High prevalence to resistance of Clarithromycin in Helicobacter pylori strains isolated in Sicily .

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INTRODUCTION

Helicobacter pylori **infection is found wordwide and constitutes a public health concern in many countries. Previous epidemiological studies have shown a high prevalence of *H. pylori* infection in Sicily [1] fig. 1. Antibiotic resistance is the main factor affecting efficacy of current therapeutic regimens. Prevalence of bacterial resistance varies in different geographic areas, and it has been correlated with the consumption of antibiotic. Particularly in Southern European countries where Clarithromycin is largely used. Clarithromycin is an integral part of first-line therapies to treat *H. pylori* infection and the resistance to this antibiotic among *H. pylori* isolates is accepted as a main explanation of treatment failure [2].**
The current European guidelines on *H. pylori* management suggest that first-line therapy should be tailored according to Clarithromycin resistance and should be advised where primary resistance is > 15-20% [3].
Resistance of *H. pylori* to Clarithromycin is mainly due transition at position A2142G and A2143G and to transversion at point A2142G, which are included in the peptidyltransferase loop of the *23S rRNA*.
Other mutations, such as A2115G, G2141A, C2147G, T2190C, C2195T, A2223G and C2694A might, also be associated with Clarithromycin resistance [4].
Although *H. pylori* from individual patients typically have either an antibiotic susceptible or resistant phenotype, both antibiotic susceptible and resistant *H. pylori* (i.e. heteroresistance) have been reported. Heteroresistance can represent infection with a single strain harbouring two different copies of 23S rRNA gene or infection with several different *H. pylori* strains[5].

AIM

The current study has been undertaken to determine the prevalence of Clarithromycin resistance in H. pylori strains isolated in Sicily and to assess the most prevalent point mutation of 23S rRNA.

METHODS

Patients and *H. pylori* strains
Clinical *H. pylori* strains were isolated from patients who visited at Endoscopy Services of the Ospedali Civili Riuniti in Sciacca (Agrigento), A.O.U.P. Paolo Giaccone in Palermo and M. Raimondi Hospital, San Cataldo (CL).
One bioptic sample was taken from the antrum and body of each patient for cultural analysis. The bioptic specimens were cultured 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.
Clinical *H. pylori* strains were identified by Gram staining urease, oxidase, and catalase tests.
Susceptibility tests
For *in vitro* susceptibility testing of the *H. pylori* strains to Clarithromycin, a suspension equal to McFarland tube no. 4 was prepared for each isolate. Mueller Hinton agar (Oxoid), with 5% of sheep-blood, was used as culture medium for determining antibiotic susceptibility and was inoculated by confluent swabbing of the surface with the adjusted inoculum suspensions, zone size breakpoint was read after 3 days and confirmed after 5 days under microaerophilic incubation [6].
DNA Extraction and determination of point mutation in *23s rRNA*
Genomic content of bacteria was extracted from a fresh culture using Roche DNA kit according to manufacturer's instruction.
We confirmed our *H. pylori* identity with Nested PCR assay for *ureaseA* gene amplification.
Presence of point mutations in *23S rRNA* were analyzed by amplification and sequenced using the kit "ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction" (Applied Biosystems) and specific primers [4].
Random Amplified Polymorphic DNA (RAPD)-PCR Amplification
One hundred nanograms of template DNA of heteroresistance strains were used for each RAPD-PCR reaction.
The PCR-amplified fingerprinting patterns were visualized by ethidiumbromide staining under a short-wavelength ultraviolet light source [5].

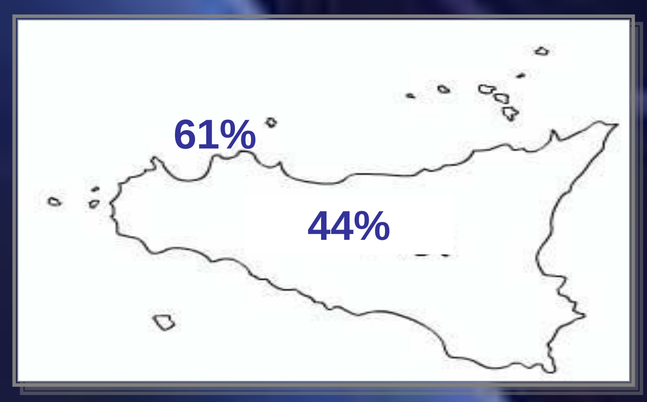

Fig. n°1 Prevalence of *H. pylori* infection in Sicily

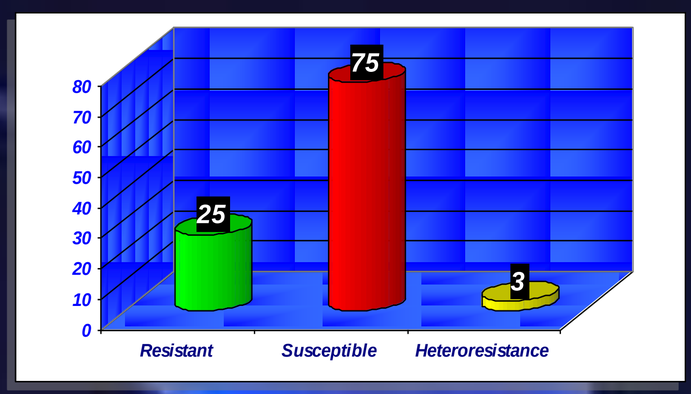

Fig n°2. Prevalence of resistance to Clarithromycin of *H. pylori* strains isolated in Sicily

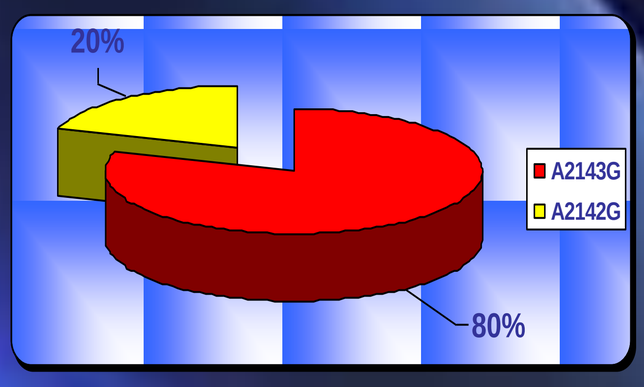

Fig n°3. Percentage of the most frequent *23S rRNA* gene point mutation of the Sicilian *H.pylori* strains.

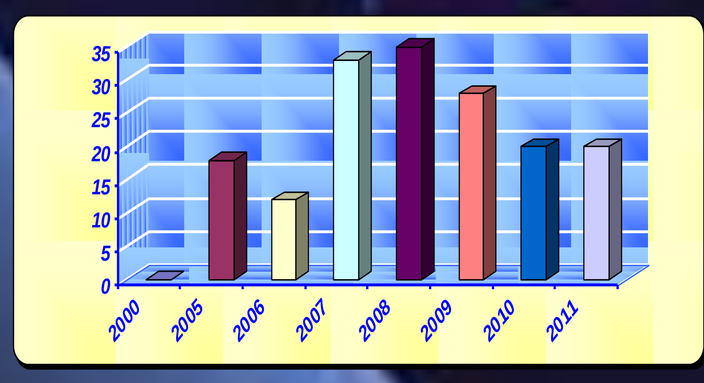

Fig n°4. Change in resistance rates of the H. p. strains isolated from 2000 to 2011

RESULTS

The assessment of Clarithromycin susceptibility, evaluated by Kirby-Bauer test, shows that 75% of *H. pylori* strains are susceptible, 25 % are resistant and 3% are heteroresistant, fig.2.
To confirm the high prevalence of resistance to Clarithromycin, sequence analysis of the *23S rRNA* gene have been carried out and a strong association between the presence of *23S rRNA* gene mutation and macrolide resistance have been founded.
The predominant mutation among the 25 *H. pylori* Clarithromycin-resistant strains is at A2143G in 80% of cases, while mutation A2142G is founded in 20% of cases, fig. 3.
However point mutations C2195T and T2182C are found in 5% cases of *H. pylori* Clarithromycin-susceptible strains.
The development of resistance, detected in the period from January 2000 to June 2011, is changed, fig. 4.
Individual colonies of the heteroresistance strains have been studied phenotypically and molecularly. In particular, a mutation in position A2142G is founded in resistant strains, whereas no mutation is present in sensitive strains.
The difference between resistant and sensitive strains isolated from the same patient, has been highlighted by the RAPD PCR, as it is apparent from the profile shown in fig. 5.

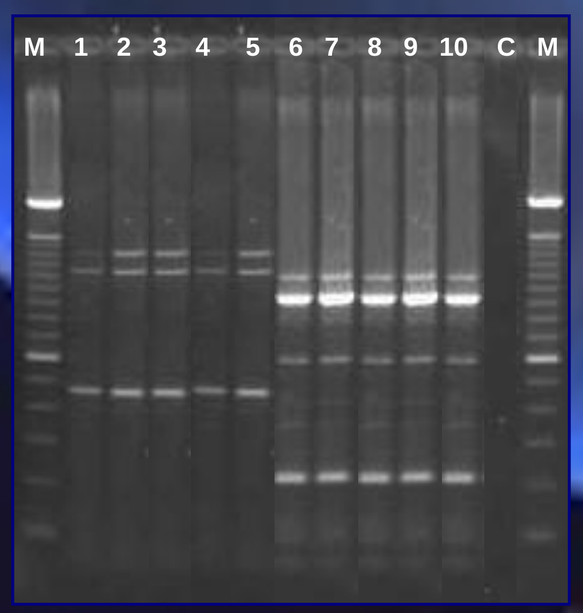

Fig n°5. RAPD PCR of two H. p. strains, sensitive (lines 1-5) and resistant (lines 6-10), M ladder 100bp, C negative control

CONCLUSIONS

Clarithromycin is the most important antibiotic included in all standard triple therapies for *H. pylori* eradication established worldwide.
Resistance to macrolide is a main cause of treatment failure in each eradication therapy, while its prevalence varies geographically, from Western to Eastern.
Our results demonstrate an high percentage (25%) of resistant to Clarithromycin bacterial strains. Resistance percentages found in Sicily are higher than those reported from other Italian regions, 16,6%[7], but lower when compared to those founded in other countries such as France, 26% [8], and Spain, 35% [9].
However the percentages found in our region have changed over the years. The percentages of resistance, which were clearly on the rise, following the directives issued guidelines defined in Maastricht, have been attenuated, certainly correlated to a controlled use of the drug. Guidelines discourage use of Clarithromycin in areas where resistance rates are more than 15-20%. In our region, unfortunately, still today, the resistance, although declining (from 35% in 2008 to 23% in 2011), remain over the threshold value. Also *De Francesco et al*., reported than in Italy, Clarithromycin resistance is present in near 10% of bacterial isolates, and it was >15% in only 3 Italian regions, begins as high as 25% in Sicily [9].
Clarithromycin resistance in *H. pylori* mainly results from point mutations in the peptidyltrasferase loop region of the *23SrRNA*. In our study, the most frequent point of the mutation is founded at position A2143G, present in 80% of cases; while A2142G is founded in only 20% of cases. This mutation predominates in *H. pylori* strains isolated from Europe and also from Japan.
Furthermore the strong association between resistance to macrolides and specific mutations in the *23S rRNA* gene is confirmed in 100% of cases [10].
Recent report indicated that other mutations might be also associated with claritromycin resistance; in 5% of our strains we found the mutation at the point T2182C and C2195T but not associated with resistance [11].
Finally, we found a different profile RAPD PCR on sensitive and resistant strains isolated from the same patient, correlated with a case of co-infection with different strains. This result suggests a stable association between different strains in persistent infections [4].

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