Fosfomycin Susceptibility Testing using the new ETEST® FO

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INTRODUCTION

Fosfomycin is a bactericidal antibiotic with a broad spectrum of activity against a wide range of Gram-positive and Gram-negative bacteria. The drug inhibits the cell wall synthesis in both Gram-positive and Gram-negative bacteria by blocking the initial step involving phosphoenolpyruvate synthetase. Oral fosfomycin is mainly used in the treatment of urinary tract infections, particularly those caused by *Escherichia coli* and *Enterococcus faecalis* and as an alternative treatment in case of multi-resistant strains.

In order to determine MICs to Fosfomycin, the ETEST® FM is already available but the reading can be difficult especially with *E. coli*. To resolve this issue, a new ETEST® with Fosfomycin, called ETEST® FO, has been developed (not FDA cleared, yet).

The purpose of this study is to compare this new ETEST® FO to the Agar Dilution reference method (AD) on a panel of *E. coli* and *E. faecalis*.

MATERIAL AND METHOD

Bacterial strains

A total of 39 selected isolates comprising 20 *E. coli*, including extended spectrum beta-lactamase and carbapenemase producers *Enterobacteriaceae* and 19 *E. faecalis*, including Vancomycin-Resistant *Enterococcus* and Vancomycin- Susceptible *Enterococcus* were tested by ETEST® FO and Agar Dilution.

QC organisms tested were *Escherichia coli* ATCC® 25922™, *Staphylococcus aureus* ATCC® 29213™ and *Enterococcus faecalis* ATCC® 29212™ following CLSI QC guidelines.

Method

The isolates were sub-cultured on Columbia agar plates supplemented with 5% sheep blood before testing. After incubation, suspensions of the isolates were prepared in 0.85% saline. These suspensions were used to inoculate both AD and ETEST® plates.

Results were read after 16-20 hours incubation at 35°C+/-2°C in ambient air for both methods.

Results were analyzed using the FDA/CLSI breakpoints for Fosfomycin (S ≤ 64µg/mL, I=128 µg/mL, R ≥ 256 µg/mL). Performance was evaluated using FDA performance criteria, essential agreement (EA, ≥ 90%), category agreement (CA, ≥ 90%), major error rate (ME, ≤3.0%) and very major error rate (VME, ≤2.0%).

RESULTS

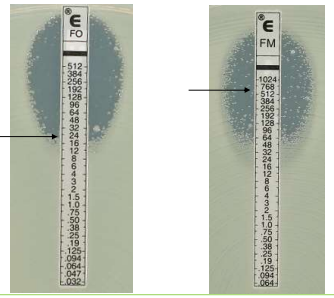
All the QC strains were within the CLSI ranges.
For the panel results, see table 1, Performance for ETEST® FO on *E. coli* and *E. faecalis*.

Table 1. Performance for ETEST® FO on E. coli and E. faecalis

Species	EA	CA	Very Major Error Rate	Major Error Rate	Minor Error Rate
Overall (39)	**97.4%** (38/39)	**97.4%** (38/39)	**0%** (0/2)	**0%** (0/37)	**2.6 %** (1/39)
E. faecalis (19)	**100%** (19/19)	**94.7%** (18/19)	**NA** (No R)	**0%** (0/19)	**5.2%** (1/19)
E. coli (20)	**95.0%** (19/20)	**100%** (20/20)	**0%** (0/2)	**0%** (0/18)	**0%** (0/20)

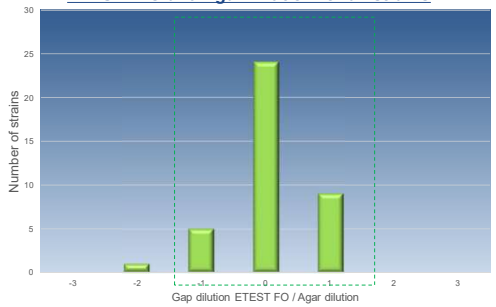
The EA and CA rates were very good whatever the species and with only one minor error. No ME or VME was found.

Figure 1 and 2 : Comparison of ETEST® FO vs ETEST® FM



E. coli
ETEST® FO MIC : 24 µg/ml
ETEST® FM MIC : 768 µg/ml (following the reading instructions)
Agar dilution MIC : 32µg/ml

Figure 3 – Essentiel MIC Agreement between ETEST® FO and Agar Dilution for all strains



CONCLUSION

This first and preliminary study shows that the new ETEST® FO is found to be substantially equivalent to the CLSI reference method. ETEST® FO could be a valuable tool for determining Fosfomycin MIC for *E. coli* harboring various resistance mechanisms and *E. faecalis* including VRE. Moreover, in comparison with the current ETEST® FM strip, this new strip brings a real reading improvement and resolves the issue for *E. coli*. ETEST FO needs clinical studies in order to be IVD cleared (FDA).

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