

Belo Horizonte, 27 June 2016

Response letter

Scientific editor Ze-Mao Gong
World Journal of Gastroenterology

Re: **ESPS manuscript NO: 27171**

Title: Detection of Helicobacter pylori resistance to clarithromycin and fluoroquinolones in Brazil: a national survey

Dear Editor,

Please, find below my replies raised by the reviewers of the manuscript:

Reviewer's code: 00183445

Reviewer's country: Poland

☐ No points to be addressed.

Reviewer's code: 00028580

Reviewer's country: Lebanon

Comments by the reviewer:

This is a well done paper examining resistance to clarithromycin and levofloxacin in Brazil. The methodology is sound and clear and the manuscript is well written. **It would have been helpful had the authors determined metronidazole resistance although this understandably would have required bacterial culture and E-test methodology. The discussion should perhaps cover this point and discuss available data on metronidazole resistance in Brazil as the combination of dual resistance to clarithromycin and metronidazole is associated with considerable decrease in the efficacy of regimens**

containing both agents.

Reply: Genotype HelicoDR test is designed to identify the most frequent mutations in the 23S gene and gyrA and it is not able of detecting gene mutations rdxA responsible for the resistance to metronidazole. But, we also agree that the consideration of dual resistance to clarithromycin and metronidazole is relevant for anti-HP therapy in Brazil. So, we added the following paragraph in the discussion: ... *“Considering the high rates of metronidazole resistance observed in Brazil^[28,29] our findings suggest that the association of this agent with clarithromycin or fluoroquinolones could promote reduction in the H. pylori eradication rates..”*

Reviewer's code: 03261349

Reviewer's country: Italy

Comments by the reviewer:

Sanches et al investigated the prevalence of antibiotic resistances to clarithromycin and fluoroquinolones in a multicentric study conducted in Brazil. Samples from gastric biopsy have been collected in the period 2012-2015. They found a resistance rate of 16.9% for clarithromycin and 13.5% for fluoroquinolones. **Main comments:** A linguistic revision is necessary. It is strange that 29 out of 519 patients with established H. pylori diagnosis tested negative at molecular analysis, taking into account that this technique has a better performance than other tests (see Ierardi E et al, J Med Microbiol 2015). The point mutations A2142C, A2142G and A2143G are the most relevant ones, since they more deeply impact on the success of antibiotic treatment (see De Francesco V et al, Ann Intern Med 2006, and Megraud F, ref 7). However, none of these mutations has been investigated in the present study. These mutations have been already reported in Brazil (see Ribeiro ML, Ann Clin Microbiol Antimicrob 2003; Lins AK, Arq Gastroenterol 2010; Suzuki RB, BMC Gastroenterol 2013). This is the most burdensome drawback. Therefore, Authors should provide sufficient discussion/bibliography demonstrating that the mutations that they have analyzed have an impact on therapeutic outcome.

Reply:

A linguistic revision is necessary.

A linguistic revision was performed.

It is strange that 29 out of 519 patients with established H. pylori diagnosis tested negative at molecular analysis, taking into account that this technique has a better performance than other tests (see Ierardi E et al, J Med Microbiol 2015).

We would like to clarify that during the recruitment process, in order to select patients probably infected by HP, we performed a rapid serological test in the endoscopy room and only included in the study those who were serologically positive. Although in a previous study by our group, this serological test showed a high positive predictive value (Helicobacter 2013; 18: 120), we considered that 29 (5.6%) patients who tested negative at molecular analysis represented probably false-positive results on the serological evaluation. We do agree the better performance of molecular test compared to other diagnostic tests.

The point mutations A2142C, A2142G and A2143G are the most relevant ones, since they more deeply impact on the success of antibiotic treatment (see De Francesco V et al, Ann Intern Med 2006, and Megraud F, ref 7). However, none of these mutations has been investigated in the present study. These mutations have been already reported in Brazil (see Ribeiro ML, Ann Clin Microbiol Antimicrob 2003; Lins AK, Arq Gastroenterol 2010; Suzuki RB, BMC Gastroenterol 2013). This is the most burdensome drawback. Therefore, Authors should provide sufficient discussion/bibliography demonstrating that the mutations that they have analyzed have an impact on therapeutic outcome.

We totally agree that the point mutations A2142C, A2142G and A2143G are the most relevant and directly related with the success of anti-HP treatment. These point mutations are equivalent to those described by us as A2146C, A2146G and A2147G since we used the denomination coming from genome sequencing of GenBank NC000921 - J99 and NC000915 - HP 26695 as cited in reference 13 of our

paper.

To turn these findings clearer we changed the following paragraph:

“Clarithromycin interacts with the peptidyl transferase in domain V of the 23S rRNA subunit, an interaction that suppresses bacterial ribosomes activity and inhibits protein synthesis^[9]. Point mutations at positions 2146 and 2147, formerly known as 2142 and 2143 (the numeration is from genome sequencing of GenBank NC000921 - J99 and NC000915 - HP 26695)^[13], of the 23S rRNA gene have been shown to lead to a modification in ribosome conformation, which consequently reduces clarithromycin affinity and leads to bacterial resistance to the drug^[9].”

Hoping that we could be able to answer all questions raised by the reviewers.

With best regards

Dr. Bruno SF Sanches