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# Antimicrobial Susceptibility Testing of 230 *Helicobacter pylori* Strains: Importance of Medium, Inoculum, and Incubation Time

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**No standardized method of susceptibility testing for *Helicobacter pylori* is currently available, so before a large agar dilution study comprising 230 *H. pylori* strains belonging to more than 80 genetically different groups was initiated, we performed a relatively small preliminary study to determine the influences of medium, inoculum density, and incubation time. Seven media were investigated and were primarily evaluated on the basis of their abilities to support growth both semiquantitatively and qualitatively; Iso-Sensitest agar supplemented with 10% horse blood was found to be well suited for the purpose; this was closely followed by Mueller-Hinton agar with 10% horse blood, Mueller-Hinton with 10% sheep blood, and finally, 7% lysed horse blood agar. Investigations of two inoculum densities and two incubation times resulted in recommendations for the use of $10^9$ CFU/ml ($10^6$ CFU/spot) as the inoculum and 72 h as the incubation time. A modest inoculum effect was noted for amoxicillin and metronidazole. By the methodology derived from our preliminary study, the susceptibilities of 230 *H. pylori* strains to six antibiotics were subsequently determined. The results were generally in accord with those of others, and apart from metronidazole, the MIC of which for approximately 25% of the strains tested was >8 μg/ml, resistance was low in Denmark. The situation might, however, quickly change when and if the number of indications for antibiotic therapy for *H. pylori* infections increase. Consequently, susceptibility testing of all *H. pylori* strains is recommended in order to survey the development of resistance, and in our hands the described methodology was relatively easy to perform and the results were easy to read.**

*Helicobacter pylori* is a fastidious microorganism, and testing of antimicrobial agents against *H. pylori* is associated with problems mostly caused by culture difficulties. Investigators are, furthermore, faced with the fact that no standardized susceptibility testing method is available for microaerobic bacteria. Consequently, parameters such as medium, size of inoculum, and length of incubation often vary, making comparisons between studies both difficult and subject to uncertainty (3, 8–11, 13, 16, 18–20).

The current indication for the treatment of *H. pylori* infections is generally restricted to peptic ulcer (5, 22). However, in view of the widespread occurrence of gastroduodenal *H. pylori* infections and the role of this organism in symptomatic acute gastritis, chronic gastritis, and the pathogenesis of gastric cancer, the potential level of consumption of antibiotics for the eradication of *H. pylori* in the future is enormous and is somewhat frightening (5, 21, 22, 27). Large-scale susceptibility studies that preferably use similar methodological approaches which could be used as a reference for future comparisons are clearly warranted, as are programs for the surveillance of resistance.

In the absence of a standard method and with the variations in the test parameters mentioned above in mind, some basic investigations were justified before large numbers of strains could be studied. The basis of all susceptibility testing is the availability of a medium that supports growth well, so the first part of our study involved an investigation of the growth-supporting properties of various relevant agar media which had been used in previous studies. Four media were selected and investigated further before a relatively small-scale agar dilution study with two inoculum sizes, six antibiotics representing six different antibiotic groups, and two lengths of incubation was carried out. Finally, the methodology developed in our preliminary study was implemented, and the antimicrobial activities of six antibiotics against 230 clinical *H. pylori* isolates were determined by the agar dilution technique with Iso-Sensitest agar with 10% horse blood as the test medium.

## MATERIALS AND METHODS

**Strains.** A total of 230 thawed Danish *H. pylori* strains isolated from gastric biopsy specimens, mainly from patients with peptic ulcer and identified according to morphology by Gram staining, motility, and oxidase, catalase, and urease reactions, were included in this study. Subsets of these 230 strains were used in the preliminary growth study (43 strains) and preliminary agar dilution study (34 strains). The *H. pylori* strains were genetically investigated with two restriction enzymes, and the material consisted of at least 80 genetically different groups. The following control strains were included on each day of the experiment of the agar dilution study to observe potential day-to-day variation: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Helicobacter pylori* CCUG 17874 (ATCC 43504), and *Campylobacter jejuni* C.I.P. 70.3 (ATCC 33560). We have used the *E. coli* and *S. aureus* control strains in many previous susceptibility studies, and the MICs of all antibiotics except metronidazole for these strains were similar to what we have determined previously.

**Media.** Iso-Sensitest (Oxoid, Basingstoke, United Kingdom) agar with 5% lysed horse blood, Iso-Sensitest agar with 10% horse blood, Mueller-Hinton agar (Difco, Detroit, Mich.) with 5% lysed sheep blood, Mueller-Hinton agar with

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TABLE 1. Distribution of 43 *H. pylori* strains according to character of growth on seven different agar media incubated for 48 and 72 h[a]

| Incubation period | Character of growth | No. of strains growing on the following media: | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Iso-Sensitest agar + 5% lysed horse blood (I) | Iso-Sensitest agar + 10% horse blood (II) | Mueller-Hinton agar + 5% lysed sheep blood (III) | Mueller-Hinton agar + 10% sheep blood (IV) | Mueller-Hinton agar + 5% lysed horse blood (V) | Mueller-Hinton agar + 10% horse blood (VI) | 7% lysed horse blood agar (VII) |
| 48 h | 0 | 24 | 9 | 12 | 16 | 10 | 8 | 15 |
| | + | 10 | 13 | 16 | 10 | 18 | 19 | 19 |
| | ++ | 5 | 14 | 13 | 14 | 11 | 13 | 7 |
| | +++ | 4 | 7 | 2 | 3 | 4 | 3 | 2 |
| 72 h | 0 | 6 | 0 | 0 | 2 | 1 | 1 | 0 |
| | + | 18 | 3 | 14 | 13 | 9 | 5 | 22 |
| | ++ | 10 | 14 | 14 | 10 | 18 | 16 | 8 |
| | +++ | 9 | 26 | 15 | 18 | 15 | 21 | 13 |

[a] Inoculum, $10^4$ CFU.

10% sheep blood, Mueller-Hinton agar with 5% lysed horse blood, Mueller-Hinton agar with 10% horse blood, and 7% heat-lysed horse blood agar (chocolate agar) were prepared by the Statens Seruminstitute, Copenhagen, Denmark (25). According to the manufacturer, only one batch of Mueller-Hinton agar base and one batch of Iso-Sensitest agar base were used to produce the agar plates. The sheep and horse blood used as supplements were 2 to 3 days old when they were added to the agar plates. Iso-Sensitest agar with 10% horse blood, Mueller-Hinton agar with 10% sheep blood, Mueller-Hinton agar with 10% horse blood, and 7% heat-lysed horse blood agar were selected for the preliminary agar dilution susceptibility tests. Iso-sensitest agar with 10% horse blood was used in the final agar dilution study with the 230 strains.

**Antibiotics.** Six different agents representing six different types of antibiotics were used in this study. All antibiotics were supplied by pharmaceutical companies as standard powders with known potencies. The following antibiotics were investigated: amoxicillin trihydrate (Yamanouchi Pharma, Glostrup, Denmark), tetracycline hydrochloride (Wyeth Lederle, Copenhagen, Denmark), clarithromycin (Abbott Laboratories, Vedbæk, Denmark), ciprofloxacin lactate (Bayer AG, Frederiksberg, Denmark), metronidazole (Rhône-Poulenc Rorer, Birkerød, Denmark), and tobramycin sulfate (Eli Lilly, Copenhagen, Denmark).

The compounds were dissolved and diluted according to the recommendations of the manufacturers, and solutions were used on the day of preparation. The agar plates used for the agar dilution study contained twofold dilutions of antibiotics ranging in concentration from 0.008 to 256 μg/ml. The stability of the antibiotics in the agar plates was 7 days when they were stored in a refrigerator.

**Procedures of the growth study.** The colonies on the 7% heat-lysed horse blood agar plates seeded heavily with *H. pylori* and incubated for 72 h were harvested, and suspensions with densities equal to that of a 0.5 McFarland turbidity standard (approximately $10^7$ CFU/ml; checked by colony counts) were prepared in sterile saline and diluted 10-fold before one loop (10 μl) was plated out onto the solid agar medium and spread in a manner enabling the semiquantitation of growth after incubation [i.e., in a triangle, so that there were three areas: (i) a well-inoculated area, (ii) a second series with successive strokes, and (iii) a third series with successive strokes]. Readings were performed after 48 and 72 h of microaerobic (5% $O_2$, 5% $CO_2$, 90% $N_2$) incubation in a $CO_2$ incubator (model 3165; Forma Scientific Inc., Marietta, Ohio) at 35°C; and growth was scored from 0 to +++, i.e., 0 is no growth, + is growth in the inoculation area, ++ is growth in the second series of successive strokes, and +++ is growth in the third series of successive strokes. Moreover, the colony sizes were estimated semiquantitatively.

**Preparation of the inocula and inoculation in the agar dilution study.** The colonies on the 7% heat-lysed horse agar plates incubated for 72 h were harvested, and the bacteria were suspended in sterile saline to densities equal to those of 0.5 and 3.0 McFarland turbidity standards. These suspensions were checked by colony counts to secure inocula of approximately $10^4$ CFU/spot ($10^7$ CFU/ml) and $10^6$ CFU/spot ($10^9$ CFU/ml). All suspensions of the strains of bacteria used in the growth study and the preliminary agar dilution study were checked by obtaining colony counts; altogether approximately 30% (76 of 230 strains) of the bacterial suspensions were checked by obtaining colony counts. Inoculation of up to 20 strains per plate, with each pin head delivering 1 μl per spot, was performed with a Denley Multipoint Inoculator A 400 (Denley, Sussex, United Kingdom). The plates were incubated microaerobically (5% $O_2$, 5% $CO_2$, 90% $N_2$) at 35°C with a $CO_2$ incubator (model 3165 Forma Scientific Inc.), and the plates were read after 48 and 72 h in the preliminary study and after 72 h in the final study. The MIC was determined to be the lowest concentration with no growth (zero colonies). No trailing (faint hazes) was observed. All MICs were determined in duplicate in one laboratory.

## RESULTS

The results of the growth study, i.e., of inoculating a fixed amount of bacteria at approximately $10^4$ CFU onto agar media, are presented in Table 1 as the distribution and character of growth of 43 *H. pylori* strains after incubation for 48 and 72 h. It is evident that growth was quite weak after 48 h, although already at this time two media (columns labeled II and VI in Table 1) differed from the rest with regard to their ability to support growth, but in general, there were large numbers of strains with growth characterized as 0, irrespective of the medium used. Prolongation of incubation by 24 h to 72 h resulted in the growth of most strains on all seven media, but especially on the media labeled II, VI, and IV in Table 1, with large numbers of strains with growth characterized as +++. It should be noted that it was not always the same strains that grew well on the different types of media and that our final choice of which media to test further was based on growth-supporting ability, quality of growth (e.g., as expressed by colony size), and an overall evaluation. Besides registering the character of growth, the colony size of each bacterial strain was estimated semiquantitatively and was regarded as an expression of the "well-being" of that particular isolate on the medium in question. The results of these estimates arranged according to decreasing colony size were as follows: Iso-Sensitest agar with 10% horse blood > Mueller-Hinton agar with 10% horse blood >> Mueller-Hinton agar with 10% sheep blood > Iso-Sensitest agar with 5% lysed horse blood > Mueller-Hinton agar with 5% lysed horse blood > Mueller-Hinton agar with 5% lysed sheep blood > 7% lysed horse blood agar. Again, the growth on the first two media (labeled II and VI in Table 1) differed from that on the rest of the media by showing larger colonies. Four different media were selected for further investigation, i.e., the media labeled II, IV, VI, and VII in Table 1. Table 2 presents the influence of both the inoculum size and the length of incubation on the MICs of the six antibiotics for 34 *H. pylori* strains with four different media. It was not possible to read all MICs after incubation for 48 h for all four media, nor was it possible to read all MICs with the low inoculum for all antibiotics after 72 h of incubation because growth was too weak. In general, the MICs obtained with an inoculum of $10^4$ CFU/spot ($10^7$ CFU/ml) were lower than the MICs obtained with an inoculum of $10^6$ CFU/spot ($10^9$ CFU/ml); moreover, prolongation of the incubation period often resulted in an increase in the MICs. The inoculum effect was particularly apparent with metronidazole, irrespective of the

TABLE 2. Influences of inoculum size and length of incubation on MICs of six antibiotics for 34 *H. pylori* strains with various test media[a]

| Antibiotic | Iso-Sensitest agar with 10% horse blood | | | | Mueller-Hinton agar with 10% sheep blood | | | | Mueller-Hinton agar with 10% horse blood | | | | 7% heat-lysed horse blood agar | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $MIC_{90}$ (μg/ml) at 48 h | | $MIC_{90}$ (μg/ml) at 72 h | | $MIC_{90}$ (μg/ml) at 48 h | | $MIC_{90}$ (μg/ml) at 72 h | | $MIC_{90}$ (μg/ml) at 48 h | | $MIC_{90}$ (μg/ml) at 72 h | | $MIC_{90}$ (μg/ml) at 48 h | | $MIC_{90}$ (μg/ml) at 72 h | |
| | $10^4$ CFU/spot | $10^6$ CFU/spot | $10^4$ CFU/spot | $10^6$ CFU/spot | $10^4$ CFU/spot | $10^6$ CFU/spot | $10^4$ CFU/spot | $10^6$ CFU/spot | $10^4$ CFU/spot | $10^6$ CFU/spot | $10^4$ CFU/spot | $10^6$ CFU/spot | $10^4$ CFU/spot | $10^6$ CFU/spot | $10^4$ CFU/spot | $10^6$ CFU/spot |
| Metronidazole | 1 | 16 | 8 | 32 | ND[b] | 4 | 1 | 16 | 1 | 8 | 16 | 32 | ND | 2 | 2 | 8 |
| Amoxicillin | 0.02 | 0.06 | 0.06 | 0.13 | ND | 0.03 | ND | 0.06 | 0.02 | 0.13 | 0.06 | 0.13 | ND | 0.06 | ND | 0.06 |
| Tetracycline | 0.25 | 0.25 | 0.5 | 1 | 0.13 | 0.25 | 0.13 | 0.25 | 0.13 | 0.25 | 0.13 | 0.5 | ND | 0.5 | 1 | 1 |
| Tobramycin | 0.13 | 0.25 | 0.13 | 0.25 | ND | 0.5 | ND | 0.5 | 0.13 | 0.25 | 0.25 | 0.5 | ND | 1 | 0.5 | 1 |
| Ciprofloxacin | 0.13 | 0.25 | 0.25 | 0.25 | ND | 0.25 | 0.13 | 0.25 | 0.13 | 0.25 | 0.13 | 0.25 | ND | 0.13 | 0.13 | 0.25 |
| Clarithromycin | 0.03 | 0.03 | 0.03 | 0.03 | ND | 0.02 | ND | 0.02 | 0.03 | 0.03 | 0.03 | 0.03 | 0.02 | 0.03 | 0.02 | 0.03 |

[a] Inocula of $10^4$ and $10^6$ CFU/spot correspond to $10^7$ and $10^9$ CFU/ml, respectively.
[b] ND, not determined owing to growth of insufficient (<50%) numbers of strains.

medium used, but was less so with the other five compounds tested.

Table 3 presents the MICs at which 90% of isolates are inhibited ($MIC_{90}$s) for the six antibiotics tested with four different media. Differences of one twofold dilution step were observed for all antibiotics except metronidazole and tobramycin, for which the differences were up to two twofold dilution steps. The MICs determined with Iso-Sensitest agar with 10% horse blood and Mueller-Hinton agar with 10% horse blood were almost identical, whereas a trend toward lower MICs was observed for the Mueller-Hinton agar with 10% sheep blood. The ranges expressed a similar trend. The MICs obtained with 7% heat-lysed horse blood agar were either equivalent to or lower than the MICs obtained with Iso-Sensitest agar with 10% horse blood for all antibiotics except the aminoglycoside tobramycin. The results of our final large agar dilution study of the activities of the six antibiotics are presented in Table 4 as the $MIC_{50}$s and $MIC_{90}$s. For one strain, the metronidazole MIC was >256 μg/ml.

The summation curves for the six antibiotics, obtained by cumulating the percentages of strains inhibited at a given MIC, are depicted in Fig. 1. The slopes of the curves for clarithromycin, ciprofloxacin, tobramycin, tetracycline, and amoxicillin were all steep, reflecting the narrow ranges of the MICs and the homogeneous susceptibilities of the strains to these five compounds. The curve for metronidazole looked quite different from the curves for the other antibiotics and clearly showed that the population of *H. pylori* strains was divided into two parts, reflecting the different susceptibilities to metronidazole. For about 70% of the strains, the metronidazole MICs were less than 2 μg/ml, and up to that point the curve had a fairly vertical slope, but after that point the curve flattened. For approximately 25% of the strains metronidazole MICs were greater than 8 μg/ml.

## DISCUSSION

Because most antimicrobial agents demonstrate excellent activities in vitro against *H. pylori* but demonstrate little or no activity when used in vivo as single agents for therapy, many question the need to test the susceptibility of *H. pylori*. The development of resistance of *H. pylori* to different antibiotics might, however, make clinicians realize that culture and susceptibility testing should be performed as often as possible. As evidence in support of the value of susceptibility testing grows (1, 6, 10, 12, 24), test results appear to be of value if they are used as follows. (i) If a strain is susceptible to a certain agent(s) of a particular combination which has empirically been effective, the clinician should maintain or choose such a combination because it is likely to succeed. (ii) If a strain is resistant to a certain agent(s) of a particular combination, the risk of failure is sizeable and the clinician should consider alternatives. (iii) To survey resistance, to enable clinicians to choose therapy empirically according to the resistance patterns of organisms in a particular geographical area.

After having gathered 230 *H. pylori* strains representing >80 genetically diverse groups, we optimized our technology concerning susceptibility testing by investigating some parameters that might influence test results. Mueller-Hinton agar supplemented with horse blood or sheep blood has been used by many investigators (8, 11, 13, 16, 18). One study comprising 97 *H. pylori* strains was carried out with Iso-Sensitest agar supplemented with 10% saponin-lysed horse blood (20). Because our experience with these media regarding *H. pylori* was limited and no medium had qualified to be the medium of choice, *H. pylori* was grown by using a defined inoculum of $10^4$ CFU on

TABLE 3. $MIC_{90}$s and MIC ranges of six antibiotics for 34 *H. pylori* strains with four different test media and incubation for 72 h[a]

| Antibiotic | $MIC_{90}$ (μg/ml [MIC range; μg/ml]) | | | |
|---|---|---|---|---|
| | Iso-Sensitest agar + 10% horse blood | Mueller-Hinton agar + 10% horse blood | Mueller-Hinton agar + 10% sheep blood | 7% lysed horse blood agar |
| Metronidazole | 32 (0.5–256) | 32 (0.5–128) | 16 (0.5–128) | 8 (0.25–32) |
| Amoxicillin | 0.13 (0.01–8) | 0.13 (0.01–4) | 0.06 (0.01–8) | 0.06 (0.01–4) |
| Tetracycline | 1 (0.06–4) | 0.5 (0.02–8) | 0.5 (0.03–4) | 1 (0.02–8) |
| Tobramycin | 0.25 (0.06–1) | 0.5 (0.02–1) | 0.5 (0.02–1) | 1 (0.02–1) |
| Ciprofloxacin | 0.25 (0.13–0.5) | 0.25 (0.03–0.5) | 0.25 (0.01–0.5) | 0.25 (0.01–0.5) |
| Clarithromycin | 0.03 (0.02–0.06) | 0.03 (0.02–0.06) | 0.02 (0.01–0.06) | 0.03 (0.02–0.25) |

[a] Inoculum, $10^6$ CFU/spot, corresponding to $10^9$ CFU/ml.

a selection of media used previously, a selection of media often recommended for susceptibility testing. The inoculum density used allowed us to estimate the sizes and appearances of single colonies. It was important to find a medium that supported growth well; only in that way could inhibition of growth be ascribed to the activity of the antibiotic and not just to growth difficulties. The results of our investigations (Table 1) indicated that supplementation with horse blood was better than supplementation with sheep blood. A similar observation has been made for liquid media, in which sheep blood apparently inhibited the growth of *H. pylori* (7). The component or components that cause the observed differences in the ability to support growth are not known, and this underlines the fact that these media are biological substances and are rather ill defined chemically. Mueller-Hinton agars with either 5% lysed sheep blood or 10% sheep blood had very similar abilities to support growth; however, the colonies were larger on the plates with 10% sheep blood. Overall, we preferred the Iso-Sensitest agar with 10% horse blood, a commercially available, semidefined medium developed for antimicrobial susceptibility testing and formulated to overcome objections directed toward the Mueller-Hinton medium by having as few undefined components as possible (23). The inoculum densities in other studies were between approximately $10^6$ and $10^{10}$ CFU/ml (8, 10, 11, 19). Stationary-phase inocula had been used and prepared by removing colonies from a solid medium and suspending the bacteria in saline or broth, as we did, or by using 48-h liquid medium cultures (20). It has been argued (20) that colonies from solid media contain a high proportion of rounded, nonviable forms, resulting in variations in inoculum densities. To overcome this all our inocula in the preliminary studies were checked by colony counting and contained approximately $10^7$ CFU/ml ($10^4$ CFU/spot) and $10^9$ CFU/ml ($10^6$ CFU/spot), equal to McFarland turbidity standards of 0.5 and 3, respectively. It is our experience that the inoculum density should be given as the numbers of CFU per milliliter and checked by colony counts, because McFarland turbidity standards are not specific enough: a McFarland no. 3 standard varied from $5 \times 10^6$ CFU/ml (10) to $1 \times 10^9$ CFU/ml, variations that might be explained by differences in the proportion of viable bacteria in a given suspension and in the media and culture techniques used.

In this study inoculum effects were observed for amoxicillin and metronidazole. The biggest problem in evaluating inoculum effects was in the studies with a low inoculum ($10^4$ CFU/spot), in which a number of strains failed to grow on two of the media even after incubation for 72 h. In other reports the ranges of incubation times were 2 to 7 days (10, 11, 26), with a majority of investigators using 72 h. In our study the results obtained after 48 h of incubation were more difficult to read, particularly with the low inoculum. An incubation time of 72 h made determination of the MICs easier and the results were unequivocal. In general, the MICs were lower after incubation for 48 h, probably reflecting the inoculum effect mentioned above. A possible lack of stability of the antibiotics in the agar media after 72 h was not investigated, but the producer stated that lack of stability was negligible with these compounds, provided that freshly prepared media were used.

The results of our agar dilution study with large numbers of strains were generally in accord with those of other investigators (13, 16, 18–20, 26, 28), although our ranges of MICs were wider. With the exception of metronidazole, resistance among Danish *H. pylori* strains is low, perhaps as a result of the restrictive attitude toward the consumption of antibiotics in Denmark.

With the advent of the various short, potent, and well-tolerated, low-dose drug regimens for the treatment of *H. pylori* infections (15), it is likely that more patients will be given eradication therapy, resulting in an increased level of consumption of antibiotics. This will no doubt affect the susceptibility patterns of *H. pylori* as well as the patterns of resistance of other bacteria in our microbial environment. The prevalence of resistance to macrolide antibiotics has increased, with approximately 10% of *H. pylori* strains in Belgium and France being resistant (14, 15). Tetracycline resistance has been reported in the United Kingdom and Norway (2, 17), and in some countries the prevalence of metronidazole resistance is 90% (4, 17).

On the basis of our investigations we conclude that the semidefined medium Iso-Sensitest agar with 10% horse blood satisfactorily supported the growth of *H. pylori* and was well-suited for susceptibility testing purposes. Mueller-Hinton agar with 10% horse blood also worked well. The inoculum density of $10^9$ CFU/ml ($10^6$ CFU/spot) and the incubation time of 72 h

TABLE 4. Antimicrobial activities of six antibiotics for 230 *H. pylori* strains determined by agar dilution method with Iso-sensitest agar plus 10% horse blood[a]

| Antibiotic | MIC (μg/ml) | | |
|---|---|---|---|
| | 50% | 90% | Range |
| Metronidazole | 2 | 64 | 0.03–>256 |
| Amoxicillin | 0.02 | 0.06 | 0.01–2 |
| Tetracycline | 0.25 | 0.5 | 0.02–4 |
| Tobramycin | 0.13 | 0.25 | 0.02–4 |
| Ciprofloxacin | 0.25 | 0.25 | 0.03–8 |
| Clarithromycin | 0.03 | 0.06 | 0.01–1 |

[a] Inoculum, $10^6$ CFU/spot, corresponding to $10^9$ CFU/ml.

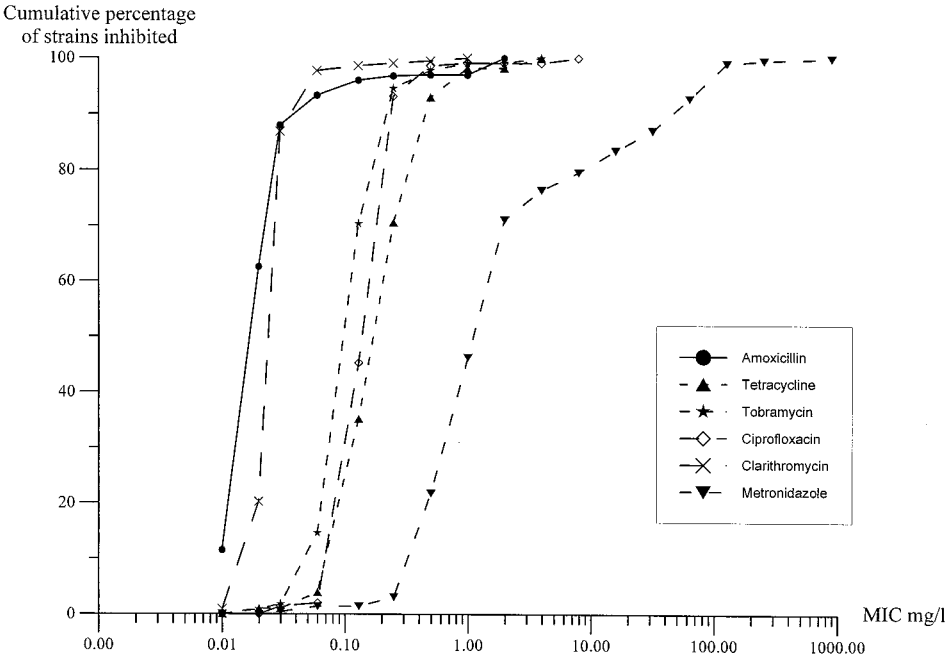


FIG. 1. In vitro activities of six antibiotics against 230 clinical isolates of *H. pylori*.

made MIC determinations easy to read and the results were unequivocal. Before general recommendations can be made, further susceptibility test studies involving more laboratories and different batches of Iso-Sensitest and Mueller-Hinton agar bases must be performed. In vitro results need to be correlated to the in vivo outcome.

Owing to the anticipated increase in the number of patients who in future will receive treatment for their *H. pylori* infections, we recommend that susceptibility tests, in principle, be carried out for all *H. pylori* strains and that programs to survey the development of resistance be implemented.

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