Comparison of Methods for Finafloxacin MIC Testing at Acidic and Neutral pH

A. Engelhardt, A. Yusof, P. Ho, C. Johansson, K. Sjöström
AB bioMérieux, Solna, Sweden

AB bioMérieux
Dalvägen 10
169 56 Solna, Sweden
etest@abbiodisk.se

F1-2039

OBJECTIVES

MIC testing of aerobic bacteria with finafloxacin (FIN) at acidic and neutral pH was studied by comparing Etest®, CLSI agar dilution (AD) and broth microdilution (BMD) methods.

INTRODUCTION

Finafloxacin is a novel broad spectrum fluoroquinolone that exhibits optimal activity at slightly acidic conditions (pH 5-6) where other fluoroquinolones lose some of their activity. Hence, finafloxacin is intended for therapy of bacterial infections associated with an acidic environment such as *H. pylori* eradication and complicated urinary tract infections.

Studies, thus far, have also shown that finafloxacin retains additional various positive features of other marketed fluoroquinolones, including a good safety profile.

MATERIALS AND METHODS

Bacterial strains

Test strains: *A. anitratus* (1), *E. coli* (8), *E. cloacae* (2), *K. pneumoniae* (2), *P. vulgaris* (1), *P. rettgeri* (1), *P. stuartii* (1), *P. aeruginosa* (3), *S. aureus* (13), *S. haemolyticus* (3), *S. marsescens* (2), *S. saprophyticus* (1) and *S. warneri* (1).

Quality control strains: *E. coli* ATCC® 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213.

Reagents

Finafloxacin powder (MerLion Pharmaceuticals Pte. Ltd, Singapore); Etest Finafloxacin (FIN) MIC range 0.002 – 32 µg/mL (AB bioMérieux, Solna, Sweden); Mueller Hinton agar and broth (BBL, Maryland, USA) at pH 5.8 and 7.2.

Procedure

Etest was used according to the manufacturer's instructions and tested at both pH 5.8 and pH 7.2. AD and BMD were performed using the CLSI procedures and tested at both pH 5.8 and pH 7.2. The MIC was read at complete inhibition of growth.

CONCLUSIONS

- MIC testing of finafloxacin with Etest, agar and broth dilution reference methods provides substantially equivalent results at both neutral and acidic pH (EA ±1 dilution 88-100 %), and demonstrates higher activity of FIN at slightly acidic pH.
- Etest agreement with CLSI methods was lower at pH 5.8 primarily due to Etest being more efficient in detecting the resistant subpopulations.
- Etest MIC values at pH 5.8 were approximately 2-4 dilutions lower than those at pH 7.2.
- Etest with a wide concentration range (15 dilutions) comprise a useful MIC tool for drug development studies with FIN and for future studies with *H. pylori*.

RESULTS

Table 1. Etest MIC for different species at acidic and neutral pH

Species	N	MIC range (µg/mL)	
		pH 5.8	pH 7.2
A. anitratus	1	0.25	1
E. coli	8	0.016 – 32	0.094 – 32
E. cloacae	2	0.012 – 32	0.047 – 32
K. pneumoniae	2	0.023 – 0.5	0.19 – 1.5
P. vulgaris	1	0.19	0.5
P. rettgeri	1	0.5	3
P. stuartii	1	0.094	0.38
P. aeruginosa	3	0.38 – 4	3 – 32
S. marcescens	2	0.5 – 32	2 – 32
S. aureus	13	0.023 – 3	0.094 – 32
S. haemolyticus	3	0.023 – 0.032	0.094 – 0.125
S. saprophyticus	1	0.19	0.38
S. warneri	1	0.064	0.25

Finafloxacin activity at acidic pH was 2-4 dilutions greater than at neutral pH

Illustrations of Etest Finafloxacin results at neutral and acidic pH

Figure 1. *P. stuartii* CDC 2083

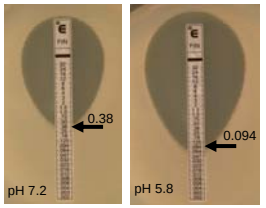


Figure 2. *S. aureus* ATCC 29213

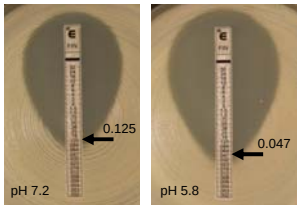
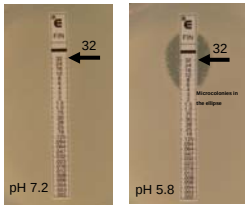


Figure 3. *E. coli* ECI 2119 FQR



MIC endpoints were generally clear-cut for most organisms. Macro- and microcolonies were occasionally seen in Etest FIN inhibition ellipses for a few *E. coli* and *E. cloacae* strains, such colonies are not specific to finafloxacin. The MIC was read at complete inhibition of growth.

Table 2. Comparison of MIC methods at acidic and neutral pH

Comparator	Regression analysis		% EA ± 1 dil.
	Equation	r	
AD vs. BMD, pH 5.8	y = 0.94 x + 0.27	0.99	100
AD vs. BMD, pH 7.2	y = 0.97 x + 0.36	0.98	100
Etest vs. AD, pH 5.8	y = 1.11 x - 0.45	0.97	87.5
Etest vs. AD, pH 7.2	y = 0.99 x + 0.28	0.97	100
Etest vs. BMD, pH 5.8	y = 1.05 x - 0.27	0.97	87.5
Etest vs. BMD, pH 7.2	y = 0.97 x + 0.55	0.98	97.9

EA = Essential Agreement

Figure 4. Comparison of Etest MICs at acidic and neutral pH

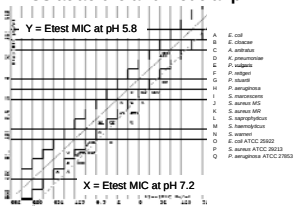


Table 3. Quality control results and tentative QC ranges for Etest FIN (µg/mL)

Strain	Etest (N 20)		Agar dilution (N 8)		Broth dilution (N 8)		Etest Tentative QC	
	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2
E. coli ATCC 25922	0.008-0.012 **Mode: 0.012**	0.047-0.094 **Mode: 0.047**	0.008-0.016 **Mode: 0.008**	0.032-0.064 **Mode: 0.064**	0.008-0.016 **Mode: 0.008**	0.032-0.064 **Mode: 0.064**	**0.008-0.032**	**0.032-0.125**
S. aureus ATCC 29213	0.032-0.047 **Mode: 0.032**	0.094-0.125 **Mode: 0.125**	0.032 **Mode: 0.032**	0.125 **Mode: 0.125**	0.032-0.064 **Mode: 0.064**	0.125 **Mode: 0.125**	**0.032-0.125**	**0.064-0.25**
P. aeruginosa ATCC 27853	0.38-0.75 **Mode: 0.5**	2-4 **Mode: 3**	0.25 **Mode: 0.25**	2-4 **Mode: 4**	0.25-0.5 **Mode: 0.5**	4 **Mode: 4**	**0.25-1**	**2-8**