Antibiotic resistance profiles in *Helicobacter pylori* strains isolated in Sicily (Italy)

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INTRODUCTION

Helicobacter pylori **colonizes the human stomach and is responsible for chronic gastritis, peptic ulcer disease and gastric mucosa-associated lymphoma curable after eradication of microorganism by therapy. The treatment with two antimicrobial agent clarithromycin and amoxicillin (or metronidazole) and an acid suppressor, such as a proton pump inhibitor, is recommended by various groups [1], but resistance to these antibiotics is a major predictive factor for therapeutic failure.**
Investigation on the susceptibility of *H. pylori* to antibiotics is one of the main factors associated with successful eradication therapy. Growing resistance is related to the patterns of antibiotic consumption, and may vary within patient groups according to the geographic region. The geographic area and *H. pylori* primary resistance are clinically important, and should be considered in the choice of eradication regimens.
The evolution of infection versus more severe gastric pathology has been related to virulence bacterial factors, the most frequently associated to microbial pathogenicity are two protein VacA and CagA, encodes by their genes [2, 3].
vacA **is a polymorphic gene encoding a vacuolating cytotoxin, it is present in all strains and comprises two well characterized variable parts, the s region, which may exists as an s1 or s2 allele, and m region which may occurs as m1 or m2 allele. Another polymorphic site has been identified in this site, the i region, also in this case present as i1 or i2 allele.**
cagA **(cytotoxin associated gene A) encodes an high molecular weight protein associated to the presence of a PAI [4]. CagA contains the five-aminoacid- motives EPIYA which are classified as A, B, C or D depending on aminoacidic sequence flanking them. Usually the C region could be repeated more than once, while the D region circulates only in Asian countries. The EPIY-C acts as primary CagA phosphorilation site whereas epidemiological studies have indicated a correlation between disease severity and increased number of EPIYA-C motives [5].**

AIM

In North Italy and many European countries *H. pylory* antibiotic susceptibility has been evaluated. As for Sicily, to our knowledge, no data are available for this aspect aim of this study was to evaluate antibiotic resistance pattern of one hundred strains isolated in three sicilian hospitals. Moreover, virulence–associated gene polimorphism of the same strains was carried out only for those isolated after our previous investigation [3].

METHODS

Clinical *H. pylori* strains were isolated from a sample of 100 patients who underwent upper gastrointestinal endoscopy at the Endoscopy Services of the Ospedali Civili Riuniti in Sciacca (Agrigento), of the Gastroenterology, Internal Medicine and Elderly Care, and Emergency Surgery Units of the University Hospital A.O.U.P. Paolo Giaccone in Palermo, and Gastroenterology Division, M. Raimondi Hospital, San Cataldo (CL).
The biopsies positive to rapid urease testing were placed on Columbia agar (Oxoid, Basingstoke, Hampshire, UK) with the addition of 7% horse blood and 0.4% Dent supplement (Oxoid). The plates were incubated at 37°C under microaerobic conditions (CampyGen; Oxoid) for 3–6 days.
***H. pylori* bacteria were identified on the basis of morphological and biochemical characteristics.**
For *in vitro* susceptibility testing of the *H. pylori* strains, a suspension equal to McFarland turbidity standard of 4 was prepared. Mueller Hinton agar (Oxoid), with 5% of sheep-blood, was used as culture medium to determinine antibiotic susceptibility and were inoculated by confluent swabbing of the surface with bacterial suspension.
For each strain sensitivity to different antibiotics was assessed, zones of inhibition were read after 3 days and confirmed after 5 days.
For virulence genotyping, DNA of each strain was used for amplify following genes: *vacA, cagA* and *23S rRNA*,using the specific primers [2,6,7].

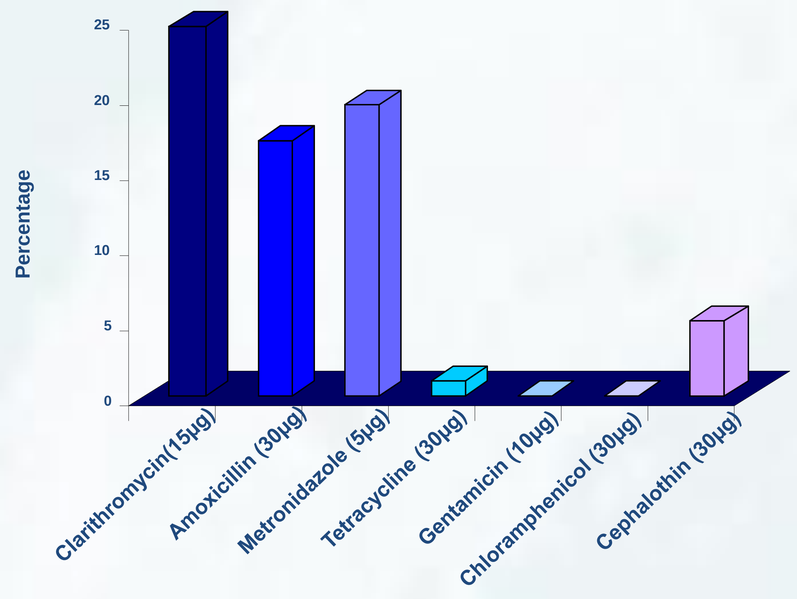

Fig. 1 Percentage of resistant *H. pylori* isolated strains

Tab 1 Virulence-associated *H. pylori* genotypes in patients with different gastrointestinal diseases. All patients were affected by chronic gastritis inactive (CG), active (CGA), and active and associated with a gastric or duodenal peptic ulcer (CGA+PU).

Genotype	Number (%) of genotypes from patients with			Number from all patients, n=100
	CG, n=54	CGA, n=29	CGA+PU, n=17	
cagA+	19 (35,2)	15 (51,7)	14 (82,3)	48
cagA−	35 (64,8)	14 (48,3)	3 (17,7)	52
vacAs1	26 (48,1)	20 (69)	14 (82,3)	60
vacAs2	28 (51,9)	8 (27,5)	3 (17,7)	39
mixed vacAs	0	1 (3,5)	0	1
vacAm1	12 (22,2)	15 (51,7)	8 (47)	35
vacAm2	42 (77,8)	13 (44,9)	9 (53)	64
mixed vacAm	0	1 (3,5)	0	1
vacAi1	20 (37)	16 (55,1)	11(64,7)	47
vacAi2	34 (63)	12 (41,3)	6 (35,3)	54
mixed vacAi	0	1 (3,5)	0	1

RESULTS

Antibiotic resistance assays, carried out by agar diffusion test, showed that the isolated strains were mainly resistant to the three most commonly used antibiotic. Results are represented in fig. 1. Clarithromycin resistance was confirmed by sequencing of the *23S rRNA* which is the target for the antibiotic.
The 15% of the analysed strains were resistant to more than one antibiotic. Interestingly and worryingly two strains were resistant to four antibiotics (amoxycillin, tetracycline, metronidazole, cephalotin) for one strain and amoxycillin, clarithromycin, metronidazole, cephalotin for the second one. No *H. pylori* strains was resistant to gentamicin and chloramphenicol.
Molecular analysis, carried out by *23S rRNA* sequencing revealed, for all resistant strains, a point mutation either in position A2142G or A2143G. Probably In two patients there was co-infection with a resistant and a sensitive strain, also correlated to the presence of two peaks in position 2142.
For all the isolated strains molecular analysis has been carried out to test genes known to be virulence- related. In particular, *vacA* and *cagA* were analysed. Results are shown in tab. 1.
Moreover, the EPIYA region was analysed revealing an high percentage of strains with ABC motif (fig. 2) well related with the higher amount of patients showing an inactive chronic gastritis and the absence of patients affected by gastric cancer.

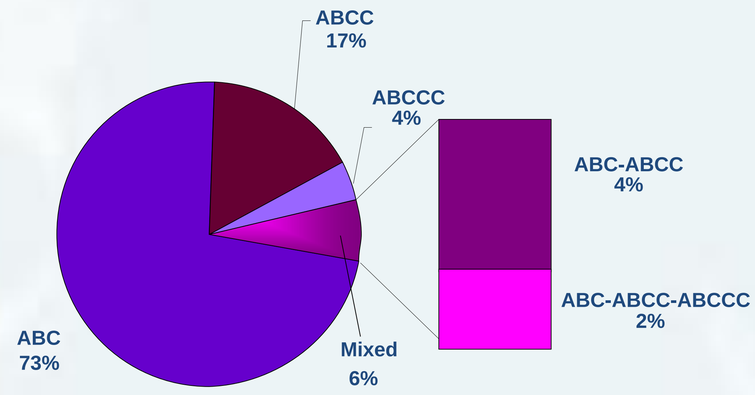

Fig. 2 Percentage of *EPIYA* motives in *cagA*-positive strains.

CONCLUSIONS AND PERSPECTIVES

H. pylori **still represents a great challenge for clinicians as therapy success is rapidly decreasing. This phenomenon is related to resistance acquiring particularly to clarithromycin and metronidazole. Our results demonstrate a high percentage of bacterial strain resistant to clarithromycin, amoxicillin, and metronidazole, first line drugs used in eradicating therapy. Resistance percentage found in Sicily are higher than those reported for other Italian regions [8], but lower when compared to those found in other countries such as France [9] and Spain [10].**
According to Maastricht III [1] an epidemiological surveillance is always recommended in order to choose the suitable antibiotic therapy. Moreover antibiotic-susceptibility testing should be carried out, when possible, for every patient before starting eradicating therapy.
DNA typing of virulence genes revealed the same genotype distribution found in other Italian regions and France [3] but a correlation between genotype and pathology severity or antibiotic resistance seems not to be possible at the moment.
The relationship between the number of EPIYA-C repeats and severity of the *H.pylori*-associated pathology is confirmed by our results, and the EPIYA analysis seems to be a promising prognostic tool in the course of *H.pylori* infection.

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