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Helicobacter pylori infection: susceptibilty to antimicrobials and eradication rate in pluritreated pangastritis patients

KEYWORDS: Helicobacter pylori Pluritreated patients Pangastritis

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ABSTRACT *Aim of our study was to evaluate the utility of the culture and the subsequent susceptibility testing in a group of 100 pluritreated patients with pangastritis undergone several therapy cycles.*

Out of 100 patients, culture and susceptibility testing was obtained in 62 patients (62%) whereas in 38 (38%) no H. pylori growth was detected. The culture-positive group was treated following the antibiogram results whereas the culture-negative group was empirically treated. In the first group, the eradication rate was 77% (48/62) whereas in the second group, 32/38 subjects (84%) were eradicated with empirical or standard therapy.

The eradication rate of the patients with antrum prevalent pangastritis was higher than in the patients with corpus-fundus or diffuse pangastritis (96%, 75% and 61% respectively).

The difference between the two groups (77% and 84% respectively; p=0.72), even if not statistically significant demonstrates that a successful eradication can be achieved even without antibiogram.

Helicobacter pylori (Hp) resistance is a primary drawback in achieving eradication.

Treatment regimens for *Hp* used in the past are declining in efficacy and the treatment of *Hp* infection is bedevilled by drug-resistant strains. Antimicrobial susceptibility testing has therefore been proposed as a logical first step in treatment failure but controlled trials suggested that it may not always be essential for clinical management (Miwa, Nagahara, Kurosawa, Ohkusa, Ohkura, Hojo, Enomoto, & Sato, 2003). Infections in some clinical trials, even with correct use of drugs combination, are not eradicated in 10-20% of patients (Wu, Hu, Kuo, & Kuo, 2014).

Aim of the present study was to evaluate the eradication success in pluritreated patients with pangastritis.

100 pangastritis patients, 82 women (mean age 56yrs) and 18 men (mean age 55yrs), previously treated with more than 2 eradication attempts at Gastroenterology ward at Policlinico Umberto I° in Rome (Italy) were included in the study. All of them resulted to be positive to both ^{13}C-Urea Breath Test (UBT) and histology.

They were divided in three groups: antrum prevalent pangastritis (AP), corpus/fundus prevalent pangastritis (CP) and diffuse pangastritis (DP), according to the part of stomach mostly involved in the disease . Forty-eight out of 100 (48%) individuals showed AP, 16/100 (16%) CP and 36/100 (36%) DP. The most predominant pattern of gastritis was the *Hp* positive gastritis (pattern A), especially in those patients with AP (26/48, 54%). No individuals were included in the pattern C (alterations absence without gastritis and *Hp*).

Table 1. Characteristics of the 100 patients with pangastritis and eradication rate

Pangastritis type, N° patients (tot. 100)	Mean age (range)	Sex	N° of eradicant therapies	Pangastritis pattern, N° patients	Eradication rate N° patients Total %
AP (antrum prevalent), 48	53.4 (22-75)	40F-8M	2-6	A, 26 B, 4 C, 0 D, 10 E, 8	A, 24 B, 4 C, 0 **96%** D, 10 E, 8
CP (corpus/fundus prevalent),16	59.7 (55-61)	16F	3-6	A, 8 B, 2 C, 0 D, 0 E, 6	A, 6 B, 2 C, 0 **75%** D, 0 E, 4
DP (diffuse), 36	54.4 (37-69)	26F-10M	2-9	A, 18 B, 4 C, 0 D, 6 E, 8	A, 10 B, 2 C, 0 **61%** D, 4 E, 6

62 patients were *Hp* positive whereas 38 were *Hp* neg-

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ative

Legenda: A= gastritis *Hp* positive; B= gastritis *Hp* positive with metaplasia;

C= alterations absence (no gastritis, no *Hp*); D= regenerating hyperplasia (biliary reflux); E= gastritis *Hp* negative .

The culture test was performed separately on gastric biopsy specimens drawn from the different sites of stomach.

The culture and the susceptibility tests for *Hp* were performed following our previous research (Mascellino, Porowska, Nicosia, Oliva, Boccia, & Severi, 2010.) The antibiotics considered were the following: metronidazole (MZ), levofloxacin (LEV), tetracycline (TE), chlaritromycin (CLA) and amoxycillin (AMX).

Genotypic susceptibility testing for resistance to CLA and TE was carried out as previously described (Glocker, Berning, Gerrits, Kusters & Kist, 2005). . Considering the presence or absence of *Hp* growth in the cultures, patients were assigned respectively to antibiotic susceptibility-tailored therapy or empirically-tailored therapy. In the empirically therapy arm, an association of two antibiotics that were never used before or AMX (1gr) and MZ (250mg), were both given twice a day for two weeks combined with Pump-Proton Inhibitors (PPIs) (40 mg once a day) following the gastritis patterns .

In the antibiotic susceptibility therapy arm, a triple therapy has been used consisting of PPI and two antibiotics following the antibiogram results.

Hp eradication was tested by a validated UBT three months after therapy completion.

Out of 100 patients, culture and susceptibility testing was obtained in 62 patients (62%) whereas in 38 (38%) no *Hp* growth was detected. All the biopsies were urease positive in both groups.

One-hundred twenty-two strains of ***Hp*** were isolated in the 62 positive subjects including the three stomach regions. The most efficacious antibiotics resulted to be AMX with a percentage of susceptibility of 98% (120/122 strains) and TE with 97% (118/122) whereas the most ineffective antibiotic was MZ with only the 56% of susceptibility (68/122).

As for MIC distribution , our isolates resulted to be highly resistant to MZ with 12 strains having a MIC value >256 mcg/ml. AMX showed a MIC value <0.12 mcg/ml for all strains except two with MIC <1mcg/ml. MICs of CLA and LEV ranged from 0.12 to 128 mcg/ml whereas MIC of TE ranged from 0.12 to 32 mcg/ml.

In the 62 patients positive to ***Hp,*** the eradication rate was 77% (48/62) whereas in the 38 patients with no growth of ***Hp***, it was 84% (32/38).

Following the pattern of pangastritis, the eradication rate in AP patients was greater than in CP and even more than in DP patients in both groups (with and without ***Hp)*** (96% vs 75%, p=0.14 and 96% vs 61%, p=0.01 respectively). The difference between the eradication rate in AP patients respect to DP ones was statistcally significant (Table 1).

The real time PCR assay was used for detection of the mutations in the regions 23S rRNA and 16S rRNA (conferring resistance to CLA and TE respectively) and compared with E-test method.

Table 2 about here.

As far as CLA is concerned, 8 patients showed heteroresistance: in the antrum there was the wild type (susceptible) together with strains carrying the mutation A21444G that were resistant by E-test, whereas in the corpus only the wild type was present which resulted to be susceptible also by phenotipic test. Forty-four patients had both the wild type and the mutant one contemporaneously. Out of these 44 subjects, 10 resulted to be resistant to CLA and 34 susceptible through E-test method. Ten patients showing only the mutations in the 23S genes resulted to be fully resistant to CLA also by phenotypic test. As far as TE is concerned, 52 patients appeared susceptible through both E-test method and PCR assay whereas in 10 no specific ***Hp*** DNA has been detected by molecular tests.

We can deduce that a mixed infection with resistant and susceptible strains contemporaneously may be seen by a real-time PCR but through E-test the susceptible bacteria were primarily found. In cases with mixed infections or with contaminations or when live bacteria were no longer available, PCR is strongly superior than bacterial culture and phenotypic testing. This method resulted to be applicable both to DNA extracted from live bacteria and to DNA extracted from fresh or frozen ***Hp***-infected gastric biopsy samples (De Francesco, Zullo, Giorgio, Saracino, , Zaccaro, Hassan, Ierardi, Di Leo, Fiorini, Castelli, Lo Re, & Vaira, 2014). The TE assay is a bit less sensitive than CLA assay; that's the reason why we had a CLA result for all strains but not a TE result for all.

Our findings are in line with other data (Wu et al., 2014). There is evidence that increasing ***in vivo*** the dosage of MZ administered, an improvement of therapy outcome, when treating MZ–resistant strains, is generally found (Jenks, 2002). In fact the ***in-vitro*** results of MZ may overestimate its rate of resistance because of the microaerophilic atmosphere in which ***Hp*** grows.

In 8 patients a different pattern of resistance to CLA was detected when considering the different districts of the stomach . Consequently, in order to avoid misclassification of a strain as sensible where only one biopsy region was investigated, three biopsy sites from each patient should always be considered.

The fact that our pangastritis patients underwent multiple cycles of therapy worsened the outcome of the antibiotic therapy. ***Hp*** eradication in fact continues to be a challenge in the patients with pangastritis who appear to be the most difficult ones to be cured (O'Connor, Vaira, Gisbert, & O'Morain, 2014).

In our study, the ultimate ***Hp*** eradication rate corresponded to 80% (80/100): 48/62 (77%) in the patients where culture and subsequent antibiotic assays could be obtained and 32/38 (84%) in those where no ***Hp*** strains were detected. In the latter group that in any case resulted positive to UBT and underwent an empiric or standard therapy, there was either no ***Hp*** growth or presence of dormient bacteria or coccoid forms (that are enable to grow) or very low numbers of bacteria (too low to be cultured).

In our study the lower detection of ***Hp*** (62/100 patients,

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62%) than in other ones (Wu et al., 2014) is probably due to our selected population. In these patients, ***Hp*** infection is considered quite characteristic because the bacteria are able to colonize a stomach with reduced acid secretion and virulence and persistence mechanisms may be different respect to patients with normal acid secretion.

In conclusion our research seems to demonstrate that a successful eradication can be achieved even without antibiogram. In literature, this question is still controversial (O'Connor et al., 2014). Following our results, we can say that in highly selected group of pangastritis patients, guidelines for culture of ***Hp*** and susceptilbility-based therapies do not apply and should be reconsidered being economically demanding, time consuming and not available in many hospitals.

Table 2. Strains genotyping for Clarithromycin (CLA) and Tetracycline (TE) resistance through real time PCR assays by hybridization probes and comparison with E-test method.

N° of patients (Total 62)	***CLA resistance testing, PCR***	***CLA resistance testing, E-test***	***TE resistance testing, PCR***	***TE resistance testing, E-test***
8*	*Antrum*: Wild type+A2144G *Corpus* : Wild type	R S	Wild type	S
10	Wild type+A2144G	R	Wild type	S
34	Wild type+A2144G	S	Wild type	S
10	A2144G	R	Negative	6 S 4 R

*** Eight patients with heteroresistance to CLA**

A2144G is the mutation in the region 23S of *Hp* chromosome conferring resistance to CLA.

R: Resistant; S: Susceptible.

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