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EUCAST zone diameter breakpoints for fosfomycin and *Escherichia coli*

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Introduction

EUCAST has defined clinical MIC breakpoints for fosfomycin and Enterobacteriaceae (S≤32, R>32 mg/L), but zone diameter breakpoints have been lacking. There are several methodological problems with antimicrobial susceptibility testing of fosfomycin, both by MIC determination and disk diffusion. Disk diffusion is particularly challenging due to the varying, but frequent, presence of colonies within the inhibition zones. Agar dilution is recommended as the reference method for fosfomycin, since broth microdilution may give unreliable results (ISO standard 20776-1).

Objective

The objective of this study was to establish zone diameter breakpoints for fosfomycin and *Escherichia coli* using agar dilution as reference.

Methods

Agar dilution (including 25 mg/L glucose-6-phosphate, G6P) and disk diffusion of fosfomycin were performed on *E. coli* according to international standards using Mueller-Hinton (MH) agar. Several modifications of the EUCAST disk diffusion method were evaluated: lower inoculum (10^7, 10^6 and 10^5 CFU/mL), shorter incubation time (6 and 8 h), different content of glucose-6-phosphate (0, 50, 100, 200 and 400 µg) in the 200 µg fosfomycin disk and with specific reading instructions in case of colonies within the inhibition zone. Disks from several manufacturers were evaluated (BD, Liofilchem, Mast and Oxoid/Thermo Fisher Scientific). The various modifications were tested at two laboratories and the proposed method was then validated by disk diffusion testing of 17 isolates with varying fosfomycin MICs (consensus MICs from 3 repeated tests) at 9 different laboratories using disks from Mast and Oxoid on local MH agar (Agrincons, BBL, Bio-Rad and Oxoid represented). Whole Genome Sequencing (WGS) was also performed on the 17 selected isolates. Additional validation was performed by routine testing of consecutive isolates of *E. coli*.

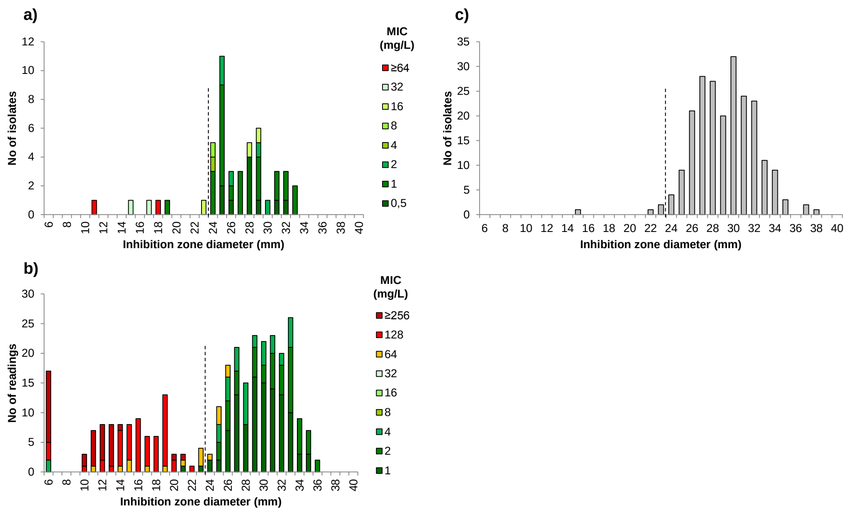

Figure 1. Inhibition zone diameter distributions for *E. coli* and fosfomycin 200 µg (with 50 µg glucose-6-phosphate) when ignoring all isolated colonies within the inhibition zones.
a) 47 isolates tested once.
b) 17 isolates tested at 9 different laboratories.
c) Results from routine testing of local consecutive isolates of *E. coli* (n=218).
MIC values are shown as coloured bars. Grey bars = no MIC. EUCAST zone diameter breakpoints are shown as dotted lines.

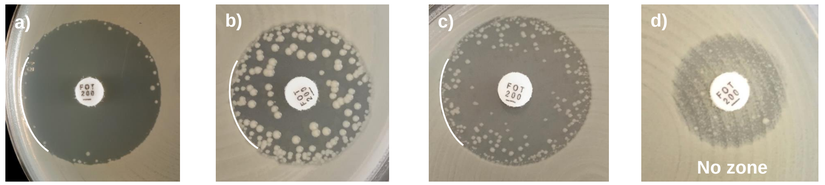

Figure 2. Examples of fosfomycin 200 µg inhibition zones for *E. coli* with EUCAST instructions for reading: Ignore isolated colonies within the inhibition zone and read the outer zone edge.
a-c) Ignore all colonies and read the outer zone edge.
d) Record as no inhibition zone.
EUCAST Breakpoint Table v 7.0, 2017.

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Results

Reproducible results and good correlation between agar dilution and disk diffusion were obtained when colonies within the inhibition zone were ignored for fosfomycin 200 µg disks with 50 µg G6P (**Figure 1a**). Lowering the inoculum and shortening the incubation time also improved results, but was considered less useful in a routine laboratory. Increasing the G6P content did not improve the test, but results were more reproducible with 50 µg G6P than without G6P. All isolates with the *fosA* gene according to WGS had fosfomycin MICs ≥128 mg/L and these were separated from wild-type isolates when isolated colonies within the inhibition zones were ignored.

Validation of the reading instructions (**Figure 2**) at 9 laboratories confirmed that the test results were reproducible (**Figure 1b**). Zone diameter breakpoints of S≥24, R<24 mm were proposed for *E. coli* and these were also supported by routine testing of consecutive *E. coli* (**Figure 1c**).

Conclusions

EUCAST has developed zone diameter breakpoints for fosfomycin and *E. coli* according to standard disk diffusion methodology with specific reading instructions. These were published in the EUCAST Breakpoint Table 7.0, January 2017. Disk diffusion for fosfomycin will be further evaluated for other Enterobacteriaceae, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Acknowledgements for participation in the validation study:
Emma Jonasson, Växjö Central Hospital, Sweden
Onur Karatuna, Acibadem Labmed Medical Laboratories, Istanbul, Turkey
Helge Kolstad, Haukeland University Hospital, Bergen, Norway
Karolina Näslund, Kalmar Hospital, Sweden
Annarita Mazzariol, Dipartimento di Patologia e Diagnostica, Verona, Italy
Ørjan Samuelsen, University Hospital of Northern Norway
Marta Tato Diez, Hospital Universitario Ramón y Cajal, Madrid, Spain