included. 178 isolates were cultured: 128 from patients without prior eradication therapy, 50 from patients after failed eradication. Primary resistance to clarithromycin, levofloxacin and metronidazole were 17.2%, 9.4% and 10.2%, respectively. Secondary resistance to clarithromycin, levofloxacin and metronidazole were 64%, 18% and 44%, respectively. Prior eradication was associated with a higher risk of clarithromycin as well as metronidazole resistance (OR = 8.1; 95% CI 3.8–17.1 and OR 5.7; 95% CI 2.5–13, respectively).
Conclusion: Primary resistance to both clarithromycin and levofloxacin was markedly lower in Southern Austria than recently reported.

1. Introduction

Helicobacter pylori is a common and potentially curable cause of many gastric diseases like peptic ulcer disease and is associated with the development of gastric cancer (Malfertheiner et al., 2012). The classic triple treatment for *H. pylori* including proton pump inhibitor (PPI), amoxicillin and clarithromycin (or metronidazole as alternative) was proposed by the first Maastricht conference in 1997 (Malfertheiner et al., 1997) and has been used as a the standard eradication regimen worldwide since then. However, successful eradication of *H. pylori* is substantially impaired by rising antimicrobial resistance. The most important cause of treatment failure remains resistance to antibiotics (Megraud et al., 2013). Clarithromycin resistance has a particularly negative effect on the efficacy of eradication therapy. In the case of clarithromycin resistance the rate of success of the clarithromycin-containing triple therapy is very low, in the range of 10–30% (Fischbach and Evans, 2007). Recently, sequential therapy consisting of amoxicillin, clarithromycin and metronidazole was shown to be more efficacious than a 14-day triple therapy with amoxicillin and clarithromycin (Liou et al., 2013).

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Resistance to antibiotics varies significantly in different regions of Europe (Glupczynski et al., 2001). A recent European study revealed clarithromycin primary resistance to be the highest in Austria among all 18 participating countries, namely 36.6%. Resistance to levofloxacin was found in 23.3% of Austrian isolates, exceeded only by 3 other countries (Megraud et al., 2013). Current guidelines recommend abandoning the classic triple treatment (PPI, clarithromycin and amoxicillin) as first line eradication regimen if clarithromycin primary resistance exceeds 20% (Malfertheiner et al., 2012). Accordingly, the recommendations for empiric eradication regimens have been changed by scientific societies in Austria after the 2013 publication by Megraud et al. (http://www.oeggh.at/images/downloads/Helicobacter_Slides_OEGGH_Stand_2014%2002-22.pdf). However, in the European multicentre study Austria was represented by a single centre in the capital (Megraud et al., 2013). This does most probably not allow inference on regional resistance patterns. Therefore, we aimed to determine *H. pylori* primary and secondary resistance to antibiotics in southern Austria. In addition, we evaluated host and bacterial factors associated with resistance to antibiotics.

2. Material and methods

2.1. Patients

In this prospective multicentre study patients without previous *H. pylori* eradication undergoing routine gastroscopy were recruited for the study from February 2014 to February 2015 (primary resistance group). In addition, patients with failed previous eradication therapy who underwent routine gastroscopy for follow up were included (secondary resistance group). The study was conducted at Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, four hospital-based, outpatient endoscopy units in Styria, four private practices in Styria and one hospital-based, outpatient endoscopy unit in Carinthia. The study was approved by the local ethics committee (votum number: 26-047 ex 13/14 Medical University of Graz). All patients provided written informed consent and participated on a voluntary basis.

2.2. Study design

All patients were submitted to routine gastroscopy. Two additional biopsies, one from antrum and one from corpus respectively, were taken and put immediately into a transport medium (Port-Pyl, Marcy-líEtoile, France). The transport medium was sent to the microbiology laboratory bioMerieux at the Section of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Medical University of Graz for culture and susceptibility testing. For each patient the participating centres provides additional information on a standardised questionnaire (patient's age, gender, country of origin, clinical symptoms, treatment with antibiotics in the past 6 months, history of eradication therapy.)

2.3. Determination of resistance by phenotypic methods

Biopsies were streaked onto a selective medium (Pylori agar, bioMerieux, Marcy-líEtoile, France). The agar plates are incubated in a microaerobic atmosphere in an incubator at 35 °C and 7.5% CO_2 (Hera cell, Kenko Laboratory products, Vienna, Austria). *H. pylori* isolates were identified by colony morphology, characteristic spiral morphology on Gram staining and positive biochemical reactions (cytochrome oxidase, catalase and urease) as described before (Almeida et al., 2014).

The MIC for amoxicillin, clarithromycin, rifampin, levofloxacin, tetracycline and metronidazole was determined by Etest® (bioMerieux, Marcy-líEtoile, France) and interpreted according to the guidelines of the European Committee of Antibiotic Susceptibility Testing (EUCAST) and was expressed as mg/L. *H. pylori* DSM 10242 (Leibniz Institute DSMZ—German Collection of Microorganisms and Cell Culture, Braunschweig, Germany) was used as a quality control of the susceptibility assay. Additionally, a second microbiologist, blinded to previous results, repeated susceptibility testing. Isolates were stored at −80 °C.

2.4. Determination of resistance by genotypic methods

DNA extraction was carried out with the NucliSENS® easyMAG system (bioMérieux, Marcy-líEtoile, France). For the detection of point mutations in the 23S *rRNA* and *gyrA* genes PCR were performed as previously described (Agudo et al., 2010; Khademi et al., 2014; Wang et al., 2001). The DNA sequence of the fragments was determined (Mix2Seq-Kit, Eurofins Genomics, Ebersberg, Germany) and compared with *H. pylori* 26695 sequence (Genbank accession no. AE000511.1) to find the point mutations responsible for the corresponding antimicrobial resistance.

2.5. Determination of virulence genes

Detection of *cagA*, *vacA* and *babA2*—genotype was performed by PCR amplification as previously described (Atherton et al., 1995; Atherton et al., 1999; Gerhard et al., 1999; Tummuru et al., 1993). The amplifications products were separated on a 2% aragose gel stained with ethidium bromide and transilluminated with ultraviolet light for visualization.

2.6. Data analysis

Quantitative variables were expressed as mean ± standard deviation. For statistical analysis Student's *t*-test, Cramer V test and Fisher's exact test were used as appropriate. Significant variables and variables that showed a trend towards significance ($p < 0.1$) were subsequently included in a binary logistic regression analysis to determine independent risk factors for resistance to each antibiotic. A p value of less than 0.05 was considered to indicate statistical significance. The statistical software package SPSS 20.0 (Chicago, IL, USA) was used.

3. Results

A total of 1266 patients were included in the study. Of these, 114 had received a prior eradication therapy. In contrast, 1152 patients had never received eradication therapy. From 178 patients *H. pylori* isolates were cultured (average age 52.4 ± 14.8 years, 54.5% female). A total of 128 patients had never received eradication therapy (primary resistance group), whereas in 50 patients eradication therapy had been attempted in vain (secondary resistance group). Clinical and demographic characteristics of all patients are presented in Table 1.

None of the 178 isolates were resistant to amoxicillin and tetracycline. Primary resistance to clarithromycin, levofloxacin and metronidazole were 17.2%, 9.4% and 10.2%, respectively. Secondary resistance to clarithromycin, levofloxacin and metronidazole were 64%, 18% and 44%, respectively (Table 2). There were no significant differences in clarithromycin, levofloxacin, metronidazole and rifampin primary resistance neither between the two regions Styria and Carinthia nor between urban and rural residents.