

Comparison of the reading of Clarithromycin E-test on *Helicobacter Pylori* by eye and with microscope

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Background

- Antibiotic resistance in *H. pylori* is an increasing problem worldwide with regional differences in resistant rates (1,2). Antibiotic susceptibility testing still mainly relies on culture and phenotypic drug susceptibility testing that can be both time consuming and uncertain. Visual inspection and determination of MICs on the E-test strips leaves room for personal interpretation and is challenged by the formation of almost invisible microcolonies of resistant strains that can be left undetected. The aim of this study was to compare the results of reading the E-test by eye and with a microscope.

Method

Aim of study and patients

- The study was conducted on 15 previously E-tested *H. pylori* strains collected from Danish patients with an indication for gastroscopy between January 2017 – 2019. The strains had all been routine E-tested and were registered in the system as susceptible to clarithromycin. The strains were re-cultivated and E-tested for clarithromycin. E - tests were read by eye and microscope at the third, fourth and fifth day from making the E-test, to reveal a possible difference in results.

Culture and susceptibility testing of *H. pylori* strains from gastric biopsies

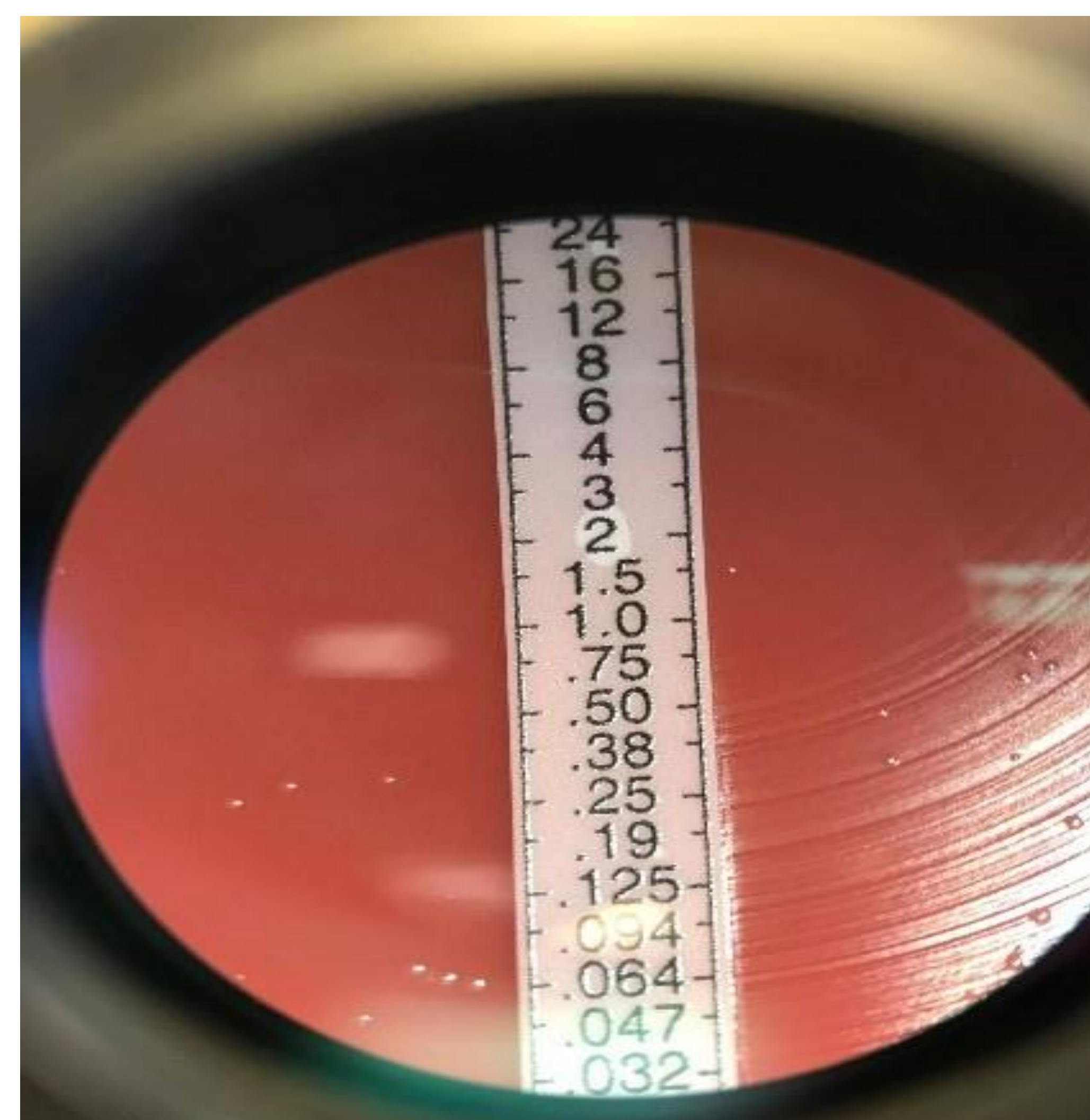
- Biopsies were cultured at 37 °C under microaerobic conditions (4% O₂, 10% CO₂ and 86% N₂) for up to 7 days. Isolated *H. pylori* strains were stored at -80 °C until susceptibility testing. All isolates were E-tested on chocolate agar plates using E- test (bioMérieux) Clarithromycin (0.016-256 mg/L). The clinical breakpoint for *H. pylori* for clarithromycin is > 0,5 mg/L (3)
- All E-tests were read by eye and with microscope. Microcolonies were isolated and subcultivated on a new agar plate, and E-tested once again. Figure 1 shows the result after e-testing the subcultivated microcolonies of one of the susceptible strains which formed small resistant microcolonies on the agar plate. Reading of the E-test on the third, fourth and fifth day revealed no significant difference in results.

Conclusion

- Clarithromycin resistant microcolonies were found in 3 of the 15 strains. This study revealed the possibility of overlooking clarithromycin resistant microcolonies when reading the E-test by eye. Prescription of clarithromycin to a patient from which a sample of *H. pylori* with resistant microcolonies has been collected, can result in selection of the resistant clones and treatment failure as a result. This should be taken into consideration when performing susceptibility testing for clarithromycin on *H. pylori* with E-tests.

Results

Figure 1: Clarithromycin resistant microcolonies in *H. pylori* strain seen with a microscope



A) E-test for clarithromycin on a strain previously registered as sensitive shows resistant microcolonies by microscopy. Break-point for clarithromycin resistance is MIC of 0.50 (3)



B) The resistant microcolonies were isolated from the agar plate, subcultivated and a new E-test was done. The new E-test showed growth of clarithromycin resistant *H. pylori*.

References

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The authors declare no
conflicts of interests