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Short communication

Fosfomycin, from susceptibility to resistance: Impact of the new guidelines on breakpoints

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ARTICLE INFO

Article history:

Received 27 February 2020

Accepted 8 July 2020

Available online 15 July 2020

Keywords:

Fosfomycin

EUCAST

Urinary tract infection

Klebsiella spp.

ABSTRACT

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The fosfomycin breakpoint using the disc diffusion method (DDM) changed in the 2019 CA-SFM/EUCAST guidelines v2 (24 mm versus 19 mm). We assessed its impact on categorization of Enterobacterales recovered from urine samples in emergency departments. A total of 7749 and 2348 strains were tested using the DDM and the broth microdilution method (BMD), respectively. The DDM with the 19-mm breakpoint was in accordance with the BMD. Using the 24-mm breakpoint, the overall rate of fosfomycin resistance in Enterobacterales increased by three-fold (5.6% vs 18.1%, $P < 0.01$) and reached 2.8% and 86.5% in *E. coli* and *K. pneumoniae*, respectively. French guidelines for the management of community-acquired UTI remain appropriate. The accuracy of the methods for routine fosfomycin susceptibility testing should be assessed. The role of fosfomycin in the treatment of documented CA-UTI due to Enterobacterales other than *E. coli* should be evaluated considering its rate of resistance and recent data reporting low accuracy.

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1. Introduction

Successive versions of the CA-SFM/EUCAST guidelines (Antibiogram committee of the French society of microbiology/European committee on antimicrobial susceptibility testing) introduced several changes over the past years. Breakpoints for new molecules (e.g., ceftolozane–tazobactam, ceftazidime–avibactam, dalbavancin) were rapidly integrated prior to their availability, allowing clinical laboratories to anticipate their therapeutic use. Other changes were implemented as consequences of the emergence of resistance mechanisms (e.g., carbapenemase detection in Enterobacterales), or better knowledge or detection of resistance mechanisms (e.g., linezolid and *Staphylococcus aureus*, glycopeptides and *Enterococcus* spp.).

In harmonization with the EUCAST guidelines, the 2019 CA-SFM/EUCAST guidelines v2 introduced a modification in the fosfomycin breakpoint for Enterobacterales using the disc diffusion method (DDM) (24 mm versus 19 mm previously), while the MIC breakpoint remained unchanged (32 µg/mL) [1–3]. This change could affect the rate of Enterobacterales reported as susceptible to fosfomycin using the DDM and consequently the role of the molecule in the empirical treatment of community-acquired urinary tract infections (CA-UTI).

We aimed to assess the impact of the updated breakpoint for fosfomycin on the categorization of Enterobacterales clinical strains collected from urine samples. This study, part of the GMC12 project, was carried out by the GMC study group.

2. Materials and methods

Six French clinical laboratories were enrolled in the study. All Enterobacterales isolates collected from adult urine samples in emergency departments, between September 1, 2017 and August 31, 2018, were included. A single strain exhibiting the same antibiotic susceptibility profile was included per patient. For all strains, demographic data (age, sex), inhibition diameter of fosfomycin, resistance to at least a third-generation cephalosporin (3GC-R), and mechanism of resistance to 3GC were recorded. Bacterial identification was performed using conventional biochemical methods (e.g., VITEK 2) or MALDI-TOF mass spectrometry as recommended by the manufacturers.

Antibiotic susceptibility testing (AST) was performed using the DDM or BMD methods in three different centers. The DDM was performed according to the 2019 CA-SFM/EUCAST guidelines v1 and interpreted using the breakpoints of 19 mm (2019 CA-SFM/EUCAST guidelines v1) and 24 mm (2019 CA-SFM/EUCAST guidelines v2) [1,2]. The BMD was performed using Vitek2 (BioMérieux,

Marcy-l'Étoile, France) or Microscan (Beckman coulter, Brea, California, United States) systems as recommended by the manufacturers and interpreted using the breakpoint of 32 mg/L (2019 CA-SFM/EUCAST guidelines v2) [2]. A phenotypic-based approach was used to distinguish ESBL-producing Enterobacterales (ESBL-PE) and cephalosporinase-overproducing Enterobacterales (COPE) as previously described [2]. Statistical analysis was performed using the Chi² test and the IBM SPSS Statistics version 20.0.0 (IBM Corp., Armonk, NY, USA).

3. Results

Overall, 7749 and 2348 strains were included in the DDM and BMD groups, respectively. *E. coli* accounted for 71.7% and 79.4% of all tested strains in the DDM and BMD groups, respectively. In both groups, ESBL-PE accounted for 9.0% and 7.7% of the strains, respectively.

Using the DDM, the median inhibition diameter of fosfomycin was 20 mm [19–22] in *K. pneumoniae*, 23 mm [20–27] in *E. cloacae* complex, 30 mm [28–33] in *E. coli*, 31 mm [29–35] in *C. koseri*, and 34 mm [28–37] in *P. mirabilis* (Fig. 1). Considering the 19-mm breakpoint, the rates of fosfomycin resistance were 5.6%, 0.9%, and 21.7% for overall, *E. coli*, and *K. pneumoniae* strains, respectively (Table 1). The reported rates of fosfomycin resistance were not significantly different between the DDM using the 19-mm breakpoint and the BMD groups for overall strains and each species (Table 1).

In comparison to the 19-mm breakpoint, using the 24-mm breakpoint led to a significant increase in the overall reported rate of fosfomycin resistance (18.1% vs 5.6%, $P < 0.01$) (Fig. 2). A significant increase (about three-fold) in the reported rate of fosfomycin resistance was observed for each species ($P < 0.01$). It increased from 0.9% to 2.8% in *E. coli*, from 10.1% to 17.0% in *P. mirabilis*, and from 21.7% to 86.5% in *K. pneumoniae* ($P < 0.01$). In ESBL-PE, this rate reached 33.1% ($P < 0.01$). The rate of fosfomycin resistance for strains collected from urine samples of women increased from 4.7% to 15.5% ($P < 0.01$).

Considering *E. coli*, 5340 and 1865 strains were included in the DDM and BMD groups, respectively. Of them, 7.5% and 7.7% were ESBL-PE, respectively. Considering the strains collected from urine samples of women, the rate of fosfomycin resistance increased from 1.0% to 3.0% ($P < 0.01$) when using the breakpoint of 24 mm in comparison to the 19-mm breakpoint (Fig. 2). Overall, 5.1% of the ESBL-PE were resistant to fosfomycin using the 24-mm breakpoint.

4. Discussion

The harmonization of the fosfomycin breakpoint between the CA-SFM/EUCAST and the EUCAST guidelines resulted in a strong

Fig. 1. Distribution of the inhibition zone diameter of fosfomycin among five species of Enterobacterales recovered from adult urinary samples in emergency departments: a: *E. coli* and *K. pneumoniae*; b: *P. mirabilis*, *E. cloacae* complex, and *C. koseri*.

Table 1

Rate of fosfomycin resistance according to the DDM (19-mm breakpoint) and to the microdilution breakpoint.

	DDM 19-mm breakpoint		Microdilution		P
	Nb. of strains	Nb. of resistant strain (%)	Nb. of strains	Nb. of resistant strain (%)	
Overall	7449	414 (5.6)	2348	115 (4.9)	0.21
Bacterial species					
<i>E. coli</i>	5340	47 (0.9)	1865	16 (0.9)	0.91
<i>Klebsiella</i> spp.	1080	224 (20.7)	257	57 (22.2)	0.61
<i>K. pneumoniae</i>	869	189 (21.7)	186	41 (22.0)	0.64
<i>K. aerogenes</i>	89	16 (18.0)	30	8 (26.7)	0.30
<i>K. oxytoca</i>	100	16 (16.0)	34	7 (20.6)	0.54
<i>K. variicola</i>	22	3 (13.6)	6	1 (16.7)	0.85
<i>P. mirabilis</i>	405	41 (10.1)	75	6 (8.0)	0.57
<i>C. koserii</i>	180	2 (1.1)	42	1 (2.4)	0.52
<i>E. cloacae</i> complex	230	29 (12.6)	43	7 (16.3)	0.51
<i>M. morgani</i>	84	57 (67.9)	27	27 (100)	0.03
Susceptibility to 3GC					
3GC-S	6625	315 (4.8)	2109	75 (3.6)	0.02
ESBL-PE	673	71 (10.5)	181	28 (15.5)	0.07
COPE	151	25 (16.6)	58	12 (20.7)	0.48

DDM: disc diffusion method; Nb.: number; 3GC: third-generation cephalosporin; ESBL-PE: ESBL-producing Enterobacterales; COPE: cephalosporinase-overproducing Enterobacterales. Statistically significant difference ($P < 0.05$) are in bold.

Fig. 2. Rate of fosfomycin resistance according to the breakpoints of 19 and 24 mm: a: according to Enterobacteriales species, mechanism of third-generation cephalosporin (3GC) resistance, and sex; b: among *E. coli* strains and according to mechanism of 3GC resistance and sex.

increase in the reported rate of fosfomycin resistance among Enterobacteriales strains recovered from adult urine samples in the emergency departments. However, *E. coli* isolates, including ESBL-PE, remained highly susceptible to fosfomycin. The overall rate of fosfomycin resistance was still below the 20% threshold of risk levels retained by the French guidelines for the empirical treatment of uncomplicated cystitis [4]. Furthermore, the rate of fosfomycin resistance in Enterobacteriales causing CA-UTI was likely overestimated as some strains might have been collected from healthcare-associated infections and some might

be associated to urinary tract colonization. However, using the BMD and the DDM with the 19-mm breakpoint, the 1% rate of fosfomycin resistance in *E. coli* recovered here was comparable to previous reports focusing on uncomplicated cystitis in women and to previous epidemiological findings [5,6].

The EUCAST epidemiological cut-off (ecoff) for fosfomycin in Enterobacteriales is only available for *E. coli* and *P. mirabilis* species [7]. However, as reflected by the distribution of the inhibition zone diameter found here, the MIC₅₀ distribution of fosfomycin in *Klebsiella* spp. and *Enterobacter* spp. appears heterogeneous among

reports but higher than in *E. coli* [8–10]. The MIC₅₀ distributions among *Klebsiella* spp. could be related to a high rate of acquired-resistance or a baseline intrinsic resistance within this genus as suggested by the *in vitro* model of bladder infection and pharmacodynamics analyses [11–13]. Furthermore, in a retrospective cohort of UTI treated with fosfomycin in a British teaching hospital, *Klebsiella* spp. infections were more likely than *E. coli* to fail treatment and more likely to be reported as fosfomycin-resistant in cases of relapse following treatment [14]. Consequently, a limited activity of fosfomycin against *K. pneumoniae* causing UTI was suggested [11–14].

Considering these findings, the 24-mm breakpoint is therefore more accurate for detecting fosfomycin resistance than the 19-mm one using the DDM. Indeed, an inhibition zone diameter below 24 mm was correlated with a higher risk of high-level resistance and regrowth [11]. However, several Enterobacterales strains (mainly within the *Klebsiella* and *Enterobacter* genus) would be reported susceptible using the BMD but resistant using the DDM and the 24-mm breakpoint. The reference method for fosfomycin susceptibility testing is the agar dilution method (ADM), which is not a good option for routine AST in clinical laboratories as it is laborious and time-consuming [15]. Categorical agreements between DDM or BMD and ADM are low, but DDM seems to be more accurate than BMD [16–20]. The BMD previously showed MIC values higher than those obtained by ADM [15–17]. It is therefore surprising that we found a lower rate of reported resistance to fosfomycin using the BMD in comparison to the DDM with the 24-mm breakpoint. However, these previous studies display a variability in methodology, for instance in terms of breakpoint and use of G6P. As BMD is the base of most automated systems for AST, the reliability of this method should be assessed in comparison to a reference.

Finally, several changes in AST guidelines were implemented over the past years, and further changes are even expected. To improve microbiologists' and physicians' comprehension, we suggest CA-SFM/EUCAST guidelines include a summary list of the major changes with synthetic explanations.

5. Conclusion

The change in fosfomycin breakpoint has affected the reported rates of resistance in Enterobacterales species collected from urine samples in emergency departments without any impact on the empirical treatment of CA-UTI recommended by the French guidelines. However, due to the discrepancies in the rates of reported resistance between the DDM and the BMD methods for fosfomycin susceptibility testing, their reliability should be assessed in comparison to a reference method. Considering the high-level of reported resistance in *Klebsiella* spp. and *Enterobacter* spp. and the recent findings suggesting a low accuracy of fosfomycin for the treatment of UTI due to *Klebsiella* spp., the role of fosfomycin in the treatment of documented CA-UTI due to Enterobacterales other than *E. coli* should be assessed.

Funding

No funding.

Disclosure of interest

The authors declare that they have no competing interest.

Author's contribution

EF conceptualized the study, analyzed the data, and wrote the original article. PL wrote the original article. All authors included strains and approved the final version of the article.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments.

Acknowledgments

Members of the GMC study Group. The authors are grateful of Prof. Marc Vasse (Head of the Department of Clinical Biology, Hôpital Foch, Suresnes), Prof. Raymond Ruimy (Head of the Department of Microbiology, Hôpital Larchet, Nice), Dr Marina Salvucci (Head of the Department of Biology, Hôpital F. Quesnay, Mantes-la-Jolie, France), Prof. J.-M. Pawlotsky (Head of the Department of Microbiology, Hôpital H. Mondor, Créteil, France), Prof. Laurent Mereghetti (Head of the Department of Microbiology, Hôpital Bretonneau, Tours, France), Prof. A. Le Monnier (Head of the Department of Microbiology, Hôpital Paris Saint-Joseph, Paris, France), Dr. N. Fortineau (Head of the Department of Microbiology, Hôpital Bicêtre, Le Kremlin-Bicêtre, France), Prof. V. Cattoir (Head of the Department of Microbiology, Hôpital Pontchaillou, Rennes, France) for supporting this work.

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