Comparison of in-vitro susceptibility of Fosfomycin against drug resistant uropathogens by various susceptibility testing methods.

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Abstract

Urinary tract infections are one of the most common bacterial infections causing high morbidity and mortality. Alarming rise of multidrug resistant uropathogens worldwide, forced the clinician to rethink about the old drugs like Fosfomycin for its therapeutic management. Our objective was to compare agar dilution (AD), disc diffusion (DD) and E test method for antimicrobial susceptibility testing (AST) of Fosfomycin against different drug resistant uropathogens. Consecutive 181 uropathogens were tested for Fosfomycin AST using AD, DD and E test. Results were interpreted using Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. *Escherichia coli* was found as the most common (62.4%) uropathogen followed by *Klebsiella* spp. (21%). Considering agar dilution as gold standard, 6.1% of isolates were resistant to Fosfomycin. Following CLSI breakpoints, the susceptibility of *E.coli*, *Klebsiella* spp., other *Enterobacterales* and *Pseudomonas aeruginosa* were 92.9%, 92.1%, 100 %, 100%; whereas using EUCAST breakpoints the susceptibility rates were 85.7%, 86.9%, 92.9%, and 100%, respectively. The essential agreement, categorical agreement, major error, and very major error for E-test/ DD for all the organisms were 91.2%/Not Applicable, 95%/93.9%, 1.8%/4.7%, 9.1%/9.1%, respectively. This result supports very low resistance of *Enterobacterales* against Fosfomycin; hence should be considered as a valuable option to treat multidrug resistant uropathogens. DD observed to be a convenient method for Fosfomycin AST comparing AD.

Introduction

Urinary tract infection (UTI) is one of the most frequent bacterial infection in both hospital and community settings, causing significant morbidity and economic burden (Foxman et al. 2003; Foxman 2002; Griebing 2005). *Escherichia coli* (*E.coli*) is the most common uropathogen accounting for 70–90% followed by other *Enterobacterales* (e.g., *Klebsiella pneumoniae*, *Proteus mirabilis*, etc.), non-fermenters (*Pseudomonas aeruginosa*, *Acinetobacter* spp.) and Gram-positive cocci (GPC) (e.g., *Enterococcus* spp.) etc. (Foxman 2014; Gupta et al. 2011). Alarming rise of multidrug resistance (MDR), especially the carbapenem-resistant Gram-negative bacilli (GNB), is a pressing concern worldwide. The increasing trend of resistance to many antibiotics and the lack of newer antimicrobials in the pipeline have renewed the interest of clinicians for the usage of Fosfomycin (FP) as a viable alternative (Raz 2012; Perdigao-Neto et al. 2014; Mezzatesta et al. 2017).

FP is derived from phosphonic acid. It inactivates UDP-N- acetylglucosamine-3-enolpyruvyltransferase enzyme, that ligates phosphoenolpyruvate to the 3-hydroxyl group of UDP-N-acetylglucosamine during peptidoglycan synthesis (Falagas et al. 2016). FP is recommended as first-line agent for empirical therapy of uncomplicated UTI (Gupta et al. 2011). It has bactericidal activity against many GNBs e.g. *E. coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp., *Serratia* spp. and GPC e.g., *Enterococcus faecalis* (Falagas et al. 2016). *In-vitro* studies showed adequate FP concentrations in serum, prostate, lungs, inflamed tissues, bone, cerebrospinal fluid, and abscess fluid; hence, it could become a valuable alternative for many of the MDR *Enterobacterales* isolates other than *E.coli* (Demir, Buyukguclu 2017).

CLSI approved disk diffusion (DD) test and agar dilution (AD) methods for *in-vitro* testing of FP against uropathogenic *E. coli* and *Enterococcus faecalis* (*E. faecalis*) (CLSI 2019). EUCAST recommends DD and AD methods for uropathogenic *E. coli* and AD or broth microdilution methods for other members of *Enterobacterales* (EUCAST 2019). On the other hand, E-test always becomes an attractive method over AD or DD being easy to perform and can provide quantitative MIC results.

Hence, this study was aimed to determine FP's activity against the clinically relevant uropathogens other than *E.coli* and *E. faecalis* comparing different AST methods e.g. AD, DD, and E-test.

Materials And Methods

Bacterial Isolate collection

A total of 181 consecutive urinary isolates (173 GNB and 8 GPC) from clinically suspected UTI patients attending a tertiary care hospital over one year were collected and included in this study. The study had been approved by the institutional ethical committee. Species identification was performed using a matrix-assisted laser-desorption ionization time-of-flight mass

spectrometry (MALDI-TOF) (VITEK-MS system, BioMérieux, Marcy-l'Étoile, France). Isolates were stored in -80°C in glycerol until further testing.

Antimicrobial susceptibility testing (AST)

Antibiotic susceptibility testing for all GNBs and GPCs was determined by DD method (Himedia, India) following CLSI guidelines. Standard American type culture collection (ATCC) control strains (*E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853) within acceptable limits were used as quality control strains for the drugs tested. Breakpoints for *E. coli* were used to interpret FP susceptibility for all other GNB in the absence of interpretive criteria. Extended-spectrum lactamase (ESBL), carbapenem resistance (CR), and high-level aminoglycoside resistance (HLAR) were interpreted according to CLSI guidelines (CLSI 2019).

Isolates susceptible to all the tested antibiotics were categorized as pan-sensitive (PS), non-susceptibility to at least one agent in three or more antimicrobial as multidrug-resistant (MDR), non-susceptibility to at least one agent in all but two or fewer antimicrobial categories as extremely drug-resistant (XDR), and resistance to all antimicrobials as pan drug-resistant (PDR). Isolates not fitting to any of the above pattern were categorised as others (Magiorakos et al. 2012).

Fosfomycin (FP) susceptibility

Susceptibility testing of FP was performed by AD, DD, and E-test methods. Results were interpreted applying CLSI and EUCAST breakpoints/MIC. MIC₅₀ and MIC₉₀ for each species were determined by calculating the MIC that would inhibit 50% or 90% of the isolates, respectively. Using CLSI breakpoints (≤ 64 µg /mL: susceptible, 128 mg/L: intermediate, ≥ 256 mg/L: resistant), MICs results of E-test (Himedia, India) were interpreted. For DD test, FP (200µg) (Himedia, India) was used and result was interpreted using CLSI breakpoints (zone diameters ≥ 16 mm: susceptible, 13-15mm: intermediate and ≤ 12 mm: resistance) (CLSI 2019). AST results were also interpreted using EUCAST breakpoints (for AD: <32 mg/L: susceptible, ≥ 32 mg/L: resistant; for DD: ≥ 24 mm: susceptible, < 24 mm: resistant). In case of any disagreement, AD method was used for reporting the test result (EUCAST 2019). ATCC control strains *E. coli* ATCC 25922 and *E. faecalis* ATCC 29212 were used as quality control strains.

Agreement analysis

Using AD as the reference method, agreement rates were calculated for different methods. Essential agreement (EA) was defined as an E-test MIC equal to or within ± 1 dilution of the AD MIC. Categorical agreement (CA) was met when E-test or DD interpretive criteria agreed (susceptible/intermediate/resistant) with AD results. A minor error (mE) was defined as E-test or DD with a susceptible or resistant result when AD result was intermediate or when E-test or DD results were intermediate, and AD was susceptible or resistant. A major error (ME) occurred when E-test or DD results were resistant, and AD was susceptible and was calculated only for susceptible isolates. Very major errors (VME) occurred when E-test or DD results were susceptible, and AD was resistant and was calculated only for resistant isolates (CLSI 2008).

Statistical analysis

Data were analysed using SPSS software v.20.0 (SPSS Inc., Chicago, IL) by χ^2 test. Fisher's exact test was used when data were scarce. Significance was set at $P < 0.05$ using two-sided comparisons. The correlation between E-test and DD against AD was calculated using the Pearson method.

Results

Among the 181 urinary isolates, 151 belonged to *Enterobacterales*. *E. coli* was the most common bacterial isolate [62.4% (113/181)] followed by *Klebsiella spp.* [21% (38/181)], *Pseudomonas aeruginosa* [7.7% (14/181)], *Enterobacter spp.* [2.8% (5/181)], *Citrobacter spp.* [1.1% (2/181)] and *Proteus mirabilis* [0.6% (1/181)]. Among GPCs, *Enterococcus faecium* [2.8% (5/181)] was found commonest followed by *E. faecalis* [1.7% (3/181)].

AST of FP by different methods

Considering AD as a gold standard, more than 85% of isolates were observed susceptible to FP. The results of AD, DD, and E-test following CLSI and EUCAST breakpoints had been shown in the table (Table 1).

The percentage of susceptible, intermediate, and resistant isolates by AD method following CLSI breakpoints was 93.4%, 0.5%, and 6.1%, respectively. Regardless of any of the methods, isolates found more susceptible to FP following CLSI breakpoints than EUCAST breakpoint. (Table 1). Nitrofurantoin resistance was observed much higher than FP (27% against all uropathogens and as high as 50% among *Klebsiella spp.*) On the contrary, resistance to FP was found less than 10% in most of the resistant phenotypes i.e. MDR, XDR, PDR, ESBL, and Carbapenem-resistant GNB (CR-GNB) isolates (Table 2). Isolates of *Enterococcus spp.* with HLAR were observed to exhibit 25% resistance against FP. Colistin had the same MIC₅₀ and MIC₉₀ (0.5 µg/mL) in colistin susceptible strain. The overall MIC₅₀ of 8 µg/mL and MIC₉₀ of 64 µg/mL was observed in AD whereas E-test showed lower MIC₅₀ value of 0.5 µg/mL and MIC₉₀ of 64 µg/mL. The MIC_{50/90} rates of E-test in *Pseudomonas aeruginosa* were similar to gold standard AD, whereas difference between two methods was seen in other organisms with higher MIC₉₀ in E-test (Tab.1).

Agreement analysis by different methods

The rates of EA, CA, mE, ME, and VME were assessed against all the organisms for DD/ E test. Applying CLSI breakpoints, EA, CA, mE, ME, and VME for E-test/DD were found 91.2%/NA, 95%/93.9%, 2.8%/1.1%, 1.8%/4.7% and 9.1%/9.1%, respectively. Using EUCAST breakpoints, CA, ME, and VME for E-test/DD were found 88.9%/80.1%, 6.3%/16.3% and 52.4%/52.4%, respectively.

Performance of the E-test for *Klebsiella spp.* was found good in comparison to *E. coli*, considering the VME result. Similar results were also observed for *Enterococcus spp.* Lastly, isolates of *Pseudomonas aeruginosa* had shown no mE, ME, or VME using CLSI breakpoints of *E.coli* (Table 3).

A Scatter diagram was plotted using the FP AST result of 181 isolates by E-test and DD and compared with AD result.(Fig. 1a, 1b) One hundred out of 142 isolate with MIC ≤16 mg/L by AD showed an excellent correlation with E-test. However, a poor correlation was seen between the results of the E-test and AD result for the isolates with MIC between 64-128 mg/L (Fig. 1a). Comparing the results of DD method with AD, a good categorical agreement was observed for the isolates with MIC ≤64 mg/L. Few isolates (8 out of 142) with MIC ≤ 16 mg/L by AD were classified as resistant by DD (Fig. 1b).

The correlation plot between MIC results of E-test and AD values was drawn for the comparative analysis. Pearson R² value was found 0.6518, which indicates a good correlation between both methods.

Discussion

Emerging drug resistance among GNB with limited therapeutic options is a growing concern worldwide, resulting in increased morbidity, mortality, more extended hospital stay, and higher hospital cost (Foxman et al. 2003; Foxman 2002; Perdigao-Neto et al. 2007; Falagas et al. 2016). With the spread of multidrug resistance, FP will be a potential option to this antimicrobial armamentarium (Falagas et al. 2016; Behera et al. 2018). Currently, the therapeutic indication for FP is limited to *E. coli* and *E. faecalis* from the urinary samples, a subset of microorganisms causing UTIs (CLSI 2019). However, the main interest lies in assessing the *in-vitro* action of FP against infections caused by other microorganisms (Karageorgopoulos et al. 2012; Pasteran et al. 2012). AD is recommended as the gold standard method to determine FP susceptibility, but its use is primarily confined due to complexity to perform the test (CLSI 2019; EUCAST 2019). DD and E-test are easier to perform and take equal time to produce results.

In this study, we determined FP's activity against 181 urinary isolates from patients causing significant bacteriuria. FP displayed consistent activity with overall susceptibility being 93.4% and 88.4% against the tested isolates following both CLSI and EUCAST criteria, respectively. MIC_{50/90} of *E. coli* (8/64 mg/L) by AD method was found lower than MIC_{50/90} of *Enterococcus spp.* (8/256 mg/L). So, the clinical use of FP against *Enterococcus spp.* will be helpful using susceptibility testing. Although few studies demonstrated good activity even in the presence of ESBL genes with MIC₉₀ ranging between 2–4 mg/L, most of Asian isolates exhibited higher MIC up to 128 mg/L, and our study also showed a concordant result with them (Ruiz Ramos, Salavert Lleti 2019).

For other *Enterobacterales* including *Klebsiella* spp., the MIC_{50/90} values were more found distributed i.e. 8/64 mg/L. The increased MIC values against FP among these isolates might be due to the patient population chosen from the hospital setting. Studies on *K. pneumoniae* isolates containing KPC carbapenemases showed 39%-100% susceptibility against FP. FP also found to exhibit activity against strains containing *mcr-1* plasmid for colistin resistance. Hence, the study results were worth looking at FP action against various resistant isolates of *K. pneumoniae* (Falagas et al. 2016; Behera et al. 2018; Jiang et al. 2015; Vardakas et al. 2016). More *in-vitro* studies including both community and hospital isolates are required to validate the breakpoints for *Enterobacterales* other than *E. coli* before the routine usage.

Using breakpoints of *E. coli*, FP susceptibility against *P. aeruginosa* was observed considerably higher than other drugs. In the current study, MIC_{50/90} value for FP ranged lower (8/32 mg/L) using both CLSI and EUCAST breakpoint in contrast to the report by Smith et al. 2020, where it was 64/256 mg/L and 124/256 mg/L, respectively (Smith et al. 2020). FP resistance in *P. aeruginosa* isolates might be due to any of the mechanisms like salvage pathway of peptidoglycan synthesis, presence of *Fos* metalloenzyme, which acted on *MurA* gene to block FP action, or *glpT* mutation, etc. Studies documented different results on the clinical and microbiological cure of UTI after completion of oral therapy (Smith et al. 2020). In the current study, only 22 urinary isolates *P. aeruginosa* were analysed. Hence, studies with a significant number of *P. aeruginosa* isolates targeting different clinical diagnoses need to be planned to establish the validity of these breakpoints and also its effect on clinical outcomes.

Our study found high resistance of Nitrofurantoin among both *E. coli* and *Klebsiella* spp. which was similar to the results shown in other studies also (Ramirez-Castillo et al. 2018; Hirsch et al. 2015).

Susceptibility to FP was ranged from 91.3%-100% among different XDR and PDR GNBs. It highlights the importance of using FP as an alternative therapeutic agent for clinically difficult management to treat infections. Similar results were observed among the isolates with ESBL, CR, and HLAR reiterating the potential use of FP in these infections (Butcu et al. 2011; Mezzatesta et al. 2017).

Our study result showed more discrepancy by DD method in comparison to E-test comparing the various agreement rates. Categorical agreement for DD and E-test was 93.9% and 95%, respectively. Both major (4.7% and 1.8%) and very major errors (9.1% and 9.1%) were more in DD method in comparison to E-test. Although E-test does not have CLSI recommendations, it can be considered a potential alternative to determine quantitative FP MICs in light of the absence of an approved CLSI guideline-based AD method.

It should also be noted that FP did appear to perform well against *P. aeruginosa*, *Klebsiella* spp. and *Enterococcus* spp. (Table 3). Previous investigations have shown poor agreement between agar dilution and E-test for isolates of *P. aeruginosa* and *Enterobacter* spp (Perdigao-Neto et al. 2014). Higher error rates were observed following EUCAST breakpoints than the CLSI. This was probably due to the stringent breakpoints, which was followed up by the EUCAST guidelines. As per our study result, E-test for FP susceptibility testing showed favourable results against *Enterobacterales* and *P. aeruginosa* following CLSI breakpoints.

This study has several limitations. The study included relatively small number of isolates. Further, all the isolates were from a single centre. This might limit application of our findings in other geographical areas due to difference in the mechanism of antimicrobial resistance. Our study result showed good activity of FP against multidrug resistant microorganisms. The current study results can be used in a larger study targeting geographically diverse isolates to validate the results.

Conclusion

In the current study, good activity of FP was observed against urinary *Enterobacterales* and *P. aeruginosa*. *In-vitro* studies to detect antibiotic resistance and comparison of various AST methods are the fundamental before any therapeutic application. Therefore, it is imperative to implement a method that delivers early, easy, less expensive, and reliable results. Here, the gold standard testing method for FP i.e., AD, is not a real option for many clinical microbiology laboratories. As per our study result, alternative methods such as E test and DD provide similar results, which may be acceptable due to low ME and VME rates. Indeed, the best performance of E-test and DD was achieved when interpreted according to the CLSI breakpoints rather than EUCAST. Although our study has limitations, including a small number of isolates and sampling bias for MDR isolates with more

FP susceptible isolates, our results suggest that other phenotypic methods may be useful in the interpretation of FP susceptibility in case of *Enterobacterales* and *P. aeruginosa* using breakpoints as indicated for *E. coli* and *E. faecalis* yielding acceptable and timely results.

Declarations

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Author contribution:

RB : Writing original draft, Formal analysis,

SM: Conceptualization, Project administration, Writing -review & editing

AmK: Supervision, Project administration, Visualization

NA: Data curation

ST: Validation, Methodology

AP: Investigation, Data curation

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SS: Editing

BKD: Review & editing

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References

1. Foxman B, Klemstine KL, Brown PD (2003) Acute pyelonephritis in US hospitals in 1997: hospitalization and in-hospital mortality. *Ann Epidemiol* 13(2):144–150. doi:10.1016/s1047-2797(02)00272-7
2. Foxman B (2002) Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med* 113 Suppl 1A:5S-13S. doi:10.1016/s0002-9343(02)01054-9

3. Griebbling TL (2005) Urologic diseases in America project: trends in resource use for urinary tract infections in women. *J Urol* 173(4):1281–1287. doi:10.1097/01.ju.0000155596.98780.82
4. Foxman B (2014) Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect Dis Clin North Am* 28(1):1–13. doi:10.1016/j.idc.2013.09.003
5. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Moran GJ, Nicolle LE, Raz R, Schaeffer AJ, Soper DE, Infectious Diseases Society of A, European Society for M, Infectious D (2011) International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin Infect Dis* 52 (5):e103-120. doi:10.1093/cid/ciq257
6. Raz R (2012) Fosfomycin: an old–new antibiotic. *Clin Microbiol Infect* 18(1):4–7. doi:10.1111/j.1469-0691.2011.03636.x
7. Perdigao-Neto LV, Oliveira MS, Rizek CF, Carrilho CM, Costa SF, Levin AS (2014) Susceptibility of multiresistant gram-negative bacteria to fosfomycin and performance of different susceptibility testing methods. *Antimicrob Agents Chemother* 58(3):1763–1767. doi:10.1128/AAC.02048-13
8. Mezzatesta ML, La Rosa G, Maugeri G, Zingali T, Caio C, Novelli A, Stefani S (2017) In vitro activity of fosfomycin trometamol and other oral antibiotics against multidrug-resistant uropathogens. *Int J Antimicrob Agents* 49(6):763–766. doi:10.1016/j.ijantimicag.2017.01.020
9. Falagas ME, Vouloumanou EK, Samonis G, Vardakas KZ (2016) Fosfomycin *Clin Microbiol Rev* 29(2):321–347. doi:10.1128/CMR.00068-15
10. Demir T, Buyukguclu T (2017) Fosfomycin: In vitro efficacy against multidrug-resistant isolates beyond urinary isolates. *J Glob Antimicrob Resist* 8:164–168. doi:10.1016/j.jgar.2016.11.011
11. CLSI (2019) Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: approved standard, vol M100. edn. CLSI, Wayne, p 29
12. EUCAST (2019) The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf. 6 January 2019
13. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 18(3):268–281. doi:10.1111/j.1469-0691.2011.03570.x
14. CLSI (2008) Clinical and Laboratory Standards Institute. Development of in-vitro susceptibility testing criteria and quality control parameters; approved guideline third edition, vol M23, 3rd edn. CLSI, Wayne
15. Behera B, Mohanty S, Sahu S, Praharaj AK (2018) In vitro Activity of Fosfomycin against Multidrug-Resistant Urinary and Nonurinary Gram-Negative Isolates. *Indian J Crit Care Med* 22(7):533–536. doi:10.4103/ijccm.IJCCM_67_18
16. Karageorgopoulos DE, Wang R, Yu XH, Falagas ME (2012) Fosfomycin: evaluation of the published evidence on the emergence of antimicrobial resistance in Gram-negative pathogens. *J Antimicrob Chemother* 67(2):255–268. doi:10.1093/jac/dkr466
17. Pasteran F, Lucero C, Rapoport M, Guerriero L, Barreiro I, Alborno E, Veliz O, Corso A (2012) Tigecycline and intravenous fosfomycin zone breakpoints equivalent to the EUCAST MIC criteria for Enterobacteriaceae. *J Infect Dev Ctries* 6(5):452–456. doi:10.3855/jidc.2238
18. Ruiz Ramos J, Salavert Lleti M (2019) Fosfomycin in infections caused by multidrug-resistant Gram-negative pathogens. *Rev Esp Quimioter* 32(Suppl 1):45–54
19. Jiang Y, Shen P, Wei Z, Liu L, He F, Shi K, Wang Y, Wang H, Yu Y (2015) Dissemination of a clone carrying a fosA3-harboring plasmid mediates high fosfomycin resistance rate of KPC-producing *Klebsiella pneumoniae* in China. *Int J Antimicrob Agents* 45(1):66–70. doi:10.1016/j.ijantimicag.2014.08.010
20. Vardakas KZ, Legakis NJ, Triarides N, Falagas ME (2016) Susceptibility of contemporary isolates to fosfomycin: a systematic review of the literature. *Int J Antimicrob Agents* 47(4):269–285. doi:10.1016/j.ijantimicag.2016.02.001

21. Smith EC, Brigman HV, Anderson JC, Emery CL, Bias TE, Bergen PJ, Landersdorfer CB, Hirsch EB (2020) Performance of Four Fosfomycin Susceptibility Testing Methods against an International Collection of Clinical *Pseudomonas aeruginosa* Isolates. J Clin Microbiol 58 (10). doi:10.1128/JCM.01121-20
22. Ramirez-Castillo FY, Moreno-Flores AC, Avelar-Gonzalez FJ, Marquez-Diaz F, Harel J, Guerrero-Barrera AL (2018) An evaluation of multidrug-resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. Ann Clin Microbiol Antimicrob 17(1):34. doi:10.1186/s12941-018-0286-5
23. Hirsch EB, Raux BR, Zucchi PC, Kim Y, McCoy C, Kirby JE, Wright SB, Eliopoulos GM (2015) Activity of fosfomycin and comparison of several susceptibility testing methods against contemporary urine isolates. Int J Antimicrob Agents 46(6):642–647. doi:10.1016/j.ijantimicag.2015.08.012
24. Butcu M, Akcay SS, Inan AS, Aksaray S, Engin DO, Calisici G (2011) In vitro susceptibility of enterococci strains isolated from urine samples to fosfomycin and other antibiotics. J Infect Chemother 17(4):575–578. doi:10.1007/s10156-011-0212-7

Tables

Table 1: Species wise distribution of Fosfomycin MIC_{50/90} along with Fosfomycin and Nitrofurantoin resistance rates.

ism	Fosfomycin MIC _{50/90} (mg/L) (MIC range) (mg/L)		Fosfomycin resistance(%)															NIT resistance(%)
			AD			E-test						DD						
	AD	E-test	CLSI			EUCAST		CLSI			EUCAST		CLSI			EUCAST		
			S	I	R	S	R	S	I	R	S	R	S	I	R	S	R	
113)	8/64(8-512)	0.25/64(0.125-1024)	92.9	0.9	6.2	86.7	13.3	92.9	0.9	6.2	89.4	10.6	92.2	0	8.8	88.5	11.5	14.2
spp. (38)	8/64(8-512)	8/128(0.125-1024)	92.1	0	7.9	86.9	13.1	86.8	5.3	7.9	89.5	10.5	84.2	0	15.8	50	50	50
er rales (7)*	8/16(8-16)	0.125/64(0.125-128)	100	0	0	100	0	87.5	12.5	0	75	25	100	0	0	87.5	12.5	28.6
s spp. (8)	8/256(8-512)	0.125/512(0.125-1024)	87.5	0	12.5	87.5	12.5	87.5	0	12.5	75	25	75	12.5	12.5	62.5	37.5	25*
nonas sa (14)	8/32(8-64)	8/32(0.125-64)	100	0	0	92.9	7.1	100	0	0	92.9	7.1	100	0	0	92.9	7.1	NA*
181)	8/64(8-512)	0.5/64(0.125-1024)	93.3	0.6	6.1	88.4	11.6	91.7	2.2	6.1	88.4	11.6	90	0.6	9.4	79.6	20.4	27.1

**Proteus mirabilis* and *Pseudomonas aeruginosa* were excluded as they are intrinsically resistant to Nitrofurantoin

DD: Disc diffusion, AD: Agar dilution, (NIT) Nitrofurantoin, S: Susceptible, I: Intermediate, R: Resistant, CLSI: Clinical and Laboratory Standards Institute, EUCAST: European Committee on Antimicrobial Susceptibility Testing, MIC: Minimum inhibitory concentration

Table 2: Characterization of urinary isolates into different resistance phenotypes and distribution of FP resistance

Resistance phenotype	Fosfomycin Sensitive (%)*
PS (30)	29(96.7)
MDR (95)	87(91.6)
XDR (23)	21(91.3)
PDR (2)	2(100)
ESBL (137)	128(93.3)
Non-ESBL (36)	35(97.2)
CRGNB (89)	80(89.9)
Non-CRGNB (84)	83(98.8)
HLAR (4)	3(75)
Non-HLAR (4)	3(100)
Others (31)	31 (100)

* Fosfomycin intermediate category is included in Fosfomycin susceptible

Extended-spectrum lactamase (ESBL), Carbapenem-resistant GNB (CRGNB), High-level aminoglycoside resistance (HLAR), Pan-sensitive (PS), Multi-drug resistant (MDR), Extremely drug-resistant (XDR) and Pan-drug-resistant (PDR).

Table 3: *In-vitro* antibacterial activity of FP against 181 urinary isolates using E-test and DD and categorization of errors compared with AD method

Organism	CLSI (%)									EUCAST (%)					
	DD					E-test				DD			E-test		
	CA	mE	ME	VME	EA	CA	mE	ME	VME	CA	ME	VME	CA	ME	VME
<i>E. coli</i> (113)	94.7	0.9	3.8	14.3	91.2	95.6	1.8	1.9	14.3	87.6	6.1	53.3	88.5	5.1	53.3
<i>ebsiella spp.</i> (38)	92.1	0	8.6	0	89.5	92.1	5.3	2.9	0	55.3	47.1	25	94.7	5.9	25
<i>Enterobacterales</i> (8)	100	0	0	0	87.5	87.5	12.5	0	0	87.5	12.5	0	87.5	12.5	0
<i>terococcus spp.</i> (8)	75	12.5	14.3	0	100	100	0	0	0	75	28.6	0	87.5	14.3	0
<i>monas aeruginosa</i> (14)	100	0	0	0	92.9	100	0	0	0	85.7	7.7	100	85.7	7.7	100
Total (181)	93.9	1.1	4.7	9.1	91.2	95	2.8	1.8	9.1	80.1	16.3	52.4	88.9	6.3	52.4

EA: Essential agreement, CA: Categorical agreement, mE: Minor error, ME: Major error, VME: Very major error, DD: Disc diffusion, CLSI: Clinical and Laboratory Standards Institute, EUCAST: European Committee on Antimicrobial Susceptibility Testing

Figures

		AD Fosfomycin MIC (µg/mL)					
		≤16	32	64	128	256	≥512
E-test Fosfomycin MIC (µg/mL)	≤16	130*†	6	7	1	0	0
	32	6	8	2	0	0	0
	64	1	4	0	0	1	0
	128	2	0	0	0	1	1
	256	1	0	0	0	2	1
	≥512	2	0	0	0	2	3

(a)

		AD Fosfomycin MIC (µg/mL)					
		≤16	32	64	128	256	≥512
DD Fosfomycin zone diameter (mm)	≥ 24 mm	120*	14	9	1	0	0
	23mm - 16 mm	14	4	0	0	1	0
	15 mm- 13 mm	0	0	0	0	0	1
	≤ 12 mm	8	0	0	0	5	4

(b)

Figure 1

Correlation of Fosfomycin minimum inhibitory concentration and zone diameters values of E-test and disc diffusion compared to agar dilution

DD: Disc diffusion, AD: Agar dilution, MIC: Minimum inhibitory concentration

Scattergram of FP MICs obtained by (a) MICs obtained by E-test and (b) zone diameters of DD compared with AD for 181 isolates. Essential agreement highlighted in green (perfect agreement) and yellow (± 1 log₂ dilution). The solid horizontal and vertical lines represent the clinical breakpoint value established by EUCAST. The blue, orange and red colour represent the clinical breakpoint value established by CLSI i.e. susceptible, intermediate and resistant respectively. Control strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 are represented by an * and †, respectively.