Brief Original Article

Tigecycline and intravenous fosfomycin zone breakpoints equivalent to the EUCAST MIC criteria for *Enterobacteriaceae*

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Abstract

Introduction: Tigecycline and intravenous (i.v.) fosfomycin could be alternative therapeutic options for the treatment of carbapenemase-possessing *Enterobacteriaceae* bacterial infections. However, routine laboratories are forced to test these drugs using minimum inhibitory concentration (MIC) methods as zone breakpoints are not available for the disc diffusion technique.

Methodology: Clinical and Laboratory Standards Institute methods for agar dilution and disc diffusion were compared to determine tentative zone breakpoints that best correlate to tigecycline and i.v. fosfomycin MIC breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing. A total of 195 *Enterobacteriaceae* with defined mechanisms of resistance were tested in duplicate assays. Half of the strains were characterized as carbapenemase producers (KPC-2, OXA-48, OXA-163, VIM-1, VIM-2, IMP-8, NDM-1).

Results: Corresponding zone diameters of susceptible ≥15mm, resistant ≤12mm and susceptible ≥17mm, resistant ≤15mm for the 50µg fosfomycin plus 50µg glucose-6-phosphate and 200µg fosfomycin plus 50µg glucose-6-phosphate discs, respectively, allowed categorization of the strains with an acceptable level of error (< 10% minor errors, < 1.5 % major errors, < 1% very major errors and categorical agreement > 90%). For the 15µg tigecycline disc, the best performance was achieved with the corresponding zone diameters of susceptible ≥21mm and resistant ≤16mm, which eliminated the very major and major errors but not the minor errors (34.4%).

Conclusions: Based on these results, tigecycline and fosfomycin can be included in the routine panel of antibiotics for susceptibility testing by disc diffusion to provide fast and reliable information for the selection of treatment alternatives, especially for strains with extreme resistance, as carbapenemase producers.

Key words: tigecylcine; fosfomycin; disc diffusion; KPC; carbapenemase

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Introduction

The emergence of carbapenemase-possessing *Enterobacteriaceae* has resulted in extremely limited therapeutic options. Tigecycline, a broad-spectrum glycylcycline, may be a beneficial and safe adjunctive treatment in the management of life-threatening infections caused by carbapenem-resistant *Klebsiella pneumoniae* [1]. Clinicians have also reconsidered the potential value of old antibiotic compounds as intravenous (i.v.) fosfomycin (disodium salt) in the treatment of multidrug resistant Gram-negative bacterial infections [2].

Updated susceptibility data are vital in guiding the selection of treatment with these agents; however, interpretation of these data depends on the availability of reliable breakpoints. The European Committee on Antimicrobial Susceptibility Testing/European Medicines Agency (EUCAST/EMA) has defined both tigecycline and fosfomycin i.v. MIC breakpoints for *Enterobacteriaceae* as follows: tigecycline; susceptible ≤ 1.0 mg/L and resistant ≥ 4.0 mg/L; fosfomycin i.v.: susceptible ≤ 32 mg/L and resistant ≥ 64 mg/L [3], On the other hand, the Clinical and Laboratory Standards Institute (CLSI) has yet to set values; breakpoints are available only for fosfomycin trometamol, an oral formulation intended for *Escherichia coli* urinary tract infection treatment [4].

Considering that MIC is a time-consuming method, that several automated systems have not yet included tigecycline and fosfomycin, and that the Etest has shown conflicting results [5,6], disc diffusion seems to be the most practical alternative in routine labs to evaluate susceptibility to these agents. EUCAST has defined tigecycline zone breakpoints only for *E. coli* (susceptible ≥ 18mm and resistant ≤

Table 1. Comparison of categorical agreement and errors using different disc zone breakpoints

Disc	Breakpoint	Source	CA(%)	VME(%)	MaE(%)	MiE(%)
Tigecycline 15µg	S≥18 R≤14	EUCAST	43.1	4.6	0	52.3
Tigecycline 15µg	S≥21 R≤17	Hope et al. [8]	65.6	0	2.1	32.3
Tigecycline 15µg	**S≥21 R≤16**	**This work**	**65.6**	**0**	**0**	**34.4**
Fosfomycin 200/50µg [a]	S≥14 R≤10[b]	Lu et al. [9]	95.3	4.1	0.6	0
Fosfomycin 200/50µg	S≥14 R≤13[b]	Lu et al. [9]	94.7	4.7	0.6	0
Fosfomycin 200/50µg	S≥16 R≤13[c]	Lu et al. [9]	96.2	2.8	1	0
Fosfomycin 200/50µg	**S≥17 R≤15[d]**	**This work**	**92.3**	**0**	**0.6**	**7.2**
Fosfomycin 50/50µg [e]	**S≥15 R≤12[d]**	**This work**	**90.6**	**0**	**1.1**	**8.3**

CA, categorical agreement; VME, very major errors; MaE, major errors; MiE, minor errors. S, susceptible; R, resistant.
a200µg fosfomycin plus 50µg glucose-6-phosphate, bbreakpoints for Enterobacteriaceae. , c breakpoints for Klebsiella pneumonia, d colonies within the zones of inhibition should not be considered in the zone diameter determination

14 mm) [3]. Unfortunately, *Klebsiella pneumoniae,* which is more likely to develop extreme drug resistance (XDR, *i.e*., resistance to all but two or fewer antimicrobial categories) than *E. coli* [7], is not included in these standards. A recent publication showed that, for *Enterobacteriaceae*, tigecycline zone breakpoints of susceptible ≥ 21mm and resistant ≤ 17mm produced the best agreement with the EUCAST MIC categorization [8]. Fosfomycin i.v. faces a more complex scenario because discs containing different loads (200 µg of fosfomycin plus 50 µg glucose-6-phosphate and 50 µg fosfomycin plus 50 µg glucose-6-phosphate) are available (glucose-6-phosphate is required for induction of the transport system necessary for entry of fosfomycin into bacterial cells). Fosfomycin zone breakpoints corresponding to EUCAST MIC were recently proposed by Lu *et al*. for the 200 µg of fosfomycin disc (susceptible ≥ 14mm and resistant ≤ 10mm or susceptible ≥ 14mm and resistant ≤ 13mm for species of *Enterobacteriaceae*) [9].Thus there is an urgent international need to have tigecycline and fosfomycin cutoffs validated in more than one center, by using strains belonging to a presumably different bacterial population, especially XDR isolates, and establishing zone breakpoint corresponding to EUCAST MIC cutoff values for the 50 µg fosfomycin disc.

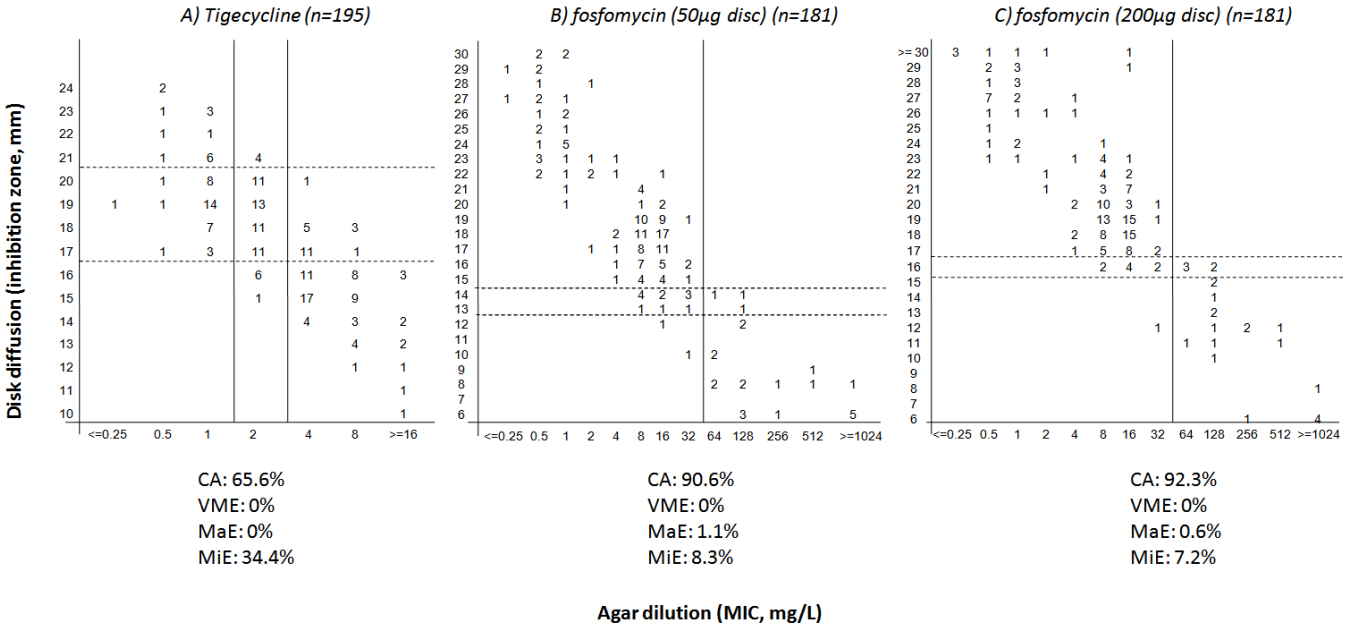
The present study was designed to i) optimize the recently proposed tigecycline and fosfomycin zone breakpoints by challenging them with a panel of XDR strains, and ii) determine fosfomycin i.v zone breakpoints by the CLSI diffusion method for the 50 µg fosfomycin disc that best correlates with the EUCAST MIC breakpoints.

Methodology

A panel of 195 *Enterobacteriaceae* (158 *K. pneumoniae*, 24 *Enterobacter* spp., 6 *Serratia marcescens*, 3 *Citrobacter freundii*, 2 *Klebsiella oxytoca,* 1 *Escherichia coli,* and 1 *Providencia rettgeri*) were tested for tigecycline. A subset of 181 isolates was further evaluated for fosfomycin. The strains were submitted to the National Reference Laboratory because they either had an XDR phenotype (50% of the isolates) or required a tigecycline/fosfomycin MIC confirmation. The resistant mechanisms were characterized by PCR and DNA sequencing and included (n): KPC (70), CTX-M-2 plus porin loss (21), OXA-163 (3), IMP-8 (3), VIM-2 (2), VIM-1 (1), NDM-1 (1), OXA-48-like (1), ESBLs (CTXM-2, PER-2, SHV-2, SHV-5, SHV-18) (76), narrow-spectrum beta-lactamases (6), and wild type isolates (including ATCC quality control strains) (12). The isolates were from clinical sources and they were single isolates from each patient. The strains were recovered from 65 hospitals located in 25 cities from 17 Provinces (2007-2010 period).

The MIC of fosfomycin (Sigma) and tigecylcine (Pfizer) were determined by the agar dilution method using Mueller-Hinton medium (Difco, Becton-Dickinson) according to CLSI recommendations [10]. For fosfomycin, the agar was supplemented with 25 mg/L of glucose-6-phosphate (Sigma) [4]. Simultaneously, 15 µg tigecycline (Oxoid), 200 µg fosfomycin plus 50 µg glucose-6-phosphate (Becton-Dickinson) and 50 µg fosfomycin plus 50 µg glucose-

Figure 1. Scattergrams showing antimicrobial susceptibility results obtained with disc diffusion (in mm) and agar dilution



6-phosphate (Oxoid) discs were tested following the method outlined by CLSI using Mueller-Hinton medium (Difco, Becton-Dickinson) [11]. Strains were tested in duplicate assays and the average result was recorded.

Results and discussion

The results obtained by challenging the panel of strains with different breakpoints are shown in Table 1. By error minimization analysis we did not find a tigecycline zone breakpoint that could reduce errors to acceptable levels (< 10% minor errors –MiE-, < 1.5% major errors-MaE-, < 1% very major errors-VME- and categorical agreement-CA- > 90%). Tigecycline EUCAST zone breakpoints for *E. coli* led to unacceptable levels of errors. When we challenged the panel of strains included in this study with the zone breakpoints recommended by Hope *et al*. [8], we observed a larger amount of MaE (2.1%) and MiE (32.3%) with respect to those previously reported by this author (CA 75.5%, VME 0.6%, MaE 0.6%, MiE 23.3%). These observed differences could be due to the different brands of Mueller-Hinton agar used. Even so, this zone breakpoint was one of the two cutoff values that displayed the best performance. We found an alternative zone breakpoint of susceptible ≥ 21 mm and resistant ≤ 16 mm that showed a similar number of total errors but eliminated the MaE (Figure 1 and Table 1). As the elimination of a potential agent for treatment may be critical for XDR strains, the reduction of MaE errors was prioritized in this study; thus this breakpoint was further selected. Clinical labs using disc diffusion on a daily basis could properly categorize true tigecycline susceptible and resistant strains by using a zone breakpoint of susceptible ≥ 21 mm and resistant ≤ 16 mm (VME and MaE 0%) (Mueller-Hinton agar from Difco, BD, New Jersey, US). Strains with halos between 17-20 mm could fall in any of the three MIC categories, although most of the isolates had a trend toward susceptibility by the reference (MIC) method, as has been also observed in other study using the BSAC method [12]. We observed in this series that 40% of strains with intermediate halos showed susceptibility by MIC, and only 7% resulted true resistant. Thus strains with halos of 17-20 mm must be confirmed by MIC before removing tigecycline prematurely as a therapeutic option.

As panels can introduce bias, we analyzed the impact of the selected tigecycline zone breakpoints in a clinical scenario by examining the halos distribution of nosocomial *Enterobacteriaceae* obtained by 92 Hospitals (WHONET–Argentina Network; n = 5605, year 2009-2010). In this setting, the actual number of isolates that will require a definition by MIC (halos 17-20 mm) was 34%, not very different from that observed for the panel, while the remaining 66% will be properly classified as true

susceptible (halos ≥ 21 mm, 63% of the strains) or true resistant (halos ≤ 16 mm, 3%).

For the 200 µg fosfomycin disc, when we challenged the panel of strains included in this study with the zone breakpoints recommended by Lu *et al*. [8], we observed a similar amount of VME (4.1%) to those previously reported by this author (VME 3.7%) (Table 1). For this disc load, we found an alternative zone breakpoint of susceptible ≥ 17 mm and resistant ≤ 15 mm that reduced VME to an acceptable level (0%) (Figure 1 and Table 1). On the other hand, fosfomycin 50 µg disc zone breakpoints of susceptible ≥ 15 mm and resistant ≤ 12 mm produced the best agreement with EUCAST MIC (i.v. formulation) categorization (Figure 1 and Table 1). Proper interpretation of the fosfomycin breakpoints proposed here requires that colonies within the zones of inhibition should not be considered in the zone diameter determination. Based on our results, the disc diffusion method, regardless of the disc load (50 µg or 200 µg), resulted highly reliable for MIC categorization with EUCAST breakpoints for i.v. use. In summary, for fosfomycin i.v., targeting optimal therapy can be done with the CLSI disc method using the zone breakpoints proposed here with no need to confirm target isolates using an MIC method.

This paper presents some limitations that deserve mention: i) a high proportion of *Klebsiella* isolates were included in this study. As these bacteria very frequently tend to become XDR, it is likely that the current number of bacterial species that require a tigecycline and fosfomycin test in the clinical laboratory will be quite similar; ii) the cutoff values were obtained with Mueller-Hinton agar from Difco. It was recently reported that different Mueller-Hinton agars could affect tigecycline categorization among *Enterobacteriaceae* [13]. Although limited by the low number of strains tested, Difco media showed a trend toward larger inhibition zones in the report by Torrico *et al*. [13]. Thus users of Mueller-Hinton agar other than Difco can report with high confidence those strains categorized as susceptible with the zone breakpoints proposed in this study.

Based on the results of this work, tigecycline and fosfomycin can be included in the routine panel of antibiotics for susceptibility testing by disc diffusion to provide fast and reliable information for the selection of treatment alternatives for XDR strains.

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