**Helicobacter pylori** susceptibility testing by disc diffusion

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The bacterium *Helicobacter pylori* is found in c. 40% of the population and is responsible for the development of duodenal disease. Triple treatment with a proton-pump inhibitor or bismuth salt plus two antibiotics is now commonplace in all patients diagnosed. As antibiotic resistance reduces treatment efficacy, it is time to consider routine susceptibility testing to guide individual patient treatment and surveillance of antibiotic resistance. There are no published nationally agreed standards for disc diffusion testing of *H. pylori*. After reviewing the literature, we recommend the following method for disc diffusion tests. A suspension of cultures equivalent to McFarland Standard no. 4 (10\textsuperscript{8} cfu/mL) should be used on Mueller–Hinton or Columbia agar base with 5–10% blood, using a metronidazole disc strength of 5 µg and a clarithromycin disc strength of 2 µg. Anaerobic pre-incubation of plates is unnecessary. A *H. pylori* control susceptible to metronidazole (e.g. NCTC 12822) should be used. Zone sizes with the Mueller–Hinton agar base for metronidazole testing are <16 mm resistant, 16–21 mm intermediate and >21 mm susceptible. We suggest that isolates in the intermediate zone should be re-tested by Etest. Zone sizes with the Columbia agar base for metronidazole testing are <10 mm resistant and >10 mm susceptible. Co-infection with two strains, which may be a mixture of isolates susceptible and resistant to metronidazole leading to conflicting susceptibility results, occurs in 5–10% of patients. Zone sizes with Mueller–Hinton agar and Columbia blood agar for clarithromycin testing are resistant no zone and susceptible any zone.

**Introduction**

The bacterium *Helicobacter pylori* is found in c. 40% of the population and is responsible for the development of duodenal ulcer disease.\textsuperscript{1,2} The infection also has a causative role in gastric ulcer disease, some cases of non-ulcer dyspepsia and gastric adenocarcinoma. *H. pylori* is classed as a Class 1 carcinogen by the World Health Organisation.\textsuperscript{3,4} Many groups, including the US National Institutes of Health, the *H. pylori* European Study Group and the European Society of Primary Care Gastroenterology,\textsuperscript{5,6} now recommend treatment of *H. pylori* in all patients with duodenal ulcer, as it is well established that eradicating *H. pylori* infection cures ulcer disease\textsuperscript{6,7} and it is a cost-effective strategy.\textsuperscript{8} Because of the association of *H. pylori* with adenocarcinoma, treatment of all patients diagnosed...
with *H. pylori* has become common practice, even though the benefits in non-ulcer dyspepsia are small. Triple therapies based on a proton-pump inhibitor with clarithromycin plus amoxicillin or metronidazole are the most commonly recommended treatment regimes for *H. pylori* infection. As antibiotic resistance in *H. pylori* has now become an important therapeutic issue, we feel that it is now time to review the routine laboratory methods available for ascertaining resistance. We will focus on metronidazole, as resistance to this antibiotic is more common and susceptibility testing causes more problems with this antibiotic than with amoxicillin, clarithromycin or tetracycline.

**Does resistance to antimicrobials make a difference to clinical outcome?**

Two meta-analyses, including over 60 studies, indicate that *H. pylori* resistance significantly lowers eradication rates. In the presence of nitroimidazole resistance, a drop in efficacy of 15–50% was found for triple therapies based on bismuth or proton-pump inhibitors and containing a nitroimidazole. In the face of increasing problems with metronidazole-resistant strains, recent reviews have suggested that empirical treatment should start with a triple regimen, including clarithromycin, with a back-up regimen based on metronidazole, unless resistance to clarithromycin is above 15%, in which case the order should be reversed. Although clarithromycin resistance is low in most communities, when present it also significantly reduces eradication and is often associated with metronidazole resistance. In a selection of strains from the UK, dual resistance to clarithromycin and metronidazole has increased from 2.9% in 1995 to 11.7% in 1998. In a recent German study, 46% of 554 isolates from patients in whom one or more therapies to eradicate *H. pylori* had failed were resistant to both clarithromycin and metronidazole.

**Why is empirical treatment commonplace in the face of antibiotic resistance?**

Increasingly, physicians are turning to indirect tests for the diagnosis of *H. pylori* and then instituting empirical treatment when tests are positive. Culture of *H. pylori* to undertake susceptibility testing is uncommon, and a survey in 2000 by the Public Health Laboratory Service (PHLS) of its laboratories in England and Wales found that only seven of 49 laboratories undertook routine culture and susceptibility testing. Investigators undertaking initial clinical treatment studies doubted the clinical relevance of susceptibility testing when a triple therapy regimen, including two antibiotics, was being used. The NCCLS has published guidance on susceptibility testing by agar dilution and the BSAC on susceptibility testing by the Epsilometer test (Etest), but there are no published internationally agreed susceptibility methods by disc diffusion for *H. pylori*. The primary and secondary resistance increasingly ascribed to metronidazole, clarithromycin and amoxicillin add strong weight to the view that the time has come to consider seriously routine susceptibility testing as a guide to individual patient treatment and the surveillance of antibiotic resistance both nationally and internationally to guide empirical treatment.

**What method should be used in the routine diagnostic laboratory?**

Agar dilution has been considered the gold standard (NCCLS) and has been used in large studies on stored strains, but it is more technically demanding and is therefore probably an unrealistic option for everyday practice. As resistance to clarithromycin is due to point mutations occurring in the *H. pylori* 23S rRNA gene, there are now assays to detect these directly in clinical samples, such as gastric biopsy specimens. However, the exact mechanisms of resistance to metronidazole in *H. pylori* remain to be determined. Therefore, culture methods will continue to be required until responsible gene mutations are identified, and PCR technology can be developed and used cost-effectively in routine diagnostic laboratories.

The other two options for susceptibility testing are the Etest and the disc diffusion test. Both diffusion methods have the advantage of allowing the visualization of resistant sub-populations of bacteria within zones of inhibition. Which of these two tests should be used? Results with Etest were initially greeted with great optimism and were considered superior to those with disc diffusion. However, recently there has been some doubt expressed as to its superiority.

In a multicentre European study, the correlation between Etest and agar dilution for testing metronidazole was not as good as the excellent results seen with clarithromycin and amoxicillin. The European Study Group multicentre study analysed the factors that influence Etest results for *H. pylori* susceptibility testing. Disc diffusion was not studied. There were discrepancies between Etest and agar dilution for metronidazole and lack of reproducibility in some isolates. They proposed a standardized Etest method using a suspension of a 2 day fresh culture inoculated with 10% horse blood. They suggest microaerobic incubation at 37°C for 3 days, as 5 days gave greater variability. Culture methods will continue to be required until responsible gene mutations are identified, and PCR technology can be developed and used cost-effectively in routine diagnostic laboratories.
**Helicobacter pylori** disc diffusion testing

No standardized methods have been proposed for disc susceptibility testing. Therefore, we have reviewed the studies that have directly compared Etest or agar dilution with disc diffusion (Table 1). Although these 13 metronidazole studies used various inocula, media and incubation conditions, many conclusions can be drawn about the susceptibility testing methods employed, which can also be used for other antibiotics.

**Culture medium**

Several types of medium (GAB-camp agar, Vestfold charcoal agar, An aerogenic medium, brucella blood agar, Wilkins-Chalgren agar) were used in single studies only, and we would not recommend their use without further work. Taylor et al. investigated their susceptibility testing method because the prevalence of metronidazole resistance observed was very high (80%). They attributed the falsely high rate of resistance to the medium, which was Columbia blood agar supplemented with X and V factors and menadione. The X factor and menadione, used as growth supplements, inhibited metronidazole activity. This study highlights the need for using a generally agreed susceptibility medium. Mueller–Hinton with 5–10% sheep or horse blood was used in six of the 13 studies (three studies with a disc strength of 5 μg metronidazole) and this is also recommended by the European Study Group and the BSAC for Etests and the NCCLS for agar dilution tests. The largest study, including 263 isolates, used Columbia agar with 7% horse blood, with excellent results. We recommend that Mueller–Hinton agar is used, as it is a specialized sensitivity medium, with 5–10% sheep or horse blood. However, if this is not available, Columbia agar with 5–10% sheep or horse blood may be used as an alternative. Studies are needed to show equivalence between the two media.

**Age of cultures**

Henriksen et al. tested 57 isolates on Vestfold charcoal medium, which is based on Oxoid’s charcoal agar (CM119) and contains 10% serum and 1% Isovitalex. This group was the only one to use primary cultures in their study, which, of course, is much more representative of everyday, routine laboratory practice. They found that older primary cultures gave less reliable susceptibility results. For isolates of **H. pylori** recovered beyond 4 days of incubation, only 15 of 23 resistance tests had grown sufficiently after 24–31 h incubation and the correlation between Etest and zone diameter was only −0.742 (R² = 0.552). In contrast, for isolates recovered within 4 days of primary incubation, the correlation between the two methods was −0.937 (R² = 0.878). **H. pylori** is a helical bacterium that changes to coccoid morphology as the culture ages. Coccoid strains after 2–4 weeks of ageing are not inhibited in vitro by amoxicillin, although they remain susceptible to metronidazole. We suggest that bacteria should be harvested under microaerobic conditions but susceptible in anaerobic/ microaerophilic conditions. Some reports suggest that isolates resistant to metronidazole under microaerobic conditions become susceptible following previous anaerobic incubation. Abdi et al. undertook a detailed study of over 200 isolates. Anaerobic incubation for 24 h before microaerophilic incubation for 48 h consistently increased metronidazole activity, with larger zones in the disc test and lower MIC values in Etest. However, this would have led to a change in the laboratory report in only four of the 102 strains that were resistant in microaerophilic conditions but susceptible in anaerobic/ microaerophilic conditions. The difference in zone size was a few millimetres but led to a discrepancy as the zones were close to the cut-off point. There was a 100% agreement between disc diffusion and Etest.

**Size of inoculum**

The inoculum used in these studies varied greatly, from McFarland 0.5 to 4. The most common inoculum of McFarland 4 (10⁸ cfu/mL) was used in the larger studies. Iovene et al. examined the effect of increasing the inoculum size from 10⁴ to 10⁶ cfu/mL. They state that inoculum did not affect MIC results, but unfortunately the complete results are not given.

The European Study Group examined the effect of an inoculum between 10⁶ and 10⁹ cfu/mL on susceptibility testing by Etest and agar dilution. The lower inoculum of 10⁴ cfu/spot was too low to read MIC results at 3 days of incubation, and contamination caused a problem when tests were read at 5 days. With Etest, susceptibility results were very similar with an inoculum of 10⁶ or 10⁸ cfu/mL, and correlation with agar dilution was very good with either inoculum when read at 3 days. Therefore, we suggest that an inoculum of 10⁶ cfu/mL (equivalent to McFarland 4) is used to allow results to be read by 3 days.

**Anaerobic pre-incubation**

Some reports suggest that isolates resistant to metronidazole under microaerobic conditions become susceptible following previous anaerobic incubation. Abdi et al. undertook a detailed study of over 200 isolates. Anaerobic incubation for 24 h before microaerophilic incubation for 48 h consistently increased metronidazole activity, with larger zones in the disc test and lower MIC values in Etest. However, this would have led to a change in the laboratory report in only four of the 102 strains that were resistant in microaerophilic conditions but susceptible in anaerobic/ microaerophilic conditions. The difference in zone size was a few millimetres but led to a discrepancy as the zones were close to the cut-off point. There was a 100% agreement between disc diffusion and Etest.

Taylor et al. compared disc testing (5 μg metronidazole disc) and Etest using fastidious anaerobic agar with and without anaerobic pre-incubation. Any zone was considered susceptible. Disc testing with microaerobic 72 h incubation gave a 0.9518 correlation compared with Etest; anaerobic pre-incubation reduced this to 0.7119. The above studies indicate that anaerobic pre-incubation of plates or cultures is an unnecessary complication of the method.
<table>
<thead>
<tr>
<th>Study (Ref.)</th>
<th>No. of strains (% resistant)</th>
<th>Characteristics of subculture</th>
<th>Medium</th>
<th>Correlation$^b$ of disc with Etest or agar dilution</th>
<th>Inoculum</th>
<th>Length of incubation (days)</th>
<th>Method for microaerobic atmosphere</th>
<th>Disc content (µg)</th>
<th>Zone size breakpoint (resistance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cederbrant et al., 1993 (38)$^a$</td>
<td>20</td>
<td>2 d subculture of strains frozen at −80°C subculture of strains frozen at −70°C</td>
<td>GAB-camp agar, BBL</td>
<td>not stated</td>
<td>plates flooded with 1 mL $10^3$−$10^6$ cfu/mL and excess removed</td>
<td>2</td>
<td>BBL Campy pac</td>
<td>10</td>
<td>not stated</td>
</tr>
<tr>
<td>Hirschl et al., 1993 (42)</td>
<td>86 (19)</td>
<td>3–4 d subculture of strains frozen at −70°C</td>
<td>Mueller–Hinton, 5% sheep blood, Oxoid Mueller–Hinton, 5% sheep blood, BBL</td>
<td>$r = 0.96$ with agar dilution $q = 0.742$ with broth microdilution</td>
<td>plates flooded with 1 mL McFarland 0.5 and dried</td>
<td>3</td>
<td>not stated</td>
<td>50</td>
<td>≤45 mm</td>
</tr>
<tr>
<td>Hachem et al., 1996 (43)$^a$</td>
<td>122 (39)</td>
<td>3 d primary cultures or 3–4 d subculture</td>
<td>Vestfold charcoal anaerobic agar</td>
<td>not stated</td>
<td>plates flooded with 2 mL McFarland 0.5 and dried</td>
<td>1–2</td>
<td>Oxoid Campy gen, no catalyst</td>
<td>16</td>
<td>≤28 mm</td>
</tr>
<tr>
<td>Henriksen et al., 1997 (37)</td>
<td>57 (40)</td>
<td>3–4 d subculture</td>
<td>Mueller–Hinton, 5% horse blood, Unipath Fastidious anaerobic agar, 5% horse blood</td>
<td>not stated</td>
<td>plates flooded with 2 mL McFarland 0.5 and dried</td>
<td>3</td>
<td>Oxoid Campy gen, no catalyst</td>
<td>15</td>
<td>any zone</td>
</tr>
<tr>
<td>Smith et al., 1997 (44)</td>
<td>156 (66)</td>
<td>3–4 d subculture of frozen strains</td>
<td>Mueller–Hinton, 5% horse blood, BBL</td>
<td>not stated</td>
<td>plates flooded with 2 mL McFarland 0.5 and dried</td>
<td>3–4</td>
<td>variable atmosphere incubator, 3% H₂, 10% CO₂, 81% N₂</td>
<td>5</td>
<td>&lt;20 mm</td>
</tr>
<tr>
<td>Taylor et al., 1998 (35)</td>
<td>145 pts (48 (40))</td>
<td>not stated</td>
<td>Brucella blood agar, BBA</td>
<td>not stated</td>
<td>plates flooded with 2 mL McFarland 0.5 and dried</td>
<td>3</td>
<td>Oxoid Campy gen, no catalyst</td>
<td>15</td>
<td>any zone</td>
</tr>
<tr>
<td>Abdi et al., 1999 (41)$^a$</td>
<td>100 (60)</td>
<td>subcultured twice for 3 + 2 d from liquid nitrogen</td>
<td>Mueller–Hinton, 10% blood</td>
<td>$r = 0.94$ with Etest; $r = 0.90$ with agar dilution</td>
<td>McFarland 3–4 (10⁸ cfu/mL)</td>
<td>3</td>
<td>Oxoid Campy gen, no catalyst</td>
<td>5</td>
<td>&lt;16 mm R’</td>
</tr>
<tr>
<td>Chaves et al., 1999 (36)</td>
<td>100 (21)</td>
<td>2 d subculture of strains frozen at −70°C; no cocci present</td>
<td>McFarland 3–4 (10⁸ cfu/mL)</td>
<td>3</td>
<td>Oxoid Campy gen, no catalyst</td>
<td>5</td>
<td>&lt;16 mm R’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iovene et al., 1999 (39)</td>
<td>80 (24)</td>
<td>3–6 d subculture from primary culture</td>
<td>Mueller–Hinton, 5% horse blood</td>
<td>$r = 0.96$ with Etest</td>
<td>McFarland 3</td>
<td>3</td>
<td>Oxoid Campy gen, no catalyst</td>
<td>50</td>
<td>&lt;26 mm</td>
</tr>
<tr>
<td>van der Wouden et al., 1999 (40)</td>
<td>263 (35)</td>
<td>2–3 d primary cultures</td>
<td>Columbia agar, 7% horse blood</td>
<td>5% discrepancies compared with Etest</td>
<td>McFarland 3</td>
<td>2–3</td>
<td>Anoxomat</td>
<td>5</td>
<td>&lt;10 mm</td>
</tr>
</tbody>
</table>

Table 1. Studies of disc diffusion tests for susceptibility testing of metronidazole (resistance >8 mg/L by Etest or agar dilution)
Helicobacter pylori disc diffusion testing

Which disc content?

A small early exploratory study examined the use of different disc contents.38 Although a 1 μg metronidazole disc gave no zone, there was a good correlation between disc susceptibility with 5 and 10 μg discs and agar dilution in isolates with an MIC of >32 or <1 mg/L. Hirschl et al.42 compared both the accuracy and precision of disc diffusion using a 50 μg disc with those of agar dilution and Etest and compensated for effects generated by scales of better resolution. The correlation of the results of agar dilution with those of either Etest \( (r = 0.96) \) or disc diffusion \( (r = -0.96) \) were strong. However, they found that disc diffusion led to the underestimation of MIC values calculated from the inhibition zone diameters via linear regression. As a result of these early studies, most other centres have used lower-content discs, usually 5 μg.

Only one group used a very high metronidazole disc content of 80 μg, with a 5 day incubation.43 In a comparison of agar dilution and Etest using 122 isolates, they found that the disc test performed better than Etest, with a 96% concordance between disc \( (\approx 31 \text{ mm resistant zone}) \) and broth microdilution and a 70% concordance between Etest and agar microdilution. Overall, the results of these studies leave us uncertain which disc content to recommend. A 50 μg metronidazole disc is available but it is marketed as a diagnostic test for Gardnerella. We suggest using the 5 μg discs, as they are available more readily and the larger studies have used this content very successfully.

Zone size breakpoints

The most detailed study published has been by Chaves et al.36 and included excellent details of their methods and results, including regression analysis. They used the inoculum and methods suggested by the European Study Group.14,34 They highlighted that previous studies have found greatest problems with isolates that lie in the intermediate range of metronidazole MICs (i.e. 4–32 mg/L). Therefore, they suggested three susceptibility categories: susceptible, intermediate and resistant. They also looked at reproducibility. They concluded that any inconsistencies reported previously might be due to inoculum differences and that this problem should be minimal if the inoculum is standardized. No significant differences were found between MICs determined by agar dilution and Etest. No very major errors (strain classified as resistant by reference method and susceptible by new method) occurred with disc diffusion if the three criteria of resistant, intermediate and susceptible were used. Using a buffer intermediate zone prevented very major errors. The correlation between Etest and diffusion was \( r = 0.94 \). The zones they suggested for Mueller–Hinton with a 5 μg metronidazole disc were <16 mm resistant, 16–21 mm intermediate and >21 mm susceptible. Other studies have not considered an intermediate category, and unfortunately Chaves’ data cannot

<table>
<thead>
<tr>
<th>Weiss et al., 1998</th>
<th>Mueller–Hinton, 10% sheep blood</th>
<th>correlation of disc McFarland 5 with Etest or agar (10 µg/mL)</th>
<th>16 mm resistant, 16–21 mm intermediate, 21 mm susceptible</th>
<th>0.74 between McFarland 3–4 (3 × 10^6 cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>not stated</td>
<td>correlation coefficient ( \rho ) (strength of association between the two results).</td>
<td>Control stated. ( \rho_r ) resistant, ( \rho_i ) intermediate, ( \rho_s ) susceptible.</td>
<td>0.94 between McFarland 3–4 (3 × 10^6 cfu/mL)</td>
</tr>
<tr>
<td>Midolo et al., 1995</td>
<td>not stated</td>
<td>correlation coefficient ( \rho ) (strength of association between the two results).</td>
<td>Control stated. ( \rho_r ) resistant, ( \rho_i ) intermediate, ( \rho_s ) susceptible.</td>
<td>0.94 between McFarland 3–4 (3 × 10^6 cfu/mL)</td>
</tr>
<tr>
<td>DeCross et al., 1993</td>
<td>not stated</td>
<td>correlation coefficient ( \rho ) (strength of association between the two results).</td>
<td>Control stated. ( \rho_r ) resistant, ( \rho_i ) intermediate, ( \rho_s ) susceptible.</td>
<td>0.94 between McFarland 3–4 (3 × 10^6 cfu/mL)</td>
</tr>
<tr>
<td>100 (49) subculture of strains</td>
<td>information at −80°C</td>
<td>Horse blood agar</td>
<td>Horse blood agar</td>
<td>10% CO2</td>
</tr>
<tr>
<td>25</td>
<td>5% horse blood dilution</td>
<td>McFarland 3–4</td>
<td>McFarland 3–4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10% CO2</td>
<td>5–7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Weiss 55 not stated Mueller–Hinton, 5% horse blood dilution for metronidazole, 98% with agar dilution; 100% with Etest, correlation coefficient \( \rho \) (strength of association between the two results). Control stated. \( \rho_r \) resistant, \( \rho_i \) intermediate, \( \rho_s \) susceptible.
necessarily be extrapolated to other media. A direct comparison of Mueller–Hinton and Columbia agar is needed to determine the need for an intermediate zone for Columbia agar, which we are currently investigating. In the meantime we suggest a zone of <10 mm resistant and ≥10 mm sensitive for Columbia agar, as this was very predictive in the large series of van der Wouden et al. Reporting an isolate as intermediate susceptibility is not useful to the clinician; therefore, if a strain falls into the intermediate category of susceptibility to metronidazole (zone 16–21 mm with Mueller–Hinton), it would be sensible to determine its MIC by Etest or agar dilution.

Co-infection with resistant and susceptible strains and variants

The largest study, testing 263 isolates using Columbia agar base with 7% horse blood, was reported by van der Wouden et al. This is the medium commonly used in northern European countries. Plates were read at 2–3 days for both Etest and disc test. The inoculum was prepared from a suspension of multiple colonies from primary plates, but the exact inoculum size was not stated. A 5 µg metronidazole disc was used, and a zone of <10 mm was considered resistant. Etest and disc testing was discordant in 14 (5%) of isolates. These isolates were usually not in the intermediate zone of susceptibility. Five isolates with no zone on disc testing had an MIC of ≤5 mg/L. Two isolates with MICs of ≥32 mg/L had a zone size of 26 mm. Reproducibility using single subcultured colonies was good. However, Etests carried out using 10 separate colonies from a primary plate produced susceptible and resistant bacteria in five of 52 patients. This co-infection with susceptible and resistant isolates occurred in 10% of patients and may explain the discordant results between different tests and studies. Smith et al. discovered that differences in susceptibility were found in isolates obtained from both the corpus and antrum in five of 22 patients. The discrepancies may be due to co-infection in an individual with two different strains of distinct lineage. Co-infection with distinct strains has been confirmed by DNA fingerprinting. The discrepancy in susceptibility results may also be due to resistant variants within a single strain. This suggests that we should accept a 5–10% discordance when comparing results of susceptibility tests undertaken from purity plates arising from single colonies. To detect all resistance, it would be sensible to ask clinicians to send biopsy specimens from the antrum and corpus. When isolates are prepared for susceptibility testing, several colonies should be touched.

Clarithromycin disc susceptibility testing

As resistance to clarithromycin increases, susceptibility testing becomes more important. H. pylori strains have a bimodal distribution of susceptibility and few strains have MICs around the breakpoint, so the discrepancies seen with metronidazole susceptibility testing rarely occur with clarithromycin. Five studies have validated disc clarithromycin susceptibility testing (Table 2). The prevalence of clarithromycin resistance ranged from 0 to 13%. All have shown an excellent correlation with MICs determined by agar dilution or Etest. The medium used was Mueller–Hinton with 5–10% blood and a clarithromycin disc content of 15 or 2 µg. Smith et al. published the largest series, 156 isolates, of which 20 (13%) were resistant to clarithromycin. They commented that a clarithromycin disc content of 15 µg produced large zones and these would need to be measured to differentiate resistant and susceptible strains. With a clarithromycin disc content of 2 µg, no measurement is required, as no zone is produced with resistant isolates (MIC > 2 mg/L), so simplifying the method. Therefore, we suggest the same inoculum and incubation as for metronidazole disc testing with a 2 µg clarithromycin disc on Mueller–Hinton agar with 5–10% blood. Isolates with no zone should be reported as resistant.

Summary

- Avoid high (≥50 µg) or low (1 µg) metronidazole disc content. We recommend a disc content of 5 µg metronidazole and 2 µg clarithromycin.
- Use Mueller–Hinton or Columbia agar base with 5–10% blood.
- Use cultures of ≤4 days old for setting up susceptibility testing.
- Use an inoculum of suspension equivalent to McFarland 4 (10^6 cfu/mL).
- Using anaerobic pre-incubation of plates is an unnecessary complication.
- Include a metronidazole-susceptible control (e.g. NCTC 12822).
- Suggested zone sizes with disc diffusion for Mueller–Hinton agar plus 7–10% blood are <16 mm resistant, 16–21 mm intermediate and >21 mm susceptible.
- Interim suggested zone sizes with disc diffusion for Columbia agar plus 7–10% blood are <10 mm resistant and ≥10 mm susceptible.
- Test strains in metronidazole intermediate zone by another quantitative method.
- Expect discordant results in 5–10% of patients, due to co-infection with multiple strains.
- Suggested zone sizes with disc diffusion for clarithromycin using Mueller–Hinton agar plus 7–10% blood or Columbia agar plus 7–10% blood are resistant no zone and susceptible any zone.

Acknowledgements

We wish to thank the referees for their helpful comments.
Table 2. Studies of disc diffusion tests for susceptibility testing of clarithromycin (resistance >2 mg/L by Etest or agar dilution)

<table>
<thead>
<tr>
<th>Study (Ref.)</th>
<th>No. of strains (% resistant)</th>
<th>Characteristics of subculture</th>
<th>Correlation of disc with Etest or agar dilution</th>
<th>Inoculum</th>
<th>Length of incubation (days)</th>
<th>Method for microaerobic atmosphere</th>
<th>Disc content (µg)</th>
<th>Zone size breakpoint (resistance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weiss et al., 1998 (47)</td>
<td>55 (0)</td>
<td>not stated</td>
<td>Mueller–Hinton, 10% sheep blood</td>
<td>100% with agar dilution</td>
<td>McFarland 5 (10^8 cfu/mL)</td>
<td>3</td>
<td>Campylobacter Difco</td>
<td>15</td>
</tr>
<tr>
<td>Hachem et al., 1996 (43)</td>
<td>122 (7)</td>
<td>3–4 d subculture of strains frozen at −70°C</td>
<td>Mueller–Hinton, 5% sheep blood, BBL</td>
<td>100% with agar dilution and Etest r = 0.94 with Etest</td>
<td>McFarland 2 streaked with swab</td>
<td>5</td>
<td>not stated</td>
<td>15</td>
</tr>
<tr>
<td>Iovene et al., 1999 (39)</td>
<td>80 (10)</td>
<td>3–6 d subculture from primary culture subculture</td>
<td>Mueller–Hinton, 5% sheep blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midolo et al., 1997 (50)</td>
<td>51 (8)</td>
<td>3–4 d subculture of strains frozen at −80°C</td>
<td>Mueller–Hinton, BBL, 5% horse blood</td>
<td>100% with Clari Etest</td>
<td>McFarland 1 streaked with swab</td>
<td>3</td>
<td>Oxoid Campy pak</td>
<td>15</td>
</tr>
<tr>
<td>Smith et al., 1997 (44)</td>
<td>156 (13)</td>
<td>from 145 pts</td>
<td>Mueller–Hinton, Unipath, 5% horse blood</td>
<td>100%</td>
<td>McFarland 4 (10^8 cfu/mL)</td>
<td>3–4</td>
<td>variable atmosphere incubator</td>
<td>2</td>
</tr>
</tbody>
</table>

*aControl stated.

b r = correlation coefficient (strength of association between the two results).
References


Helicobacter pylori disc diffusion testing


