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Discrepancies between E-Test and Agar Dilution Methods for Testing Metronidazole Susceptibility of *Helicobacter pylori*

We read with interest the recently published paper by Piccolomini et al. (7). They evaluated three different methods to study the susceptibility of *Helicobacter pylori* to 20 antimicrobial agents. They found a very good correlation between E-test and agar dilution methods, as well as between broth microdilution and agar dilution. They recommended the E test as a reliable and alternative method for testing susceptibility of *H. pylori* to a wide range of antimicrobial agents in clinical practice. However, they found six major errors (6 of 71, 8.5%) and two very major errors (2 of 71, 2.8%) with the metronidazole E tests when they compared the results with those obtained by reference methods. They consider that chance is the explanation for why these errors were observed only with metronidazole.

Among the 20 antibiotics studied by Piccolomini et al., only some, metronidazole, tetracycline, amoxicillin, and clarithromycin, have been widely used in clinical practice. It is important to consider that for precisely 11.3% of the isolates, discrepancies between the E-test and agar dilution methods are with metronidazole.

We studied the in vitro activity of metronidazole against 36 *H. pylori* clinical isolates by E-test and agar dilution methods, and we found two major errors (5.5%) and three very major errors (8.3%). We found no discrepancies when amoxicillin was studied.

Although the E test is much less laborious and is easier to perform than the agar dilution method, especially for routine purposes, the results obtained with metronidazole should be confirmed by agar dilution.

Some investigators have reported an excellent correlation between E-test results and those obtained by standards methods, with no major or very major errors (2, 4). However, Von Recklinghausen et al. reported a 13.3% discrepancy, major or very major, for metronidazole when the E test was compared with agar dilution (8).

On the other hand, Piccolomini et al. studied 71 isolates of *H. pylori* and found MICs at which 90% of the isolates were inhibited (MIC_{90}s) by agar dilution of 0.25 mg/liter for azithromycin, 1 mg/liter for clarithromycin, 0.5 mg/liter for erythromycin, and 0.125 mg/liter for roxithromycin. They found 0% resistance to azithromycin and roxithromycin, 6% resistance to clarithromycin, and 8% resistance to erythromycin.

We have studied Spanish *H. pylori* clinical isolates and found that clarithromycin was the most active among the five macrolides tested (erythromycin, clarithromycin, azithromycin, roxithromycin, and midecamycin) (1). The results are shown in Table 1. Other authors have also studied the in vitro activities of macrolides. Hardy et al. found that clarithromycin (MIC_{90}, 0.03 mg/liter) is the most active of the macrolides tested, clarithromycin being 4 to 32 times more active than other macrolides (3). The MIC_{90} for erythromycin, roxithromycin, and azithromycin was 0.25, while for clarithromycin it was 0.03 mg/liter (3). Clarithromycin (MIC_{90}, 0.03 mg/liter) was also found to be significantly more active than either erythromycin (MIC_{90}, 0.125 mg/liter) or azithromycin (MIC_{90}, 0.25 mg/liter) in the study of Malanoski et al. (6).

Among the most frequently used antibiotics for *H. pylori* infection, metronidazole and clarithromycin show different percentages of resistance, depending on the population studied (5); therefore, *H. pylori* susceptibility should be determined.

TABLE 1. MIC_{50}, MIC_{90}, range, and percentage of resistance to five macrolides in Spanish *H. pylori* clinical isolates

Macrolide	MIC[a]			% Resistance
	50%	90%	Range	
Erythromycin	0.25	32	0.008–128	17.8
Clarithromycin	0.008	0.064	0.008–16	4.8
Azithromycin	0.125	32	0.125–64	10.5
Roxithromycin	0.125	16	0.008–64	12.5
Midecamycin	0.25	16	0.008–32	14

[a] Measured in milligrams per liter.

It is important that different laboratories perform *H. pylori* susceptibility tests under the same conditions, and standardization is strongly recommended so that results from different laboratories can be compared.

REFERENCES

1. **Alarcón, T., D. Domingo, I. Sánchez Romero, M. J. Martinez, J. C. Sanz, and M. López-Brea.** 1996. In vitro activity of 16 antimicrobial agents against *Helicobacter pylori* clinical isolates. Rev. Esp. Quimoter. **9:**138–142.
2. **Glupczynski, Y., M. Labbé, W. Hansen, F. Crokaert, and E. Yourassowsky.** 1991. Evaluation of the E-test for quantitative antimicrobial susceptibility testing of *Helicobacter pylori*. J. Clin. Microbiol. **29:**2072–2075.
3. **Hardy, D. J., C. W. Hanson, D. M. Hensey, J. M. Beyer, and P. B. Fernandes.** 1988. Susceptibility of *Campylobacter pylori* to macrolides and fluoroquinolones. J. Antimicrob. Chemother **22:**631–636.
4. **Hirschl, A. M., M. M. Hirschl, and M. L. Rotter.** 1993. Comparison of three methods for the determination of the sensitivity of *H. pylori* to metronidazole. J. Antimicrob. Chemother. **32:**45–49.
5. **López-Brea, M., D. Domingo, I. Sánchez, and T. Alarcón.** 1997. Evolution of resistance to metronidazole and clarithromycin in *Helicobacter pylori* clinical isolates from Spain. J. Antimicrob. Chemother. **40:**279–281.
6. **Malanoski, G. J., G. M. Eliopoulos, M. J. Ferraro, and R. C. Moellering.** 1993. Effect of pH variation on the susceptibility of *Helicobacter pylori* to three macrolide antimicrobial agents and temafloxacin. Eur. J. Clin. Microbiol. Infect. Dis. **12:**131–133.
7. **Piccolomini, R., G. D. Bonaventura, G. Catamo, F. Carbone, and M. Neri.** 1997. Comparative evaluation of the E test, agar dilution, and broth microdilution for testing susceptibilities of *Helicobacter pylori* strains to 20 antimicrobial agents. J. Clin. Microbiol. **35:**1842–1846.
8. **Von Recklinghausen, G., and R. Ansorg.** 1995. Metronidazole susceptibility testing of *Helicobacter pylori* with the PDM epsilometer test (E-test). Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis. **282:**83–85.

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Authors' Reply

We thank Drs. Alarcón, Domingo, and López-Brea for their letter concerning the discrepancies between the E-test and agar dilution methods for testing metronidazole susceptibility of *Helicobacter pylori* and their reference to our report on susceptibility testing of *H. pylori* (2).

It is commonly accepted that the emergence of primary

resistance and acquired resistance to metronidazole in *H. pylori* is usually associated with treatment failure. The differences between the resistance rates in our report and that of Alarcón et al. may reflect the variation in metronidazole usage between countries, since use of metronidazole alone can easily induce resistance to the drug in *H. pylori*. Furthermore, the methodological variables and the differences in interpretation of susceptibility test results may also contribute to the varied resistance rates.

Therefore, we essentially agree with Alarcón et al. about the necessity to standardize antimicrobial susceptibility testing for *H. pylori*. We do, however, offer the following comments.

First, in our study we had no explanation other than chance for why the major (6 of 71, 8.5%) and the very major (2 of 71, 2.8%) errors were observed only with metronidazole because the E test and the agar dilution tests were performed under the same conditions (the culture medium and the atmosphere, temperature, and duration of incubation) except for the size of the bacterial inoculum. However, Cederbrant et al. (1) showed that, in contrast to results obtained by the standard broth or agar dilution method, the E-test results were not significantly affected by inoculum density.

Second, it is commonly accepted that one of the prominent reasons for treatment failures is that *H. pylori* strains that survive after eradication therapy remain in the surface mucous gel layer more frequently than on the surface of surface mucous cells (3). This finding may indicate that the concentrations of the drugs administered cannot fully eradicate the *H. pylori* in the mucous gel layer. The drug concentrations in the mucous gel layer itself have not been determined, and this, consequently, makes the standardization of the MIC corresponding to the cutoff level defining the breakpoint for resistance impossible. For these reasons, the percentage of discrepancies found in susceptibility testing may depend on the breakpoint MIC considered, as shown in Table 1.

In conclusion, although we agree with the conclusion of Alarcón et al., we maintain that the lack of standardization of the breakpoint MIC for metronidazole is mostly responsible for determining the accuracy of the E test.

TABLE 1. Correlation between breakpoint MICs and discrepancies found in testing metronidazole susceptibility of *H. pylori*

Breakpoint MIC	No. (%) of errors[a]		
	Very major	Major	Total
>32 (our study)	2 (2.8)	6 (8.5)	8 (11.3)
≥32	2 (2.8)	0	2 (2.8)
>16	1 (1.4)	0	1 (1.4)
>8	2 (2.8)	3 (4.2)	5 (7)

[a] Discrepancies in MICs were characterized as follows: very major error, E-test result was susceptible and agar dilution result was resistant; major error, E-test result was resistant and agar dilution result was susceptible.

REFERENCES

1. **Cederbrant, G., G. Kahlmeter, and A. Ljungh.** 1993. The E test for antimicrobial susceptibility testing of *Helicobacter pylori*. J. Antimicrob. Chemother. **31:**65–71.
2. **Piccolomini, R., G. Di Bonaventura, G. Catamo, F. Carbone, and M. Neri.** 1997. Comparative evaluation of the E test, agar dilution, and broth microdilution for testing susceptibilities of *Helicobacter pylori* strains to 20 antimicrobial agents. J. Clin. Microbiol. **35:**1842–1846.
3. **Shimizu, T., H. Ota, Y. Kawakami, A. Gotoh, T. Akamatsu, and T. Katsuyama.** 1996. Immunohistochemical detection of *Helicobacter pylori* in the surface mucous gel layer and its clinipathological significance. Helicobacter **1**(4)**:**197–206.

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