27th ECCMID Vienna, Austria 22 – 25 April 2017

The congress of ESCMID

Session: P007 MIC and disc diffusion methods - revisited

Category: 3c. Susceptibility testing methods

22 April 2017, 15:30 - 16:30
P0159

EUCAST zone diameter breakpoints for fosfomycin and Escherichia coli

Erika Matuschek*[1], Mandy Wootton[2], Jenny Ahman[1], Leanne Davies[3], Robin Howe[3], Gunnar Kahlmeter[1]

[1]*Eucast Development Laboratory; Clinical Microbiology*

[2]*University Hospital of Wales; Public Health Wales - Sacu*

[3]*Public Health Wales*

Background: EUCAST has defined clinical MIC breakpoints for fosfomycin and Enterobacteriaceae (S≤32, R>32 mg/L), but there are no zone diameter breakpoints yet. There are several methodological problems with 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 20776-1). The objective of this study was to establish zone diameter breakpoints for fosfomycin and *Escherichia coli* using agar dilution as reference.

Material/methods: Agar dilution (AD, including 25 mg/L glucose-6-phosphate) and disk diffusion (DD) 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 (G6P: 0, 50, 100, 200 and 400 µg) in the 200 µg fosfomycin disk and with specific reading instructions on how to deal with the 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 DD testing of 17 isolates with varying fosfomycin MICs (consensus MICs from 3 repeated tests) at 9 additional 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.

Results: Reproducible results and good correlation between AD and DD were obtained when colonies within the inhibition zone were ignored for fosfomycin 200 µg disks with 50 µg G6P. Lowering the

inoculum and shortening the incubation time also seemed promising, but less useful in a routine laboratory. Increasing the G6P content did not improve the test results, but results were more reproducible with 50 µg 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 at 9 additional laboratories confirmed that the test results were reproducible and zone diameter breakpoints of S≥24, R<24 mm were proposed for *E. coli* (Figure 1).

Conclusions: EUCAST has developed zone diameter breakpoints for fosfomycin 200 µg with 50 µg G6P and *E. coli* according to standard disk diffusion methodology with specific reading instructions. These will be 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*.

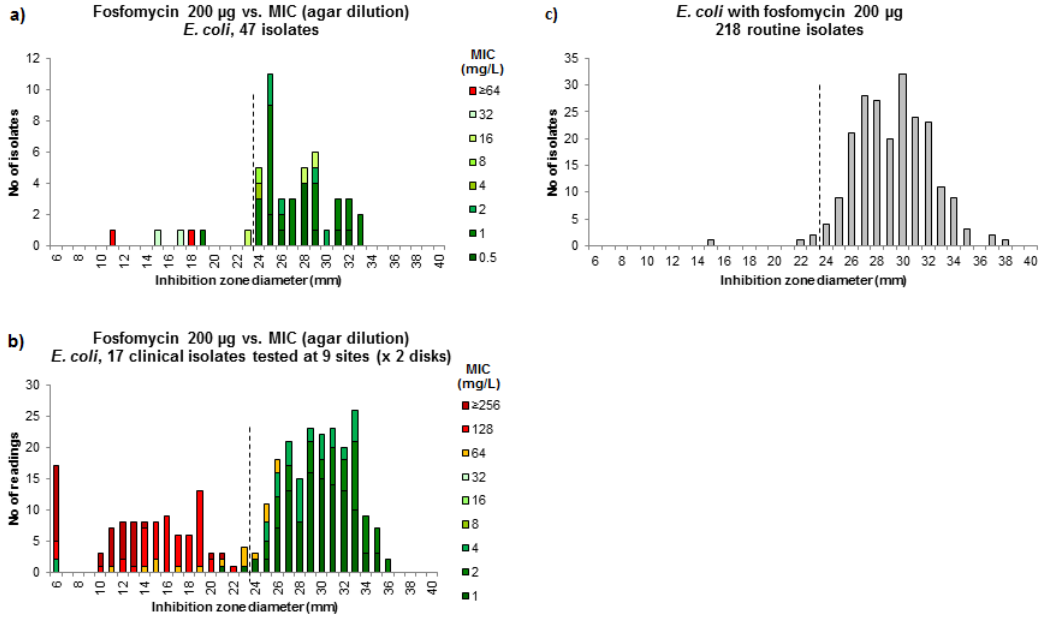

Figure 1. Inhibition zone diameter distributions for *E. coli* and fosfomycin 200 µg (with 50 µg glucose-6-phosphate) when ignoring all isolated colonies within the inhibition zones.
a) 47 isolates tested once with corresponding MICs as different colours of the bars.
b) 17 isolates tested at 9 different laboratories with corresponding MICs as different colours of the bars.
c) Results from routine testing of local isolates of *E. coli*.
The proposed breakpoint is shown as a dotted line.