Resistance to clarithromycin and genotypes in *Helicobacter pylori* strains isolated in Sicily

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The resistance of *Helicobacter pylori* strains to clarithromycin is increasing in several developed countries and their association with a genetic pattern circulation has been variously explained as related to different geographical areas. In this study we have reported: the prevalence of the resistance of *H. pylori*, isolated in Sicily, to clarithromycin; the principal point of mutation associated with this resistance; and the more frequent association between resistance to clarithromycin and *cagA*, the EPIYA motif, and the *vacA* and *oipA* genes. Resistance to clarithromycin was detected in 25 % of cases, the main genetic mutation involved being A2143G. The *cagA* gene was present in 48 % of cases and the distribution of the EPIYA motif was: ABC in 35 cases; ABCC in 8 cases; ABCCC in 2 cases; ABC-ABCC in 2 cases; and ABC-ABCC-ABCCC in 1 case. Regarding the *vacA* allele, an *s1i1m1* combination was detected in 35 % of cases, *s1i1m2* in 12 %, *s1i2m2* in 12 %, *s2i2m2* in 40 %, and a double *s1m1-m2* mosaic in 1 % of cases. The status of the *oipA* gene was 'off' in 45 % of cases and 'on' in 55 %. Resistance to clarithromycin was found to be high in Sicily, but no correlation was found among resistance to clarithromycin, the *vacA* gene and *oipA* status; a higher correlation was observed between resistant strains and *cagA*-negative strains.

INTRODUCTION

Helicobacter pylori infection is usually superficial and clinically asymptomatic, but in approximately 10–20 % of cases it can be more aggressive, being associated with other gastric pathologies (Eusebi *et al.*, 2014). Clarithromycin is the key component used in eradication therapies (De Francesco *et al.*, 2009). However, the prevalence of primary resistance to clarithromycin is increasing in several developed countries (Megraud *et al.*, 2013; De Francesco *et al.*, 2010a; Boyanova & Mitov, 2010). In Italy the overall primary resistance to clarithromycin can be detected in 35.2 % of cases, in France it can be detected in 26 % of cases, while in Spain clarithromycin resistance is present in 27.2 % of strains (Saracino *et al.*, 2012; Agudo *et al.*, 2010; Raymond *et al.*, 2010).

Clarithromycin acts by binding to the peptidyltransferase region of 23S rRNA, thereby inhibiting protein synthesis

(Gerrits *et al.*, 2006; Jones *et al.*, 2008). The resistance to clarithromycin in *H. pylori* has been shown to be due to point mutations in the peptidyltransferase region of domain V of the 23S rRNA. The most common mutation is an A-to-G transition at position 2143 (A2143G), but three point mutations – A2142G, A2144G and T2182C – have also been described (Taylor *et al.*, 1997; Alfaresi & Elkoush, 2010). The presence of the A2143G point of mutation has been shown to significantly affect the eradication rate of *H. pylori*. Consequently, investigating the distribution of each mutated genotype for resistance to clarithromycin is relevant for managing *H. pylori* in clinical practice (Sakinc *et al.*, 2012). Thus, various studies have been performed in order to identify the relationship of the mutated genotypes with the bacterial genetic factors involved in virulence and clarithromycin sensitivity (Vannarath *et al.*, 2014; Agudo *et al.*, 2010; Garcia *et al.*, 2010).

The three main *H. pylori* genes correlated with pathogenicity are: *cagA, vacA* and *oipA*. The *cagA, vacA* and *oipA* genotype markers are widely used to characterize *H. pylori* virulence in relation to disease severity (Agudo *et al.*, 2010). The *cagA* pathogenicity island is a marker of enhanced virulence, and it is associated with the

Abbreviations: CI, confidence interval; OR, odds ratio.

The GenBank/EMBL/DDBJ accession numbers for sequences of the EPIYA ABC and EPIYA ABCC motifs are KM262820 and KM262821, respectively.

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development of major gastroduodenal diseases, such as peptic ulcers and gastric cancer (Hatakeyama & Higashi, 2005). VacA is capable of causing 'vacuole'-like membrane vesicles in the cytoplasm of gastric cells, but its role in *H. pylori* pathogenesis remains unclear (Abadi & Lee, 2014). The *oipA* (outer inflammatory protein) gene encodes one of the outer-membrane proteins involved in the induction of IL-8 secretion by epithelial cells.

In patients living in Sicily it has been demonstrated that high-virulence genotypes (i.e. *cagA*-positive and *vacaA s1m1*) have been associated with the active form of chronic gastritis, but no data are available with respect to clarithromycin resistance and its correlation with the virulence factors (Chiarini *et al.*, 2009). According to the Maastricht IV/Florence consensus report, the standard triple therapy should be abandoned in areas with clarithromycin resistance ≥ 20 % (i.e. Spain, Turkey, central Italy, Alaska, China, Japan and Cameroon) (Malfertheiner *et al.*, 2012).

On the basis of this evidence, the main aims of this study were to assess and to expose high rates of resistance to clarithromycin in Sicily in relation to the distribution of the involved point mutations. Finally, we shall focus our interest on verifying a possible association between resistance to clarithromycin, point mutations and *cagA*, *vacA* and *oipA* genes.

METHODS

Patients and sample collection. In this study a total of 100 *H. pylori* clinical strains were tested. These strains were obtained from gastric biopsy samples of patients attending the endoscopy ward of the Endoscopy Services of the Ospedali Civili Riuniti in Sciacca (Agrigento, Italy), A.O.U.P. Paolo Giaccone (Palermo, Italy) and M. Raimondi Hospital, San Cataldo (Caltanissetta, Italy). Exclusion criteria for patient recruitment to the study were as follows: previous attempts to eradicate *H. pylori* and use of antibiotics or proton pump inhibitor within the 2 weeks prior to endoscopy. Each patient signed an informed-consent form before endoscopy. The clinical strains were isolated from adults whose mean age was 45 years (median 44 years, ranging from 23 to 76 years), comprising 54 females and 46 males. Antral and corpus biopsies taken from each patient were transferred to the microbiology laboratory in cold transport medium (brain–heart broth, Oxoid, supplemented with 15 % v/v glycerol and 10 % v/v FCS). A further two gastric biopsy specimens from the antrum and corpus were immersed in 10 % formalin and embedded in paraffin.

On the basis of endoscopic and histological findings, the diagnoses were: inactive chronic gastritis in 39 cases, active chronic gastritis in 28 cases, duodenal peptic ulcer in nine cases, active chronic gastritis with gastric or duodenal peptic ulcer in 13 cases, other diseases in 10 cases, and gastric cancer in only one case. Chronic inflammation was defined on the basis of the increase in lymphocytes and plasma cells in the lamina propria and activity when neutrophil infiltration of the lamina propria, pits or surface epithelium were found. Regarding microbiological analysis, biopsy samples were streaked onto Colombia agar base (Oxoid) with 7 % horse blood and 0.4 % Dent supplement (Oxoid). The plates were then incubated for 3–6 days at 37 °C in a microaerophilic atmosphere (7 % O_2, 7.1 % CO_2, 7.1 % H_2 and 79.8 % N_2), which was provided by CampyGen (Oxoid).

Identification of *H. pylori*. The isolates were presumptively identified as *H. pylori* on the basis of colony morphology and a positive

biochemical reaction to the following: cytochrome oxidase, catalase, urease and a Gram stain.

DNA extraction. The genomic DNA of the 100 *H. pylori* strains was extracted by using a High Pure Template Preparation kit (Roche), in accordance with the manufacturer's instructions. The DNA extracted was stored at −20 °C until use.

Susceptibility test. With reference to the *in vitro* clarithromycin susceptibility test of the *H. pylori* isolates, the inoculum was adjusted in opacity until a 3–4 McFarland turbidity standard was reached. It was then flooded onto Mueller–Hinton agar plates (Oxoid) containing 7 % horse blood. The E-test strip, containing clarithromycin (0.125–256 µg ml^{-1}; Abbiodisk), was placed onto the plate and then dried, according to the manufacturer's instructions. Incubation was performed in a microaerophilic atmosphere for 48–72 h at 37 °C. MIC was established according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org/clinical_breakpoints/). The breakpoint used for clarithromycin was > 0.5 µg ml^{-1}.

We used disk diffusion susceptibility testing as a simple, cheap and easy means to evaluate the susceptibility of *H. pylori* to drugs. The E-test has been recommended because it has a more stable pattern of antibiotic release and has been found to tolerate prolonged incubation. Moreover, agar or broth dilution methods are difficult to perform routinely.

Determination of point mutations in the *H. pylori* 23S rRNA gene. In order to detect mutations related to clarithromycin resistance in the 23S rRNA gene, PCR amplification methods and oligonucleotide primers derived from a known sequence of the 23S rRNA gene were used (sense, 5′-ACG GCG GCC GTA ACT ATA-3′, positions 2357 to 2375, GenBank accession number U27270; antisense, 5′-TTA GCT AAC AGA AAC ATC AAG-3′, positions 2635 to 2653, GenBank accession number U27270) (Taylor *et al.*, 1997; Kim *et al.*, 2008).

The PCR was carried out in a volume of 50 µl, according to the method of Chiarini *et al.* (2009). The amplification was performed in a Perkin-Elmer ThermoCycler 9700 under the following conditions: 95 °C for 10 min, followed by 40 cycles of 94 °C for 45 s, 50 °C for 45 s and 72 °C for 45 s, and a final extension at 72 °C for 5 min. The resulting PCR products were subjected to gel electrophoresis in a 2 % agarose gel, subsequently purified and concentrated for sequencing using Amicon Ultra 0.5 ml columns (Millipore). Purified amplicons were sequenced directly with the same forward and reverse primers as had already been used in the previous PCR, using an ABI PRISM BigDye Cycle Sequencing Ready Reaction kit (Applied Biosystems) according to the instructions supplied by the manufacturer. The analysis of products was performed using BioEdit v. 7.2.0 software.

Detection of *cagA*, the EPIYA motif, the typing of *vacA* alleles and determining *oipA* status. In order to detect the presence of the *cagA* gene and to analyse the EPIYA motif, the DNA from each strain was subjected to PCR, as described by Panayotopoulou *et al.* (2007). In determining the EPIYA motif, three different PCR products were obtained: EPIYA ABC (550 bp), EPIYA ABCC (650 bp) (GenBank accession numbers KM262820 and KM262821) and EPIYA ABCCC (750 bp). The correct sequence of the EPIYA motif was confirmed in all cases by sequencing, according to the procedure described in the previous paragraph. The signal peptide (s) and mid-region (m) of the *vacA* gene were amplified using the primers described by Atherton *et al.* (1995). Four different PCR products were obtained: *s1* (259 bp) and *s2* (286 bp) from the s-region, and *m1* (290 bp) and *m2* (352 bp) from the m-region (Atherton *et al.*, 1995). Two different PCR products were obtained from the i-region: *i1* (250 bp) and *i2* (260 bp) (Rhead *et al.*,

2007). The *oipA* status was determined in all strains by PCR and the sequencing of the signal region of the *oipA* gene, according to Yamaoka *et al.* (2002).

Statistical methods. By means of univariate analysis, the association between resistance to clarithromycin and demographic variables, gastrointestinal symptoms, *vacA*, *cagA*, and *oipA* status was assessed using the c^2 test or Fisher's exact test, as appropriate. In order to measure the association level, crude odds ratios (ORs) were calculated as the ratio between the odds of resistance to clarithromycin for 'exposed' and the odds of resistance to clarithromycin for 'not exposed'; corresponding 95 % confidence intervals (CIs) for ORs were calculated on the natural log scale as $\exp\{\ln(OR) \pm \text{SE}[\ln(OR)]\}$. Fisher's exact test was used to assess the statistical association between gene combinations and EPIYA motif. A *P*-value ≤ 0.05 was considered statistically significant.

If more than one variable was significantly associated with resistance to clarithromycin in the univariate analysis, they were included in a multivariate logistic regression, in order to compute the adjusted ORs and 95 % CI. The age variable was categorized with respect to the median of the analysed sample. All analyses were performed using v. 11.0 StataMP software (StataCorp LP).

RESULTS

Clarithromycin resistance

The resistance to clarithromycin test demonstrated that 75 patients were infected with *H. pylori*-susceptible strains and the remaining 25 with resistant strains. Nevertheless, a significant difference regarding the resistance of *H. pylori* to clarithromycin between female and male patients was highlighted. The analysis of the 23S rRNA gene also revealed a strong association between the presence of point mutations and macrolide resistance. The predominant point mutation observed among the 25 *H. pylori* resistant strains was A2143G in 20 cases (80 %) and A2142G in 5 cases (20 %). However, the point mutations C2195T and T2182C were detected in four cases (5 %) of *H. pylori* clarithromycin-susceptible strains. No point mutation in the 23S rRNA gene was found in the remaining 71 (95 %) of the clarithromycin-susceptible strains nor were any differences observed in the MIC values of the isolates with A2143G or A2142G point mutation (data not shown). The MICs of isolates are presented in Table 1.

cagA gene, EPIYA motif, *vacA* mosaicism distribution and *oipA* status

The *cagA* gene and *vacA* (s- and m-regions) genotype were assessed in all strains. The *cagA* gene was detected in 48 % of cases. The EPIYA motif ABC was found in 35 (73 %) of 48 *cagA*-positive strains, ABCC in 8 (17 %) and ABCCC in 2 (4 %), and a mixed ABC-ABCC and ABC-ABCC-ABCCC EPIYA motif was present in 2 (4 %) and 1 (2 %), respectively, of the *cagA*-positive strains. In all cases the EPIYA motif was confirmed by PCR product sequencing. Regarding the *vacA* gene, the *s1* allele was found in 60 cases, the *m1* allele in 37 cases and the *i1* allele in 48 cases. A double *vacA* mosaic combination was detected in only

Table 1. MIC of clarithromycin for 100 isolates of *H. pylori* by E-test

MIC (µg ml^{-1})	No. *H. pylori* isolates
<0.125	58
0.125	9
0.19	6
0.38	2
4	2
6	6
8	8
12	4
16	1
32	3
48	1

one case (2 %). Specifically, the *s1i1m1* combination was observed in 35 cases, *s1i1m2* in 12 cases, *s1i2m2* in 12 cases, *s2i2m2* in 40 cases and double mosaics *s1i1-i2m1-m2* were in 1 case. The distribution of *vacA* mosaicism with respect to the *cagA* gene is shown in Table 2. The absence of the *cagA* gene in six cases with the *s1i1m1* combination of the *vacA* gene was confirmed using the *cag2* 5′-GGAACCCTAGTCGGTAATG-3′ and *cag4* 5′-ATC TTTGAGCTTGTCTATCG-3′ primers, according to Argent *et al.* (2005).

There was found to be a strong association between the presence of mosaic *s1m1* and *cagA* positivity (29 cases versus 6 cases, *P*-value <0.001) and between mosaic *s2m2* and *cagA* negativity (38 cases versus 2 cases, *P*-value <0.001). The prevalence of the EPIYA pattern is shown in Table 3. The *oipA* gene was detected in all strains, thereby determining the *oipA* status by the number of CT repeats. The 'on' status was observed in 55 strains while the status was 'off' in the remaining 45. A total of 13 different *oipA* sequencing patterns were detected. The most prevalent in the 'on' frame status had six CT repeats, and this was found to be the case in other studies (Matteo *et al.*, 2010). Among out-frame status 'off' variants, the most prevalent were the patterns with eight CT repeats. The strains being studied in this research showed more

Table 2. Distribution of *vacA* mosaicism between positive and negative *cagA* strains

vacA mosaic	*cagA*-positive (48 cases)	*cagA*-negative (52 cases)	*P*-value
s1i1m1	29	6	<0.001
s1i1m2	10	2	
s1i2m2	6	6	
s2i2m2	2	38	
s1m1-m2	1	0	

Table 3. Prevalence of the EPIYA pattern among 48 *H. pylori* *cagA*-positive strains related to *vacA* genotype

EPIYA motif	*vacA* mosaic				
	s1i1m1 (29)	*s1i1m2* (10)	*s1i2m2* (6)	*s2i2m2* (2)	*s1i1-i2m1-m2* (1)
ABC	21	5	6	2	1
ABCC	4	4	0	0	0
ABCCC	2	0	0	0	0
ABC-ABCC	1	1	0	0	0
ABC-ABCC-ABCCC	1	0	0	0	0

P=0.721.

than five CT repeats in the 5′ region of *oipA* that were specific for European strains (Kauser *et al.*, 2005). The presence or absence of CTAA nucleotides may influence the frame state of the gene. CT patterns and their distribution are shown in Table 4. A close correlation was found between the status of the *oipA*, *vacA* and *cagA* genes and, indeed, the 'on' status occurred in 45 (89 %) of the *cagA*-positive strains, being associated in 27 cases (35 %) with *s1m1* mosaicism.

The relationship of clarithromycin resistance with *vacA*, *cagA* and *oipA* status

The *cagA* gene was variously distributed between susceptible and resistant strains. Both *cagA* positivity and negativity were significantly associated with susceptibility although the *P*-value was < 0.001. In addition, when we correlated *cagA*-positive to *cagA*-negative strains, a stronger association with clarithromycin susceptibility in the case of *cagA*-positive strains was observed. The number of negative strains was higher in the context of resistant strains than susceptible ones. Moreover, the association between the *cagA* gene and resistance was not statistically significant (Table 5).

Statistical analysis (Table 6) included crude ORs of resistance to clarithromycin with respect to demographic variables, gastrointestinal symptoms, *vacA*, *cagA* and *oipA* status. Only the gender variable was statistically significant in the univariate analysis, and this was used to assess the association between resistance or susceptibility to clarithromycin and *vacA*, *cagA* or *oipA* status. With only one variable significant in univariate analysis, multivariate analysis was found not to be required.

DISCUSSION

Resistance to antimicrobials is mainly associated with wide and indiscriminate use of antibiotics, and it can be considered a major cause of failure to eradicate *H. pylori* infections. Many reports have indicated that the prevalence of

Table 4. Frequency of the *oipA* CT repeat patterns according to Yamaoka *et al.* (2002)

Sequence of signal peptide-encoding region of *oipA*	No. of CT	No. of isolates
'On' status		
ATGAAAAAAGCCCTCTTA<u>CTCTCT</u><u>CTCT</u>CGTT	5	1
ATGAAAAAAGCCCTCTTG***CTAA***C<u>T</u><u>CTCTCTCTCTCGT</u>TTTGGCT	6	31
ATGAAAAAAGCTCTTTTA<u>CTCTCT</u><u>CTCTCTCTCT</u>CGTT	8	15
ATGAAAAAAGCTCTCTTA***CTAA***C<u>T</u><u>CTCTCTCTCTCTCT</u>CGTT	9	6
ATGAAAAAAGCTCTCTTA<u>CTCTCT</u><u>CTCT</u>**TT**<u>CTCT</u>CGTT	5 + 2	2
Total		55
'Off' status		
ATGAAAAAAGCTCTCTTA***CTAA***C<u>T</u><u>CTCTCTCT</u>CGTT	5	4
ATGAAAAAAGCCCTCTTA<u>CTCTCT</u><u>CTCTCTC</u>CGTT	6	4
ATGAAAAAAGCTCTCTTA***CTAA***C<u>T</u><u>CTCTCTCTCTCT</u>CGTT	7	6
ATGAAAAAAGCTCTCTTA<u>CTCTCT</u><u>CTCTCTCT</u>CGTT	7	5
ATGAAAAAAACTCTCTTA***CTAA***C<u>T</u><u>CTCTCTCTCTCTCT</u>CGTT	8	15
ATGAAAAAAGCTCTCTTA<u>CTCTCT</u><u>CTCTCTCTCT</u>CGTT	9	10
ATGAAAAAAGCTCTCTTA***CTAA***C<u>T</u><u>CTCTCTCTCTCTCTCT</u>CGTT	11	1
Total		45

Bold type indicates insertion of CTAA, italic bold type indicates insertion of TT and underlining indicates the CT repeat.

resistance varies geographically and that there is a broad range of resistance variability, depending on the use of antimicrobials. Clarithromycin is recognized as the key antibiotic for treating *H. pylori*, but, regrettably, the level of primary resistance is increasing worldwide (De Francesco *et al.*, 2010a,b).

An initial finding of this research is that the rate of *H. pylori* resistance to clarithromycin is high, occurring in 25 % of isolates throughout geographical areas in Sicily. It would appear higher than computed in previous Italian studies, but in agreement with the 23 % rate detected in the study by De Francesco *et al.* (2006). Nevertheless, the rate of *H. pylori* resistance to clarithromycin is lower than that found in other countries, such as France and Spain, and in recent Italian studies, which identified a rate of between 26 and 35 % (Cellini *et al.*, 2006; Saracino et al., 2012). Of interest, *H. pylori* resistance to clarithromycin has been found to be more frequently observed in female than in male patients. A possible explanation for this finding may be a wider use of antibiotics for curing

Table 5. Association between *cagA* gene and clarithromycin resistance

Clarithromycin resistance	cagA-positive (48 cases)	cagA-negative (52 cases)
Resistant	9	16
Sensitive	39	36

OR, 0.52; 95 % CI, 0.18–1.44.

infections, with a greater prevalence in females, with, for example, urinary tract infections (Giorgio *et al.*, 2013).

Resistance to clarithromycin in *H. pylori* results from point mutation in the peptidyltransferase loop region of the 23S rRNA gene. In this study, the most frequent point mutation (80 %) was A2143G. This mutation is predominant in *H. pylori* strains isolated not only from Europe but also from Japan. The strong association between resistance to macrolides and specific mutation in the 23S rRNA gene was confirmed in all cases. Furthermore, only the C2195T and T2182C mutations were not associated with resistance to clarithromycin in strains described in this paper (Agudo *et al.*, 2010.), but we observed that these isolates showed a higher MIC value equal to 0.38 µg ml^{-1}. The *cagA* and *vacA* genes are widely used as markers to

Table 6. Putative association of *vacA*, *cagA* and *oipA* with resistance to clarithromycin: univariate analysis

Variable	n	Resistance to clarithromycin	%	Crude OR[a]	95 % CI	P
Gender						0.028
Male	47	7	14.9	1.00		
Female	53	18	34.0	2.94	1.01; 9.25	
Age (years)						0.247
≤44	54	11	20.4	1.00		
>44	46	14	30.4	1.71	0.62; 4.75	
vacA						0.447
s1i1m1	35	12	34.3	1.00		
s1i1m2	12	1	8.3	0.17	0.02; 1.67	
s1i2m2	12	3	25.0	0.64	0.14; 2.87	
s2i2m2	40	9	22.5	0.56	0.20; 1.57	
s1m1-m2	1	0	0.0	–	–	
cagA						0.166
Negative	52	16	30.8	1.00		
Positive	48	9	18.8	0.52	0.18; 1.44	
oipA						0.202
On	55	11	20.0	1.00		
Off	45	14	31.1	1.81	0.66; 5.02	
Symptom*						0.080
GCNA	39	10	25.6	1.00		
GCA	28	8	28.6	1.16	0.39; 3.48	
PU	9	2	22.2	0.83	0.14; 4.75	
Other diseases	10	4	40.0	1.93	0.44; 8.51	
GCA+PU	13	0	0.0	–	–	
KG	1	1	100.00	–	–	

*GCNA, inactive chronic gastritis; GCA, active chronic gastritis; UP, peptic ulcer; KG, gastric cancer.
[a]Odds ratio.

characterize *H. pylori* virulence, and several epidemiological studies have reported geographical variations in the circulation of the virulence factors pertaining to microorganisms. In Sicily the *cagA* gene is present in 48 % of strains, in 73 % of the strains it is associated with the ABC EPIYA motif and in most it is associated with the presence of the *s1m1 vacA* allele.

The assessment of *vacA* gene mosaicism has revealed only three out of the four possible combinations, *s2m1* mosaicism never having been detected in the cases described in this paper. This finding, in agreement with previous studies (Atherton *et al.*, 1995), probably depends on the selective disadvantage of mosaicism, which undermines bacterial viability. The equal distribution of *s1m1* and *s2m2 vacA* is in accordance with observations in other countries [for example, in the US, where these are the most frequent combinations (Atherton *et al.*, 1995)]. However, and according to a previous study (Han *et al.*, 1998), *s2m2 vacA* mosaicism has been significantly associated with *cagA*-negative status, and we observed that *H. pylori* infections were sustained by a single *vacA* mosaic. The prevalence of *cagA* found in the strains used in this research was lower than that reported in Italy by De Francesco *et al.*, while the identified *s1* and *m1 vacA* alleles were similar to those observed in that study (De Francesco *et al.*, 2006). No correlation was found between resistance to macrolides and the 'on' status of the *oipA* gene.

Finally, this study has indicated that *cagA*-negative strains, in addition to *oipA* 'off' status, seem more resistant to clarithromycin than *cagA*-positive strains. The presence of *cagA*-positive isolates, a more virulent genotype, may damage the gastric epithelium extensively, and it is conceivable that, with inflamed mucosa, antibiotics can reach higher concentrations. Another possible explanation for resistance of *H. pylori* to clarithromycin is correlated with the possibility that antibiotic activity interferes with the metabolism of a dividing cell; *cagA*-positive strains may proliferate faster than *cagA*-negative strains, and the latter would, therefore, be more susceptible to antibiotics (van Doorn *et al.*, 2000).

In conclusion, the prevalence of *H. pylori* resistance to clarithromycin is higher in Sicily and more frequently associated with *s1m1*, *s1m2 vacA* mosaicism and with *cagA* negativity. We did not identify any relationship between resistance to clarithromycin and the status of the *oipA* gene. Using multivariate analysis, resistance to clarithromycin was significantly associated with the female sex. The results of this research indicate the advisability of monitoring the status of resistance to clarithromycin in Sicily because the efficacy of first-line therapy is expected to decrease in the future. It would be particularly useful to be able to test for resistance to clarithromycin prior to commencing conventional triple therapy. An antimicrobial susceptibility test is also recommended if the triple therapy should be chosen in Sicily, where antibiotic resistance to clarithromycin is high. Moreover, it is clear that the

inappropriate use of an anti-*H. pylori* regime will incur heavy costs in terms not only of an initial ineffective treatment but also of subsequent visits and investigations. Thus, the clinical use of alternative medicines could also be envisaged in Sicily.

ACKNOWLEDGEMENTS

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