MIC Testing of *Helicobacter pylori* using Etest® Finafloxacin and the Reference Agar Dilution Method

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OBJECTIVES

The aim of the study was to compare MIC testing with Etest Finafloxacin and the CLSI agar dilution reference method using clinical *Helicobacter pylori* isolates.

INTRODUCTION

Helicobacter pylori (HP) is a Gram negative bacteria commonly found in the gastric mucosa, where it can reside without any clinical symptoms. However, this pathogen has been associated with e.g. peptic ulcers and gastric cancer.

Since the treatment of HP infections used today is complex and can lead to side effects and cross-resistance, it is of great importance to find the most suitable drug.

Finafloxacin hydrochloride (FIN) is a novel 8-cyano fluoroquinolone that exhibits optimal activity at slightly acidic conditions, where other fluoroquinolones lose activity. The intended use of FIN is for the therapy of bacterial infections such as HP infections, since the bacteria harbours in an acidic environment.

The Etest FIN gradient is preformed, predefined and stable which makes the system suitable for testing of fastidious organisms like HP with varying growth rates.

MATERIAL AND METHODS

Strains

Test isolates: A total of 36 *H. pylori* clinical isolates, including fluoroquinolone resistant strains, were tested in quadruplicate.

Quality control strain: *H. pylori* ATCC 43504 was tested in quintuplicate.

Reagents

Finafloxacin powder (MerLion Pharmaceuticals Pte. Ltd, Singapore); Mueller Hinton agar (BBL, Maryland, USA); Etest Finafloxacin (FIN) MIC range 0.002 – 32 µg/mL (AB bioMérieux, Solna, Sweden).

Procedure

Etest was used according to manufacturers instruction and agar dilution was performed according to CLSI guidelines. Both methods were read after 3 and 5 days of incubation.

RESULTS

Figure 1. Etest FIN vs. AD; 3 days

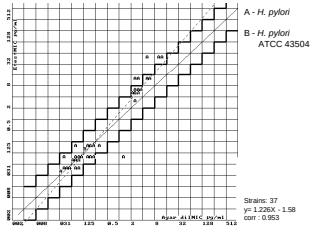
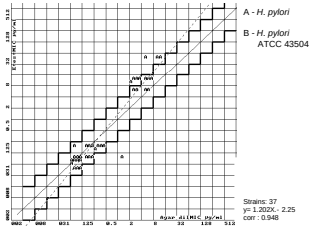
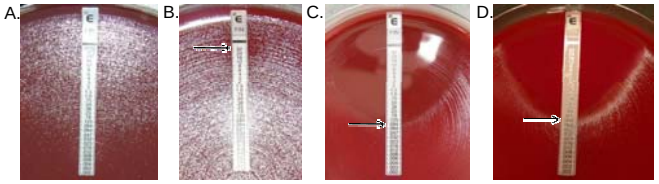


Figure 2. Etest FIN vs. AD; 5 days



Etest FIN and CLSI agar dilution method were shown to be comparable, although the correlation between Etest and AD were slightly better after 3 days (table 1), i.e. the recommended incubation time. Resistant micro/macro subcolonies were seen in the inhibition ellipse for a few isolates when tested by Etest, especially after 5 days of incubation (figure 3A and 3B). These subcolonies are not specific to finafloxacin and the clinical relevance needs to be further investigated.

Figure 3. Illustrations of Etest Finafloxacin results



A. Etest MIC >32 AD MIC 8
B. Etest MIC 32 AD MIC 4
C. Etest MIC 0.094 AD MIC 0.25
D. Etest MIC 0.094 AD MIC 0.125

CONCLUSIONS

- Etest FIN vs. CLSI agar dilution MIC results showed good agreement after 3 and 5 days of incubation.
- Recommended incubation time of *Helicobacter pylori* is 3 days.
- Etest FIN was more efficient than AD in detecting resistant subpopulations.
- Etest is a useful tool for testing new agents against *Helicobacter pylori*.

Table 1. Essential agreements Etest vs. agar dilution

Incubation time	% EA, modal MIC	
	± 1 dil	± 2 dil
3 days	83.3	94.4
5 days	77.8	94.4

Table 2. Intralaboratory reproducibility of Etest and agar dilution

Incubation time	% Reproducibility, modal MIC ± 1 dil	
	Etest (36 strains x4)	Agar dilution (36 strains x3)
3 days	93.1	97.2
5 days	90.3	97.2

Table 3. Tentative quality control ranges for Etest Finafloxacin (µg/mL)

Organism	3 and 5 days incubation		Tentative Etest QC range
	AD (n=30)	Etest (n=20)	
H. pylori ATCC 43504	0.125 - 0.25 Mode: 0.125	0.064 - 0.19 Mode: 0.125	0.064 - 0.25