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

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INTRODUCTION

Helicobacter pylori infection is found worldwide and constitutes a public health concern in many countries. Previous epidemiological studies showed a high prevalence of H. pylori infection in Sicily (1, 2). 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 (3-5). 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% (3).

Resistance of H. pylori to Clarithromycin is mainly due to 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 A2110G, G2141A, C1470T, T2198C, C2197G, A2223G and C2594A might, also be associated with Clarithromycin resistance (3-5).

Although H. pylori from individual patients typically have either an antibiotic susceptible or resistant phenotype, both antibiotic susceptible and resistant H. pylori (i.e. heterogeneous) 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 (3).

METHODS

Clinical H. pylori strains were isolated from patients who visited at Endoscopy Services of the Ospedale Civile Rizzuti in Sciacca (Agrigento), A.O.U. Paolo Giaccone in Palermo and M. Raimondi Hospital, San Cataldo (CL). One biotastic sample was taken from the antrum and body of each patient for cultural analysis. The bioplastic specimens were cultured on Columbia agar (Oxoid, Basingstoke, Hampshire, UK) with the addition of 7% horse blood and 0.4% Gent antibiotic supplement (Oxoid). The plates were incubated at 37°C under microaerobic conditions (CampyGen; Oxoid) for 5-6 days.

Clinical H. pylori strains were identified by Gram staining urease, oxidase, and catalase tests.

RESULTS

The 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.

The assessment of Clarithromycin susceptibility, evaluated by Kirby-Bauer test, shows that 75% of H. pylori strains are susceptible, 25% are resistant and 5% are heteroresistant.

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 found.

The predominant mutation among the 25 H. pylori Clarithromycin-resistant strains is A2142G in 80% of cases, while mutation A2142G is found in 20% of cases, fig. 2.

However point mutations C1470T and T2198C are found in 5% of cases of H. pylori Clarithromycin-resistant 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 found 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.

CONCLUSIONS

Clarithromycin is the most important antibiotic included in all standard triple therapies for H. pylori eradication established worldwide. Therefore, a high prevalence to resistance in H. pylori is often observed in each eradication therapy, while its prevalence varies geographically, from Western to Eastern Europe. Our results demonstrate an high percentage (25%) of resistance to Clarithromycin bacterial strains. Resistance percentages found in Sicily are much lower than reported from other Italian regions, 16.8%, but lower when compared to those found in other countries such as France, 26% (6), and Spain, 35% (7).

However the percentages found in our region have changed over the years. The percentages of resistance, which were clear on the rise, following the directives issued guidelines 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 2000 to 23% in 2011), remain over the threshold value. Also, De Franceso et al., reported, that 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 (8).

Clarithromycin resistance in H. pylori mainly results from point mutations in the peptidyltransferase loop region of the 23S rRNA. In our study, the most frequent point mutation is the A2142G, present in 80% of cases; while A2142G is found in only 20% of cases. This mutation predominates in H. pylori strains isolated from Europe and also from Japan.

Performance of the drug and the strong association between resistance to macrolides and specific mutations in the 23S rRNA gene is confirmed in 100% of cases (9).

Recent report indicated that other mutations might be also associated with clarithromycin resistance; in 5% of our strains we found the mutation at the point T2182C and C2197T but not associated with resistance (10).

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 profile among different strains in persistent infections (11).

REFERENCES