

# Comparative Evaluation of the E Test, Agar Dilution, and Broth Microdilution for Testing Susceptibilities of *Helicobacter pylori* Strains to 20 Antimicrobial Agents

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Received 7 November 1996/Returned for modification 4 February 1997/Accepted 31 March 1997

**The Epsilon test (E test; AB Biodisk, Solna, Sweden), a new quantitative technique for the determination of antimicrobial susceptibility, was compared to reference methods (agar dilution and broth microdilution) for the antimicrobial susceptibility testing of *Helicobacter pylori*. Seventy-one *H. pylori* strains isolated from patients with duodenal ulcers were tested against 20 antimicrobial agents. The E test and the agar dilution method were carried out on Mueller-Hinton agar; the broth microdilution method was performed with Mueller-Hinton broth. The E-test results showed excellent correlation with the agar dilution results, with 91.3 and 98.8% agreement within 1 and 2 log<sub>2</sub> dilution steps, respectively, in a total of 1,350 tests. The correlation between the E-test results and the broth microdilution results was slightly higher, with 91.6 and 99.1% agreement within 1 and 2 log<sub>2</sub> dilution steps, respectively, in a total of 1,317 tests. There were six major errors and two very major errors by the metronidazole E test compared to the results obtained by reference methods. Excellent agreement between E-test, agar dilution, and broth microdilution results was found for resistance to erythromycin (8%), clarithromycin (6%), and tetracycline (6%). Our results confirm that the E test is comparable to standardized methods for susceptibility testing. Therefore, the E test is a reliable and alternative method for testing *H. pylori* susceptibility to a wide range of antimicrobial agents in clinical practice.**

*Helicobacter pylori* is now accepted as a major cause of chronic type B gastritis (9, 14, 16), and there is a strong association with peptic ulceration (16, 19, 30) and gastric cancer (10, 11, 23), two of the most important diseases in the upper gastrointestinal tract. Treatment with antimicrobial agents and/or bismuth salts successfully eradicates *H. pylori* from the gastric mucosa, producing favorable clinical responses (1), but relapses frequently occur after therapy (12, 18). In fact, resistance of *H. pylori* to metronidazole and other 5-nitroimidazoles has emerged worldwide and now constitutes a major problem in therapy (1, 3, 12, 25). Therefore, the treatment of infections caused by *H. pylori* requires that special attention be given toward reliable methods for determining the in vitro susceptibility of this microorganism. Susceptibility testing of *H. pylori* is not yet either standardized or routinely performed in most laboratories. Several investigators have reported the susceptibilities of *H. pylori* to antimicrobial agents using various MIC methods (7, 12, 15, 18, 20), but no standard methods exist because of the slow growth of the bacterium combined with its requirement for numerous additives in the growth medium and a microaerophilic atmosphere. The Epsilon test (E test; AB Biodisk, Solna, Sweden), a modification of the disk diffusion and the agar dilution methods, is a recently developed technique for quantitative determination of susceptibility to antimicrobial agents (4).

The purpose of this study was to assess the reliability of results obtained by using the E-test methodology for determining the quantitative susceptibility of *H. pylori* to 20 antimicrobial agents of possible clinical relevance. To accomplish this,

we compared the results obtained by the E-test method with results obtained by the agar dilution and broth microdilution methods. In addition, agar dilution results were compared with broth microdilution results.

(Part of this study was presented at the IX International Workshop on Gastrointestinal Pathology and *Helicobacter pylori* in Copenhagen, Denmark, 16 to 19 October 1996 [24].)

## MATERIALS AND METHODS

**Bacterial strains.** Seventy-one consecutive nonduplicate clinical strains of *H. pylori* isolated from patients with duodenal ulcer or gastritis were tested. The strains were identified by Gram staining and oxidase, catalase, and urease reactions. After identification, the bacteria were stored at -80°C in aliquots of 1 ml of defibrinated sheep blood (Biolife Italiana S.r.l., Milan, Italy) supplemented with 10% (vol/vol) glycerol (Sigma Chemical Co., Milan, Italy) (27) until they were ready for use. Before they were used, the bacteria were subcultured twice on Mueller-Hinton agar (Unipath S.p.A., Garbagnate Milanese, Milan, Italy) supplemented with 5% defibrinated sheep blood (Biolife) at 37°C in a microaerophilic atmosphere (5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>; CampyGen; Unipath) for 72 h.

**Control strains.** *H. pylori* NCTC 11637 and NCTC 11638 and *Escherichia coli* ATCC 25922 were included as control organisms with each run of each method. To compare the antimicrobial susceptibility test methods, we also used 35 selected strains of *H. pylori* with known resistance patterns. These strains were isolated from patients subjected to several treatment trials: 15 strains were resistant to metronidazole (breakpoint MIC, >32 µg/ml), 8 strains were resistant to clarithromycin (breakpoint MIC, >8 µg/ml), 9 strains were resistant to tetracycline (breakpoint MIC, >16 µg/ml), and 3 strains were resistant to both metronidazole (breakpoint MIC, >32 µg/ml) and ciprofloxacin (breakpoint MIC, >4 µg/ml).

**Antimicrobial agents.** The antimicrobial agents tested against *H. pylori* included amoxicillin, amoxicillin-clavulanate (tested at a 2:1 ratio), ampicillin, azithromycin, aztreonam, cefaclor, cefotetan, ceftizoxime, ciprofloxacin, clarithromycin, erythromycin, gentamicin, metronidazole, nitrofurantoin, norfloxacin, pefloxacin, roxythromycin, tetracycline, ticarcillin, and tobramycin. The E-test strips of each antibiotic were purchased from AB Biodisk. Antibiotic powders of known potency for the agar dilution and broth microdilution MIC tests were purchased from Sigma except as follows: tobramycin was from Eli Lilly Italia S.p.A., Sesto Fiorentino, Florence, Italy; ceftizoxime was from Farmitalia Carlo Erba, Milan, Italy; aztreonam was from Menarini, Florence, Italy; pefloxa-

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TABLE 1. Comparison of antimicrobial susceptibility test results by the E test and the agar dilution and broth microdilution methods for 71 isolates of *H. pylori*

| Antimicrobial agent     | MIC <sub>90</sub> (µg/ml) |                 |                 | % Resistant <sup>a</sup> |    |    | MIC (µg/ml) (geometric mean) |       |       |
|-------------------------|---------------------------|-----------------|-----------------|--------------------------|----|----|------------------------------|-------|-------|
|                         | E test                    | AD <sup>b</sup> | MD <sup>c</sup> | E test                   | AD | MD | E test                       | AD    | MD    |
| Amoxicillin-clavulanate | 0.032                     | 0.064           | 0.064           | 0                        | 0  | 0  | 0.018                        | 0.021 | 0.022 |
| Amoxicillin             | 0.125                     | 0.125           | 0.25            | 0                        | 0  | 0  | 0.037                        | 0.056 | 0.055 |
| Ampicillin              | 0.032                     | 0.064           | 0.064           | 0                        | 0  | 0  | 0.017                        | 0.021 | 0.023 |
| Azithromycin            | 0.25                      | 0.25            | 0.25            | 0                        | 0  | 0  | 0.073                        | 0.097 | 0.108 |
| Aztreonam               | 1                         | 1               | 1               | 0                        | 0  | 0  | 0.350                        | 0.285 | 0.277 |
| Cefaclor                | 1.5                       | 1               | 2               | 0                        | 0  | 0  | 0.134                        | 0.148 | 0.153 |
| Cefotetan               | 1                         | 2               | 2               | 0                        | 0  | 0  | 0.151                        | 0.176 | 0.200 |
| Ceftizoxime             | 1                         | 1               | 2               | 0                        | 0  | 0  | 0.146                        | 0.152 | 0.173 |
| Ciprofloxacin           | 0.094                     | 0.25            | 2               | 0                        | 0  | 0  | 0.048                        | 0.076 | 0.054 |
| Clarithromycin          | 0.75                      | 1               | 0.5             | 6                        | 6  | 6  | 0.087                        | 0.107 | 0.105 |
| Erythromycin            | 0.25                      | 0.5             | 0.5             | 8                        | 8  | 8  | 0.120                        | 0.155 | 0.141 |
| Gentamicin              | 1                         | 2               | 2               | 0                        | 0  | 0  | 0.541                        | 0.562 | 0.670 |
| Metronidazole           | >32                       | >32             | >32             | 38 <sup>d</sup>          | 32 | 32 | 3.173                        | 3.529 | 2.988 |
| Nitrofurantoin          | 0.5                       | 1               | 1               | 0                        | 0  | 0  | 0.305                        | 0.296 | 0.332 |
| Norfloxacin             | 0.19                      | 0.25            | 0.25            | 0                        | 0  | 0  | 0.081                        | 0.137 | 0.108 |
| Pefloxacin              | 0.19                      | 0.25            | 0.5             | 0                        | 0  | 0  | 0.083                        | 0.098 | 0.095 |
| Roxithromycin           | 0.094                     | 0.125           | 0.25            | 0                        | 0  | 0  | 0.045                        | 0.063 | 0.062 |
| Tetracycline            | 0.125                     | 0.5             | 0.25            | 6                        | 6  | 6  | 0.081                        | 0.116 | 0.117 |
| Ticarcillin             | 0.19                      | 0.25            | 0.25            | 0                        | 0  | 0  | 0.057                        | 0.067 | 0.069 |
| Tobramycin              | 1.5                       | 2               | 2               | 0                        | 0  | 0  | 0.670                        | 0.521 | 0.746 |

<sup>a</sup> Strains were classified as resistant when the MIC was greater than the breakpoint concentration.

<sup>b</sup> AD, agar dilution method.

<sup>c</sup> MD, broth microdilution method.

<sup>d</sup> Six major errors (resistant by the E test and susceptible by the agar dilution and broth microdilution methods) and two very major errors (susceptible by the E test and resistant by the agar dilution and broth microdilution methods).

cin was from Formenti S.r.l., Milan, Italy; and ciprofloxacin was from Bayer S.p.A., Milan, Italy. Stock solutions (1,600 mg/liter) of each antimicrobial agent were stored at  $-70^{\circ}\text{C}$  until use.

**Antimicrobial susceptibility testing.** Thawed isolates were inoculated onto Mueller-Hinton agar supplemented with 5% defibrinated sheep blood and were incubated under a microaerophilic atmosphere (CampyGen) for 72 h at  $37^{\circ}\text{C}$ . Colonies were suspended in 8 ml of brucella broth (Biolife) supplemented with 2% fetal calf serum (Unipath) to achieve a turbidity equivalent to that of a no. 3 McFarland opacity standard. Serial dilutions of this bacterial suspension were made for both *H. pylori* control strains from the National Collection of Type Cultures (NCTC) and yielded colony counts of about  $0.8 \times 10^9$  CFU/ml. Before inoculation, the shapes and motilities of the organisms were tested by Gram staining and phase-contrast microscopy. Cultures showing a high proportion (>25%) of coccoid and nonmotile bacterial forms were discarded. All three susceptibility tests were performed with samples from this adjusted inoculum.

**E test.** Plates containing Mueller-Hinton agar supplemented with 5% defibrinated sheep blood were used for the E test. All antimicrobial agents except metronidazole and ciprofloxacin were tested at concentrations ranging from 0.008 to 256 µg/ml; metronidazole and ciprofloxacin were tested at 0.002 to 32 µg/ml. The 140-mm-diameter agar plates were inoculated by confluent swabbing of the surface with the adjusted inoculum suspensions. After the surface of the inoculated plates had dried at  $37^{\circ}\text{C}$  inside a microaerophilic chamber (Don Whitley Scientific Ltd., International PBI S.p.A., Milan, Italy), five E-test strips were applied onto the surface of each agar plate. The plates were incubated at  $37^{\circ}\text{C}$  under microaerophilic conditions (CampyGen). MICs were read after 72 h of incubation on the basis of the intersection of the elliptical zone of growth inhibition with the MIC scale on the E-test strip.

**Agar dilution.** Agar dilution was performed by using twofold increments (across a range of 0.008 to 64 µg/ml) of the antimicrobial agents incorporated in Mueller-Hinton agar supplemented with 5% defibrinated sheep blood. The standardized inoculum was diluted in brucella broth supplemented with 2% fetal calf serum and was delivered to the surface of the agar plates with a Steers replicator so that the final concentration was approximately  $5 \times 10^5$  CFU per spot. The plates were incubated at  $37^{\circ}\text{C}$  under microaerophilic conditions (CampyGen). After 72 h of incubation, MICs were determined in the usual manner (22).

**Broth microdilution method.** Broth microdilution trays were prepared in-house and were stored at  $-70^{\circ}\text{C}$  until use. Broth microdilution was performed in brucella broth supplemented with 2% fetal calf serum. Twofold dilutions of each antimicrobial agent ranging from 0.008 to 64 µg/ml were used. The standardized inoculum was diluted to achieve a final inoculum concentration of approximately  $5 \times 10^5$  CFU per well. The microtiter plates were incubated at  $37^{\circ}\text{C}$  under microaerophilic conditions (CampyGen). MICs were read after 72 h of incubation.

**Evaluation criteria.** Because the twofold dilution scheme for the agar dilution and broth microdilution methods was different from that for the E test, those MICs determined by the E test with one-half an increment were rounded up to the next higher dilution (e.g., 0.75 µg/ml was rounded up to 1 µg/ml), and these values were used in the comparison of the results between the E test and the conventional methods. Agreement between two of the test methods evaluated was defined as MICs that differed by 1 log<sub>2</sub> dilution or less. Discrepancies in MICs were characterized as very major (reference method result was resistant and the E-test result was susceptible) or major (reference method result was susceptible and the E-test result was resistant) errors. Calculations of very major errors have been based only on the number of resistant strains tested; likewise, major errors have been calculated only on the basis of the number of susceptible strains tested (21).

**Statistical analysis.** The significance of the differences between MICs obtained by using two methods was determined by the  $\chi^2$  test. A *P* value of less than 0.05 was considered to represent a statistically significant difference between the results of the two methods compared. Microsoft Excel, version 6.0, was used to perform statistical analysis. The mode, geometric mean, MIC at which 50% of isolates are inhibited (MIC<sub>50</sub>), and MIC<sub>90</sub> were also calculated.

## RESULTS

Antimicrobial susceptibility test results are presented in Table 1. The most active compounds *in vitro* were amoxicillin-clavulanate and ampicillin (MIC<sub>90</sub>s, 0.032 µg/ml for the E test and 0.064 µg/ml for the reference methods). The highest MIC<sub>90</sub> was observed for metronidazole (>32 µg/ml by all three methods). The E test yielded greater numbers of results indicating resistance than did the reference methods when metronidazole (*P* > 0.05) was tested: 23 (32%) *H. pylori* isolates were resistant to metronidazole by the reference methods, and 27 (38%) were resistant to metronidazole by the E test. All three methods detected the following resistance rates: 8% (6 of 71) to erythromycin and 6% (4 of 71) to both clarithromycin and tetracycline.

For amoxicillin-clavulanate, the MIC (geometric mean) by the E-test method was 0.018 µg/ml, compared with 0.021 µg/ml by the agar dilution method and 0.022 µg/ml by the broth

TABLE 2. Distribution of differences in MICs of 20 antimicrobial agents for 71 isolates of *H. pylori*: E test versus agar dilution method

| Drug (no. of strains <sup>a</sup> ) | No. (%) of E-test MICs within indicated no. of log <sub>2</sub> dilution steps of agar dilution MICs <sup>b</sup> |           |            |            |           |          |         | % Agreement <sup>c</sup> |
|-------------------------------------|---|-----------|------------|------------|-----------|----------|---------|--------------------------|
|                                     | >-2   | -2        | -1         | 0          | +1        | +2       | >+2     |                          |
| Amoxicillin-clavulanate (71)        | 1 (1.4)   | 3 (4.2)   | 22 (31)    | 37 (52.1)  | 8 (11.3)  | 0        | 0       | 94.4                     |
| Amoxicillin (65)                    | 0   | 8 (12.3)  | 27 (41.5)  | 24 (36.9)  | 4 (6.2)   | 2 (3.1)  | 0       | 84.6                     |
| Ampicillin (54)                     | 0   | 0         | 14 (25.9)  | 31 (57.4)  | 9 (16.7)  | 0        | 0       | 100                      |
| Azithromycin (70)                   | 1 (1.4)   | 4 (5.7)   | 23 (32.9)  | 26 (37.1)  | 16 (22.9) | 0        | 0       | 92.9                     |
| Aztreonam (70)                      | 0   | 1 (1.4)   | 16 (22.9)  | 28 (40)    | 22 (31.4) | 3 (4.3)  | 0       | 94.3                     |
| Cefaclor (71)                       | 0   | 1 (1.4)   | 29 (40.8)  | 22 (31)    | 18 (25.4) | 1 (1.4)  | 0       | 97.2                     |
| Cefotetan (66)                      | 1 (1.5)   | 5 (7.6)   | 23 (34.8)  | 18 (27.3)  | 17 (25.8) | 2 (3)    | 0       | 87.9                     |
| Ceftizoxime (70)                    | 0   | 0         | 18 (25.7)  | 34 (48.6)  | 18 (25.7) | 0        | 0       | 100                      |
| Ciprofloxacin (60)                  | 1 (1.7)   | 6 (10)    | 14 (23.3)  | 28 (46.7)  | 11 (18.3) | 0        | 0       | 88.8                     |
| Clarithromycin (68)                 | 2 (2.9)   | 2 (2.9)   | 15 (22.1)  | 44 (64.7)  | 5 (7.4)   | 0        | 0       | 94.1                     |
| Erythromycin (69)                   | 1 (1.4)   | 4 (5.8)   | 20 (29)    | 33 (47.8)  | 8 (11.6)  | 1 (1.4)  | 2 (2.9) | 88.4                     |
| Gentamicin (70)                     | 0   | 7 (10)    | 18 (25.7)  | 21 (30)    | 18 (25.7) | 6 (8.6)  | 0       | 81.4                     |
| Metronidazole (69)                  | 2 (2.9)   | 3 (4.3)   | 1 (1.4)    | 49 (71)    | 12 (17.4) | 1 (1.4)  | 1 (1.4) | 89.9                     |
| Nitrofurantoin (68)                 | 0   | 4 (5.9)   | 13 (19.1)  | 30 (44.1)  | 21 (30.9) | 0        | 0       | 94.1                     |
| Norfloxacin (69)                    | 2 (2.9)   | 15 (21.7) | 19 (27.5)  | 29 (42)    | 4 (5.8)   | 0        | 0       | 75.4                     |
| Pefloxacin (71)                     | 0   | 5 (7)     | 15 (21.1)  | 45 (63.4)  | 6 (8.5)   | 0        | 0       | 93                       |
| Roxithromycin (70)                  | 0   | 4 (5.7)   | 34 (48.6)  | 24 (34.3)  | 8 (11.4)  | 0        | 0       | 94.3                     |
| Tetracycline (64)                   | 0   | 6 (9.4)   | 21 (32.8)  | 30 (46.9)  | 6 (9.4)   | 0        | 1 (1.6) | 89.1                     |
| Ticaracillin (64)                   | 0   | 2 (3.1)   | 27 (42.2)  | 18 (28.1)  | 12 (18.8) | 3 (4.7)  | 2 (3.1) | 89.1                     |
| Tobramycin (71)                     | 0   | 0         | 10 (14.1)  | 26 (36.6)  | 34 (47.9) | 1 (1.4)  | 0       | 98.6                     |
| All agents (1,350)                  | 11 (0.8)  | 80 (5.9)  | 379 (28.1) | 597 (44.2) | 257 (19)  | 20 (1.5) | 6 (0.4) | 91.3                     |

<sup>a</sup> Number of strains for which MICs were within the concentration range of the E test.

<sup>b</sup> A dilution of 0 indicates number (percent) of isolates for which MICs are identical; -1 and +1 indicate  $\pm$  log<sub>2</sub> dilution difference, etc.

<sup>c</sup> Percentage of isolates within the accuracy limits of the test ( $\pm 1$  log<sub>2</sub> dilution).

microdilution method. A similar trend was observed for all other antimicrobial agents tested except aztreonam, metronidazole, nitrofurantoin, and tobramycin. MICs (geometric means) were calculated by using only on-scale results, since the majority of MICs were on scale (of the 4,260 MICs analyzed, 4,033 were on scale).

The correlation between MICs determined by the E test and the agar dilution method is presented in Table 2. Overall, 91.3% of the E-test-determined MICs were within 1 log<sub>2</sub> dilution and 98.8% were within 2 log<sub>2</sub> dilutions. Excellent agreement (100%) was found for ampicillin and ceftizoxime. The agreement ranged from 75.4 to 100%.

The correlation between MICs determined by the E test and the broth microdilution method is presented in Table 3. Overall, 91.6% of the E-test-determined MICs were within 1 log<sub>2</sub> dilution and 99.1% were within 2 log<sub>2</sub> dilutions. Excellent agreement (100%) was found for erythromycin only. The agreement ranged from 70.8 to 100%.

The correlation between MICs determined by the agar dilution and broth microdilution methods showed that 88.8% of the agar dilution method-determined MICs were within 1 log<sub>2</sub> dilution and 98.6% were within 2 log<sub>2</sub> dilutions.

Overall, of 2,667 total tests, 169 (6.3%) of the E-test MICs were  $\leq -2$  log<sub>2</sub> dilutions of the reference methods and 59 (2.2%) were  $\geq +2$  log<sub>2</sub> dilutions of the reference methods.

The E test gave a modal MIC of 0.032  $\mu$ g/ml; the agar dilution and broth microdilution methods gave the same modal MICs (0.064  $\mu$ g/ml), 1 doubling dilution higher than that for the E test. The agar dilution MICs and broth microdilution MICs showed similar distribution trends. No major error (resistant by the E test and susceptible by the reference method) or very major error (susceptible by the E test and resistant by the reference method) was found between E-test, agar dilution, and broth microdilution MICs for the 35 control strains known to be resistant.

## DISCUSSION

The efficacy of the treatment of gastric infection caused by *H. pylori* can be reduced by the occurrence of primary or acquired resistance to various drugs, especially to metronidazole (1). This has made susceptibility testing of *H. pylori* increasingly important for the search for efficient antimicrobial combinations that allow for the eradication of this bacterium from the stomach. However, up to now there are no standard methods for in vitro antimicrobial susceptibility testing for this fastidious organism. Agar or broth dilution methods have been used in most studies (2, 13, 27), but they are difficult to perform routinely. Moreover, this approach is economically impractical for clinical laboratory use when testing individual isolates. The disk diffusion method is inappropriate for microorganisms like *H. pylori*, requiring a microaerophilic atmosphere, a prolonged incubation time, and numerous additives in the growth medium.

The accuracy of the E-test MIC results for *H. pylori* that we found is in agreement with the findings of previous studies encompassing a variety of other bacteria and fungi (2, 5, 6, 8, 26).

Other investigators have reported an excellent correlation of the E-test results with those obtained by standard methods for *H. pylori*. Glupczynski et al. (13), who compared the E test with the agar dilution method to assess the in vitro activities of 12 antimicrobial agents against *H. pylori*, found that 86 and 99.5% of the results correlated within 1 and 2 log<sub>2</sub> dilution steps, respectively. Van Horn et al. (28), who compared the E test and the reference agar dilution method to evaluate the activities of five antimicrobial agents against *H. pylori*, found a correlation of 86%. Cederbrant et al. (7), who determined the susceptibilities of 20 isolates of *H. pylori* to six antimicrobial agents, found that 81% of the E-test-determined MICs were within 1 twofold dilution and 93% were within 2 twofold dilu-

TABLE 3. Distribution of differences in MICs of 20 antimicrobial agents for 71 isolates of *H. pylori*: E test versus broth microdilution method

| Drug (no. of strains <sup>a</sup> ) | No. (%) of E-test MICs within indicated no. of log <sub>2</sub> dilution steps of broth microdilution MICs <sup>b</sup> |           |           |            |            |         |         | % Agreement <sup>c</sup> |
|-------------------------------------|---|-----------|-----------|------------|------------|---------|---------|--------------------------|
|                                     | >-2   | -2        | -1        | 0          | +1         | +2      | >+2     |                          |
| Amoxicillin-clavulanate (68)        | 0   | 2 (2.9)   | 17 (25)   | 40 (58.8)  | 9 (13.2)   | 0       | 0       | 97.1                     |
| Amoxicillin (65)                    | 0   | 5 (7.7)   | 27 (41.5) | 25 (38.5)  | 8 (12.3)   | 0       | 0       | 92.3                     |
| Ampicillin (51)                     | 3 (5.9)   | 1 (2)     | 15 (29.4) | 21 (41.2)  | 11 (21.6)  | 0       | 0       | 92.2                     |
| Azithromycin (67)                   | 0   | 5 (7.5)   | 32 (47.8) | 14 (20.9)  | 14 (20.9)  | 2 (3)   | 0       | 89.6                     |
| Aztreonam (71)                      | 0   | 0         | 11 (15.5) | 35 (49.3)  | 18 (25.4)  | 4 (5.6) | 3 (4.2) | 90.1                     |
| Cefaclor (69)                       | 1 (1.4)   | 0         | 25 (36.2) | 27 (39.1)  | 15 (21.7)  | 1 (1.4) | 0       | 97.1                     |
| Cefotetan (64)                      | 0   | 8 (12.5)  | 22 (34.4) | 19 (29.7)  | 15 (23.4)  | 0       | 0       | 87.5                     |
| Ceftizoxime (68)                    | 0   | 1 (1.5)   | 31 (45.6) | 26 (38.2)  | 9 (13.2)   | 1 (1.5) | 0       | 97.1                     |
| Ciprofloxacin (59)                  | 1 (1.7)   | 4 (6.8)   | 11 (18.6) | 33 (55.9)  | 6 (10.2)   | 3 (5.1) | 1 (1.7) | 84.7                     |
| Clarithromycin (65)                 | 0   | 6 (9.2)   | 30 (46.2) | 14 (21.5)  | 10 (15.4)  | 5 (7.7) | 0       | 83.1                     |
| Erythromycin (58)                   | 0   | 0         | 21 (36.2) | 27 (46.6)  | 10 (17.2)  | 0       | 0       | 100                      |
| Gentamicin (71)                     | 0   | 5 (7)     | 21 (29.6) | 31 (43.7)  | 14 (19.7)  | 0       | 0       | 93                       |
| Metronidazole (67)                  | 0   | 0         | 12 (17.9) | 37 (55.2)  | 15 (22.4)  | 3 (4.5) | 0       | 95.5                     |
| Nitrofurantoin (68)                 | 0   | 2 (2.9)   | 16 (23.5) | 37 (54.5)  | 13 (19.1)  | 0       | 0       | 97.1                     |
| Norfloxacin (71)                    | 0   | 2 (2.8)   | 33 (46.5) | 27 (38)    | 9 (12.7)   | 0       | 0       | 97.2                     |
| Pefloxacin (71)                     | 0   | 3 (4.2)   | 30 (42.3) | 19 (26.8)  | 17 (23.9)  | 2 (2.8) | 0       | 93                       |
| Roxithromycin (69)                  | 2 (2.9)   | 8 (11.6)  | 27 (39.1) | 13 (18.8)  | 17 (24.6)  | 2 (2.9) | 0       | 82.6                     |
| Tetracycline (65)                   | 0   | 14 (21.5) | 10 (15.4) | 28 (43.1)  | 8 (12.3)   | 4 (6.2) | 1 (1.5) | 70.8                     |
| Ticaracillin (59)                   | 0   | 4 (6.8)   | 18 (30.5) | 30 (50.8)  | 7 (11.9)   | 0       | 0       | 93.2                     |
| Tobramycin (71)                     | 0   | 1 (1.4)   | 25 (35.2) | 31 (43.7)  | 13 (18.3)  | 1 (1.4) | 0       | 97.2                     |
| All agents (1,317)                  | 7 (0.5)   | 71 (5.4)  | 434 (33)  | 534 (40.5) | 238 (18.1) | 8 (2.1) | 5 (0.4) | 91.6                     |

<sup>a</sup> See footnote a of Table 2.<sup>b</sup> See footnote b of Table 2.<sup>c</sup> See footnote c of Table 2.

tions of those determined by the reference agar dilution method.

In the present study, excellent agreement between the E test and the agar dilution method (91.1%) was found. This may be due in part to the common batch of Mueller-Hinton agar and in part to the common inoculum. However, Cederbrant et al. (7) showed that, in contrast to the standard broth or agar dilution method, the results of the E test were not significantly affected by the inoculum density. The results of the E test also yielded excellent agreement compared with those of the broth microdilution method (89.7%).

In general, the MICs obtained by the E test tended to be lower than those obtained by the reference methods. This is most apparent for roxithromycin and norfloxacin (E test versus the agar dilution method; Table 2) and for clarithromycin, azithromycin, and roxithromycin (E test versus the broth microdilution method; Table 3). The underestimation of MICs by the E test has been described in previous studies (2, 7, 29). The reason for this observation in the present study is not known, since all three susceptibility tests were performed from the same inoculum.

With regard to the antimicrobial agents tested in the present study, the E test produced results comparable to those obtained by the agar dilution and broth microdilution methods: the E test had greater than 80% agreement with the reference methods except for tests with tetracycline (70.8%) by the broth microdilution. The most active compounds in vitro were amoxicillin-clavulanate and ampicillin.

When evaluating new methods for susceptibility testing, it is important to test an adequate number of resistant strains to verify the ability of the new test to detect resistance. Jorgensen (17) proposed that very major errors determined for a large sample ( $n \geq 35$ ) of known resistant isolates should be  $\leq 3\%$ . In our study, we have found an excellent correlation (100% within 1 log<sub>2</sub> dilution step) for the 35 collected strains known to be resistant; furthermore, no major or very major errors were

found between the E-test, agar dilution, and broth microdilution MICs of metronidazole, clarithromycin, and tetracycline. Among the 71 clinical isolates of *H. pylori* tested, only two very major errors and six major errors were detected. We have no explanation other than chance to explain why these errors were observed only with metronidazole.

In our experience, the E test is much less labor-intensive and is easier to perform than the agar and broth dilution methods. Also, the E test requires the material and principles of the widely used Kirby-Bauer disk diffusion susceptibility method, which allows the E test to be quickly and economically adapted into the laboratory work flow. We conclude that the E test appears to represent an excellent alternative, reproducible method for determining the antimicrobial susceptibilities of *H. pylori* strains to a wide array of antimicrobial agents.

#### ACKNOWLEDGMENTS

We thank Francis Megraud for valuable criticism of the manuscript.

This work was supported in part by a grant from the Ministero Università e Ricerca Scientifica e Tecnologica (MURST; ex quota 60 and 40%).

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