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Research Article

Fosfomycin Activity against ESBL-MDR Uropathogens

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Abstract

Background: The UTI caused by ESBL producing MDR organisms is considered an issue of great concern. Multidrug resistance results into limited therapeutic options urging the clinicians to reconsider the use of old antibiotics as Fosfomycin. This work aimed at studying the invitro activity of fosfomycin against the ESBL MDR uropathogens.

Methodology: The ESBL production among the MDR urinary isolates was screened by Chrom agar and confirmed using combined disk method (CAZ and CAZ/CAL). The confirmed ESBL producing MDR uropathogens were selected for invitro susceptibility testing against Fosfomycin using Disk diffusion Kirby Bauer method.

Results: The ESBL producing MDR uropathogens were confirmed in 44.4 % among the total collected urinary isolates (472/1063). The Fosfomycin susceptibility testing recorded a sensitivity of 69.1% among the 472 totals tested ESBL MDR isolates where the *E-Coli* showed a high incidence of 93.1%.

Conclusion: The fosfomycin showed a high invitro activity against ESBL producing MDR uropathogens. *E-Coli* showed a significantly higher susceptibility rate to Fosfomycin than other other tested species.

Keywords: Fosfomycin; ESBL; MDR; Uropathogen

Introduction

The ESBL producing pathogens constitute a serious issue worldwide nowadays that cause various infections including UTI [1]. This pattern of resistance is expressed in different Gram negative species where the klebsiella and E-Coli species represent a high proportion [2,3].

Unfortunately, besides B-lactams, the ESBL producing Gram negative uropthogens causing UTI have become associated with resistance to other antimicrobials such as fluoroquinolones, aminoglycosides developing multi-drug resistance [4,5].

The problem of multi-drug resistance is reflected by a negative clinical impact on the patient. The infections caused by these resistant strains is challenging for physicians due to limited treatment options resulting in therapeutic failure with increased complications, prolonged hospital stay and case fatality rate. Many risk factors contribute to developing infections with ESBL producing pathogens including hospitalization, previous UTI, poor diabetic control, urinary catheterization and recent exposure to antibiotics [6-8].

The magnitude of the problem and its clinical adverse effect has raised the concern about searching for proposed solutions to combat the issue of multi-drug resistance. One of the introduced approaches was the revival of old antibiotics which stayed away from the applied field of antimicrobial use for a long period with diminished selective pressure in comparison to the currently used antimicrobials, thus, preserving better activity and better susceptibility rates among the tested organisms. Fosfomycin is one of the old antibiotics that have been reintroduced to the therapeutic field. It inhibits cell wall synthesis and proved to be active against 85% to 100% of MDR uropathogens [9-11]. The majority of the studies have focused on *E-Coli* and there is limited data about the activity of fosfomycin against other MDR uropathogens [12].

The knowledge of the susceptibility rates of fosfomycin will improve its use in empirical therapy as the susceptibility results of this agent are not usually available in routine lab work [13]. Accordingly, the aim of the present study was to assess the invitro activity of fosfomycin against ESBL-producing MDR uropathogens isolated from urinary tract infected patients.

Methodology

This cross sectional study included 1605 Gram negative uropathogens isolated from urine samples of urinary tract infected patients admitted in Cairo University hospitals or patients seeking the outpatient clinic in a period of one year from May 2016 to May 2017.

Identification and susceptibility testing

All Gram negative pathogens were isolated from urinary samples on CLED agar. The identification and species differentiation of the recovered pathogens were reached using conventional biochemical techniques in the form of catalase, oxidase, TSI, LIA, Citrate, urease and MIO. All Gram negative isolates were applied for antibiotic susceptibility testing against a Gram negative panel of antibiotics using the standard disk diffusion Kirby-Bauer method. The selected group of antibiotics were placed over Mueller-Hinton agar plates already streaked by each tested isolate whose inoculum density was adjusted to 0.5 Mc Farland. The plates were then, incubated at 37°C for 16-18 hrs. The zones of inhibition were read and categorized as susceptible, intermediate or resistant according to the interpretative criteria of CLSI 2016 [14]. All antimicrobials were stored according to manufacturers' instructions.

ESBL detection

According to the susceptibility results to different antibiotics MDR Gram negative uropathogens defined as; those conferring resistance to three or more antimicrobial classes were screened for the production of ESBL using Chromogenic media for ESBL (LOT 101116042, Liofilchem, Italy, 2014), then phenotypically confirmed by combined disk method. This method uses cephalosporin/clavulanate combination (CAZ 30 µg/CAL 10 µg) (LOT 082716005, Liofilchem, Italy, 2016) to compare the inhibition zone given by cephalosporin alone (CAZ; 30µg) versus cephalosporin plus clavuanate. The 2 disks were placed 20 mm apart on the surface of the Mueller-Hinton agar streaked with the tested isolate and incubated at 37°C for 16-18hrs. ESBL production was reported on recovering ≥5mm increase in the zone diameter for combined CAZ/CAL disk versus the diameter of individual CAZ when tested alone [14].

Fosfomycin Susceptibility testing

The phenotypically confirmed ESBL producing MDR isolates were targeted for studying their susceptibility to Fosfomycin. Fosfomycin susceptibility was determined by disk diffusion method on Mueller-Hinton agar using fosfomycin disk (Fos; 200µg) (LOT 363730, Mast diagnostics, U.K., 2016). Bacterial suspension with density equivalent to 0.5Mc Farland was prepared to inoculate the Mueller-Hinton plates and Fosfomycin disk was placed. The plates were incubated aerobically at 37°C; for 16-18 hrs. The zone of inhibition around fosfomycin were interpreted as sensitive, intermediate and resistant according to the CLSI 2016 interpretative guidelines [14].

The Quality control measures were followed in this study as a part of the applied routine quality assurance system in the lab which ensures the quality of the whole technical process. This was conducted with the aid of reference Quality Control strains in the form of (*E-Coli* ATCC 25922 as a B-lactamase negative control, Klebsiella Pneumonae ATCC 700603 as an ESBL –positive control.

All the targeted ESBL producing MDR isolates were stored in 20% glycerol trypticase soy broth at -80°C for further lab testing.

Results

In the present study, a sample flow of 6353 urinary samples were delivered to the microbiology lab in the period of 1 year from May 2016 to May 2017, among which 1782 showed significant growth and 1605 urine samples recovered Gram negative uropathogens that were isolated on CLED agar and identified by conventional techniques.

The routine antimicrobial susceptibility testing revealed multidrug resistance in 1063 out of the total 1605 Gram negative urinary isolates implying a prevalence rate of 66.2%. Among the MDR isolates, the ESBL production was confirmed in 472/1063(44.4%) as shown in figure 2. Formerly, the ESBL was screened by Chrom agar that picked 468 ESBL producers with a sensitivity of 99.1%, see figure 1. The confirmed ESBL producing MDR organisms were the selected isolates to proceed for fosfomycin susceptibility testing.

The characterization of the total 472 ESBL producing MDR isolates revealed that the upper hand was for the Klebsiella species 157/472 (39.6%) and E-Coli 146/472 (30.9%) followed by Pseudomonas and Acinetobacter (18.9%) and (8.1%) respectively. The

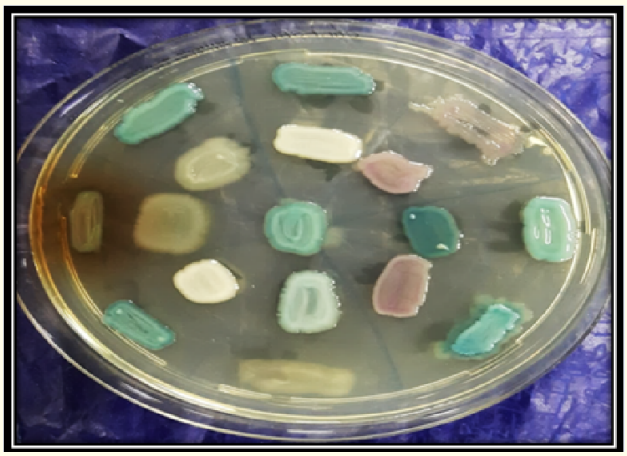

Figure 1: Chrom agar showing different ESBL-producing organisms. *E. coli*; pink to reddish, KEC group (Klebsiella, Enterobacter, and Citrobacter); metallic blue, Pseudomonas; translucent, Acinetobacter; creamy and Proteus; brown halo.

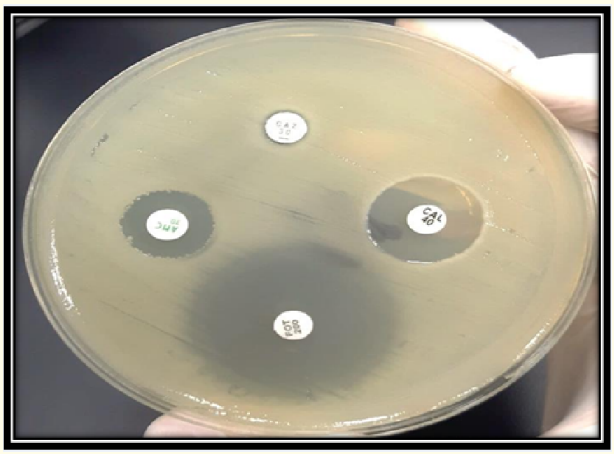

Figure 2: MHA showing positive Cephalosporin/Clavulanate combination disk test.

least recovered species were Proteus, Moragnella and Enterobacter which collectively comprised 2.5% as shown in figure 3.

Regarding the susceptibility results of the 472 ESBL producing MDR isolates against the routinely tested antibiotics, a high susceptibility rate (43.6%) was recorded for Carbapenems, unlike the quinolones which recorded a low susceptibility rate of (5.9%). Concerning Fosfomycin, it achieved the highest susceptibility rate in comparison to the other tested antibiotics as demonstrated in table 1 and figure 4, where 326/472 (69.1%) were interpreted as sensitive, 67/472(14.2%) were intermediately sensitive while 79/472 (16.7%) showed resistance.

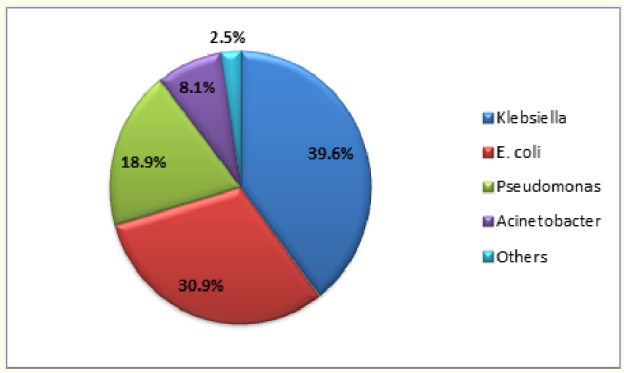

Figure 3: The characterization of isolated species among the ESBL producing MDR uropathogen.

	Number	%
Sensitive	326	69.1
Intermediate	67	14.2
Resistant	79	16.7
Total	472	100

Table 1: Susceptibility rate of fosfomycin among ESBL-producing MDR uropathogens.

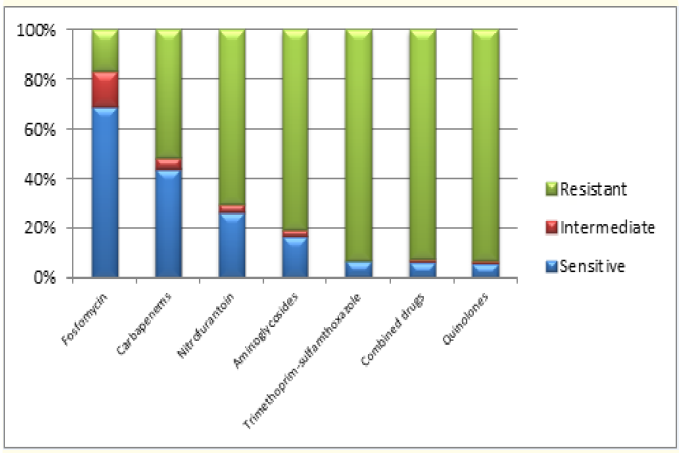

Figure 4: The susceptibility rates of fosfomycin and other antibiotics among the ESBL-producing MDR-uropathogens.

The identification of the species that were susceptible to Fosfomycin showed a statistically significant variation with P value < 0.001. The sensitivity rate was higher among the tested ESBL producing MDR *E-Coli* isolates 136/146 (93.1%) in comparison to other organisms as Klebsiella 126/187 (67.4%), *Pseudomonas* 47/89 (52.8%) and Acinetobacter 10/38 (26.3%) as illustrated in

table 2. Regarding the source of isolates, it was found that the fosfomycin was more active against outpatient isolates. Among the total 111 outpatient isolates, 83 (71%) were susceptible to fosfomycin. However, among 361 inpatient isolates, 243 (67.3%) recorded susceptibility to Fosfomycin as shown in table 3.

	Sensitive N (%)	Intermediate N (%)	Resistant N (%)	Total N (%)	P value
Klebsiella	126 (67.4)	35 (18.7)	26 (13.9)	187 (100)	< 0.001
E. coli	136 (93.1)	3 (2.1)	7 (4.8)	146 (100)	
Pseudomonas	47 (52.8)	20 (22.5)	22 (24.7)	89 (100)	
Acinetobacter	10 (26.3)	7 (18.4)	21 (55.3)	38 (100)	
Enterobacter	5 (71.4)	0 (0.0)	2 (28.6)	7 (100)	
Morganella	1 (100)	0 (0.0)	0 (0.0)	1 (100)	
Providencia	0 (0.0)	0 (0.0)	1 (100)	1 (100)	
Proteus	1 (33.3)	2 (66.7)	0 (0.0)	3 (100)	

Table 2: The susceptibility rate of fosfomycin among different species.

	Sensitive N (%)	Intermediate N (%)	Resistant N (%)	Total N (%)	P value
Inpatients	243 (67.3)	53 (14.7)	65 (18)	361 (100)	0.302
Outpatients	83 (71.0)	40 (15.5)	35 (13.5)	111 (100)	

Table 3: The susceptibility rate of fosfomycin among microorganisms isolated from inpatients and outpatients.

Discussion

The UTI caused by MDR organisms has become an important clinical problem worldwide due to its widespread both in hospitals and in the community and the limited treatment options remaining for such infection [15].

In the present study, the prevalence of MDR among the isolated Gram negative organisms recovered from urine samples was shown to be 66.2% (472/1063). (The multidrug resistance was encountered among 66.2% of the Gram negative organisms isolated from urine samples). This prevalence was consistent with a study conducted by Shrestha., *et al.* 2016 which reported a high percentage of MDR Gram negative uropathogens (55.7%) [16]. On the contrary, another study done by Khawarechareoporn., *et al.* 2013 recognized a relatively lower prevalence of MDR (41%), this can be attributed to various causes that may explain that difference, including the sample size, the spectrum of selected isolates and the variation in the compliance and adherence to antibiotic policies and infection control measures between different institutes from where the samples were collected [17].

According to the conducted study, among the total 1063 MDR Gram negative urinary isolates, the ESBL production accounted for 44.4% in which 472 isolates were confirmed phenotypically as ESBL producers by the combined disk method using CAZ/CAZ-CLAV disks. The ESBL chrom agar was done formerly for screening and picked up 468 ESBL producers implying a sensitivity of 99.1%. The prevalence result of ESBL producing MDR isolates in the present study was in agreement to other studies as those done by Paraguli., *et al.* 2017 and Ranjini., *et al*. 2015 which detected relatively similar prevalence of ESBL production (38.9% and 39.6%) respectively [18,19]. However, a study conducted by Khawarechareoporn., *et al.* 2013 detected a relatively lower prevalence of ESBL MDR Gram negative urinary isolates (24%) [17]. In the mentioned study, the Enterobactereacae was the only included group among the selected isolates, this may explain the difference in the prevalence in comparison to the present study that included a larger spectrum of Gram negatives in which *Pseudomonas* and *Acinetobacter* were represented.

The high sensitivity of the ESBL Chrom agar (99.1%) used for screening was similarly detected in a recent study in Alex, Egypt

which evaluated the ESBL Chrom agar in comparison to other different phenotypic methods in ESBL detection and the Chrom agar showed a high sensitivity 98% [20].

Identification of ESBL producing MDR species revealed that the Klebsiella species were the chief isolates accounting for 39.6% followed by Ecoli 30.9%. These results were similar to those found in Bouassida., *et al*. 2016 and Ramadan., *et al.* 2016 that aimed to detect the prevalence of ESBL producing bacteria in UTI, in which Klebsiella species were the most common isolated ESBL uropathogen of a prevalence 40.5% and *E-Coli* showed a prevalence of32.4% [21,22]. Another recent study done by Fajfr., *et al.* 2017 was conducted on 3295 urine isolates, reported that most of the isolated ESBL producing isolates (n = 374) were klebsiella species (n = 216) [23]. Although, the usually published studies state that the *E-Coli* is the originally leading pathogen for UTI, however, according to the previously mentioned studies, when it comes to the existing pattern of resistance, species type becomes different [8].

The results of susceptibility to the tested antibiotics including Fosfomycin in this study showed that the highest susceptibility was for the Fosfomycin (69.1%) followed by Carbapenems (43.6%) while the least susceptibility was observed among the quinolones. Regarding Fosfomycin, similar results were found in a recent study that included 204 MDR urinary isolates, in which fosfomycin sensitivity reached 78.4% [13]. Another study done by Cho., *et al*. 2015 was conducted on 277 ESBL producing uropathogens, revealed a high susceptibility rate of fosfomycin that was 87.7% [24].

A recent study in Egypt conducted by ElKady, 2017 on 171 Gram negative uropathogens showed that 92.4% (158/171) of the isolates were sensitive to fosfomycin [25]. In another study including 2334 Gram negative bacteria recovered from urinary samples, 92.5% (2160/2334) of the isolates were susceptible to fosfomycin [15]. The higher rates of fosfomycin sensitivity achieved by those studies may be attributed to the different criteria required for the selected isolates regarding their pattern of resistance, additionally, testing the susceptibility to fosfomycin using the MIC method.

According to this present study, the fosfomycin expressed a statistically significant higher sensitivity rate among the *E-coli* species (93.1%) in comparison to Klebsiella species (67.4%) and other tested organisms with a P value < 0.001. A study done by Fajfr., *et al*. in 2017 concluded similar results in which fosfomycin was sensitive against 97% and 80.4% of *E-Coli* and Klebsiella species respectively [23]. This can be explained that the *E-Coli* chromosome is the least harbor of FosA resistance gene, although it can be horizontally transferred by plasmid to *E-Coli* from other species. Consequently, molecular study for the genetic basis of resistance among different species is recommended [26].

In accordance, ElKady, 2017 reported a statistically significant difference between the fosfomycin sensitivity among the E-Coli (95.9%) and *Klebsiella* species (84.4%) with P value 0.01 [25]. Also, in Turkey, there was a significantly higher *in vitro* activity of fosfomycin against *E Coli* strains than other tested strains with a P value < 0.05 [15].

In conclusion, this study ascertained the high prevalence of ESBL-MDR pathogens causing UTI as lately published by many other studies where it recovered a prevalence of 44.4% among which klebsiella was the dominant species (39.6%). The revival of Fosfomycin has been introduced as one of the solutions to combat resistance in uropathogens. This was supported by the results of the present study, that evidenced a high invitro activity of fosfomycin against ESBL producing MDR pathogens with susceptibility 69.1%. The *E-Coli* topped the list by showing significantly higher susceptibility (93.1%) in comparison to other species.

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