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High Susceptibility of Fosfomycin to Uropathogenic *Escherichia coli* Isolated at Tertiary Care Hospital of Nepal

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Authors' contributions

This work was carried out in collaboration between all authors. Author BRT conceived and designed the project. Authors SW and BRK performed the experiments as guided by author BRT. Author SW reviewed the relevant literatures. Author BRT prepared the manuscript for submission. All authors read the final manuscript and provided their approval.

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ABSTRACT

Background: Antibiotic resistance in uropathogens is a worldwide problem. Empirical therapy of urinary tract infection (UTI) is based on the susceptibility patterns of locally isolated bacteria in a given time period. *Escherichia coli* (*E.coli*), the most common pathogen causing UTI has developed resistance against most of the antibiotics for empirical use. Fosfomycin is one of the best antibiotics to treat UTI, however very little information is available about the susceptibility rate of *E. coli* to fosfomycin in Nepal.
Aim: The aim of this study was to determine the fosfomycin susceptibility pattern against uropathogenic *E. coli* isolated from January to June 2016 in a tertiary care hospital of Nepal.
Methods: A total of 242 *E.coli* urinary isolates were included in this study. The isolated organisms were identified by conventional methods. The antimicrobial susceptibility was performed by modified disc diffusion method. Minimum inhibitory concentration (MIC) of fosfomycin was performed by E-test. Extended spectrum β-lactamase (ESBL) and AmpC β-lactamase production was screened and confirmed by double disc synergy test.

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Results: *E. coli* were the most common pathogen isolated and were highly resistant to common antibiotics for oral use such as fluoroquinolones, cephalosporins and cotrimoxazole. However, 98% of *E. coli* isolates were found susceptible to fosfomycin.
Conclusions: *E. coli* urinary isolates revealed a high level of resistance to all the antibiotics tested with the exception of fosfomycin. Fosfomycin showed the highest efficacy against *E. coli* and is the best choice for empirical treatment in Nepal. This study revealed that quinolones, cephalosporins and cotrimoxazole cannot be used for empirical treatment of UTI in Nepal.

Keywords: Urinary tract infection; Escherichia coli; fosfomycin susceptibility; MDR.

1. INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections occurring in both males and females of all ages. *Escherichia coli* is the predominant pathogen both in community and hospital settings accounting for 70-90% of UTI followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus* [1,2]. UTI needs empirical therapy before availability of microbiological results, which is based on the local susceptibility patterns. The Infectious Disease Society of America (IDSA) and European Society for Clinical Microbiology and Infectious Diseases (ESCMID) recommended an empirical antimicrobial regimen for treating acute uncomplicated bacterial cystitis in otherwise healthy adults [3]. Among the recommended antibiotics, several studies showed a substantial increase in antimicrobial resistance of uropathogenic *E.coli* against co-trimoxazole, ciprofloxacin and cephalosporins [4-7]. Globally accelerating antimicrobial resistance by uropathogenic *E. coli* has emphasised the use of fosfomycin to treat UTI [8]. Fosfomycin is a broad spectrum bactericidal antibiotic that interferes with bacterial cell wall synthesis, and has been recommended for the treatment of uncomplicated UTI [9,10]. An oral single dose of 3 g fosfomycin tromethamine reaches peak urinary concentration within 4 hours, and remains high for 24 to 48 hours which is sufficient to inhibit most of the uropathogens [11,12].

Since the implementation of updated guidelines by IDSA and ESCID, use of fosfomycin has increased substantially as the first line agent in the treatment of UTI [9-11]. Antibiotic resistance pattern of uropathogens should be updated periodically to ensure proper empiric treatment of UTI and to avoid the emergence of drug resistance. Little information is available about the susceptibility rate of fosfomycin to uropathogens isolated in hospitals of Nepal. Hence this study is focused on testing the susceptibility pattern of uropathogenic *E. coli* with special reference to minimum inhibitory concentration (MIC) of fosfomycin by E-test.

2. MATERIALS AND METHODS

2.1 Area of Study

The study was conducted during January to June 2016 at Norvic International Hospital, a tertiary care referral hospital located in Kathmandu with state of art facility that provides critical care services.

2.2 Organisms Selection and Processing

A total of 242 *E. coli* strains which were already isolated in routine urine cultures were included in this study. A positive culture was defined as pure growth with colony count $>10^5$ CFU/ml. All bacterial isolates were identified by standard microbiological methods [12]. The antimicrobial susceptibility of *E. coli* isolates was determined by modified Kirby Bauer's disc diffusion method as described in the Clinical and laboratory Standards Institute (CLSI) guidelines [13]. The bacterial inoculum prepared and adjusted to McFarland 0.5 turbidity standards and inoculated on the surface of Muller Hinton agar (MHA) plate using a sterile cotton swab by the lawn culture method. Several antibiotics discs (HiMedia Laboratories Pvt. Limited, India) as shown in Table 1, were tested. Plates were incubated for 18-24 hrs at 37°C., zone of inhibition was observed and the diameter of inhibitory zones was measured in millimeters (mm). The results of the measurement were interpreted as per CLSI guidelines [13]. Multi drug resistance (MDR) was determined according to the guidelines recommended by joint initiative of the European Center for Disease Prevention and Control (ECDC) and the Centers for the Disease Control and Prevention [14]. According to the guidelines, the isolates showing non-susceptibility to at least

one agent in three or more antimicrobial categories were identified as MDR. *E.coli* isolates were also screened for ESBL production by the disc diffusion method. The isolates producing a zone diameter equal to or less than 27mm against cefotaxime (30 µg) or zone diameter equal to or less than 22 mm against ceftazidime (30 µg) were screened as ESBL producers.

Phenotypic confirmatory test for ESBL was performed by double disc synergy test method as described by CLSI guidelines given elsewhere. Pre warmed and dry MHA plate was inoculated with the test organism as described above, *E. coli* ATCC 25922 was used as control strain. A ceftazidime (30 µg) disc and cefotaxime (30µg) disc along with the disc containing ceftazidime-clavulanic acid (30/10 µg) combination and cefotaxime-clavulanicacid (30/10 µg) combination were then placed at 20 to 25 mm apart. Following incubation at 35°C for 16-18 hours, a ≥5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate versus the zone diameter of the agent that was tested alone confirmed the ESBL producer.

In addition, isolates were also screened for AmpC β-lactamase production by disc diffusion method as described by CLSI and Peter-Getzlaff S, et al. The isolates resistant to ceftazidime, cefotaxime and Cefoxitin were screened as AmpC beta-lactamase producers and confirmed by cefoxitin-cloxacillin double disc synergy test (CC-DDS) method. Disks containing 30 µg of cefoxitin and 30 µg of cefoxitin plus 200 µg of cloxacillin were then placed at 20 to 25 mm apart. Following incubation at 37°C for 16-18 hours, a difference in the cefoxitin-cloxacillin inhibition zones minus the cefoxitin alone zones of ≥4 mm was considered indicative for AmpC production.

MIC of fosfomycin was tested by E-test using Ezy MIC™ strip (HiMedia Laboratories Pvt. Limited, India) with fosfomycin gradient concentrations ranging from 0.04 µg/ml to 1024 µg/ml along with 50 µg/ml glucose-6-phosphate. The lawn culture of the test inoculums was prepared on an MHA plate as described above. The Ezy MIC™ strip was placed over the lawn culture and incubated at 37°C for 24 hours. After incubation, the MIC value was noted where the ellipse intersects the MIC scale on the strip. *E. coli* 25922 was used as the control strain.

3. RESULTS

3.1 Type of Bacterial Isolates from Urine Sample

Out of 3456 routine urine cultures, 348 (10.1%) were found to be positive for bacterial infection with significant growth. The most frequently encountered bacterial pathogen was *E. coli*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Enterococcus* spp and *Proteus* spp, *Citrobacter* spp, *Acinetobacter* species, coagulase negative *Staphylococci* (CONS), *Enterobacter* spp and *Staphylococcus aureus* as given in Table 1.

Table 1. Type of bacterial isolates from urine samples

Uropathogens	No. (%)
E. coli	242 (69.5)
Klebsiella pnemoniae	36 (10.3)
Pseudomonas aeruginosa	31 (8.9)
Morganella morganii	12 (3.4)
Enterococcus spp	5 (1.4)
Proteus spp	5 (1.4)
Citrobacter spp	5 (1.4)
Acinetobacter spp	4 (1.1)
CONS	4 (1.1)
Enterobacter spp	3 (0.9)
Staphylococcus aureus	1 (0.3)

Table 1, the most frequently encountered bacterial pathogen was *E. coli*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Morganella. morganii*, *Enterococcus* spp and *Proteus* spp, *Citrobacter* spp, *Acinetobacter* species, coagulase negative *Staphylococci* (CONS), *Enterobacter* spp and *Staphylococcus aureus*

3.2 Antibiotic Susceptibility Pattern of *E. coli*

The highest resistance rate was noted for ampicillin, followed by nalidixic acid, fluoroquinolones, and cephalosporins which is given in Table 2. The greatest numbers of isolates were sensitive to fosfomycin. Very few *E. coli* isolates were fosfomycin resistant (0.8%) and intermediately resistant (1.2%).

Table 2, the highest resistance rate was noted for ampicillin, followed by nalidixic acid, fluoroquinolones, cephalosporins and

cotrimoxazole. The lowest resistance rate was noted to fosfomycin.

Table 2. Antibiotic susceptibility pattern of *E. coli*

Antibiotics used	Sensitive no. (%)
Fosfomycin (200 µg)	238 (98)
Imipenem (10 µg)	229 (94.6)
Amikacin (30 µg)	225(93.0)
Piperacillin-tazobactam (100/10 µg)	224 (92.6)
Cefoperazone-sulbactum (75/10 µg)	223 (92.1)
Nitrofurantoin (300 µg)	216 (89.3)
Gentamicin (10 µg)	208 (86.0)
Meropenem (10 µg)	159 (65.7)
Cefoxitin (30 µg)	155 (64.0)
Co-trimoxazole (1.25/23.75 µg)	133 (55.0)
Ceftriaxone (30 µg)	123 (50.8)
Cefixime (5 µg)	122 (50.4)
Cefotaxime (30 µg)	121 (50.0)
Levofloxacin (5 µg)	119 (49.2)
Ceftazidime (30 µg)	118 (48.8)
Ciprofloxacin (5 µg)	118 (48.8)
Ofloxacin (5 µg)	116 (47.9)
Nalidixic acid (30 µg)	69 (28.5)
Ampicillin	24 (9.9)

3.3 MDR Pattern of *E. coli*

MDR was detected in 221 (91.0%) of *E. coli* isolates. Only 20 (8.3%) *E. coli* were susceptible to all the antibiotics tested.

3.4 ESBL and AmpC Production in *E. coli*

Of the total 242 *E. coli* isolates, 98 (40.5%) were ESBL producers, 76 (31.4%) were AmpC producers and 54 (22.3%) were both ESBL and AmpC producers.

3.5 Fosfomycin MICs for *E. coli*

A total of 237 (97.9%) *E. coli* strains were sensitive to fosfomycin with the MICs ranging from 0.50 - 64 µg/ml, 3 (1.2%) had intermediate susceptibility with MIC value of 128 µg/ml and 2 (0.8%) were fosfomycin resistant with the MIC value of 1024 µg/ml.

4. DISCUSSION

The incidence of urinary tract infections was 10.1% in this study. Higher growth rates were reported in various studies [15,16] in Nepal. However similar rates have been reported by another study [17]. The relatively lower number of uropathogens isolation in our study is probably due to continuous implementation of hospital infection prevention activities. *E. coli* (69.5%) was the most common pathogens isolated in our study. This is in accordance with another study in Nepal [18].

On analyzing the susceptibility pattern, the highest level of sensitivity was observed to fosfomycin followed by imipenem, amikacin, pipracillin-tazobactum, and cefoperazone-sulbactum. A high level of resistance was observed for ampicillin followed by nalidixic acid, quinolones, cephalosporins and cotrimoxazole respectively. Similar results have been quoted by several other studies [19-21]. Fluoroquinolones and cotrimoxazole were suggested as a logical choice for empirical therapy of uncomplicated UTI [22,23]. However, our results showed these agents no longer remain a promising choice against *E. coli* isolates. Furthermore, our results match with another report in this regard [24].

Among the aminoglycosides, amikacin and gentamicin resistance was 7% and 14% respectively; similar finding was also noticed in other studies [25,26]. In addition, nitrofurantoin was observed only in 10.7% of the isolates; similar rates were obtained from the other report [27]. However some studies from Saudi Arabia and Bahrain have reported a high resistance rate to nitrofurantoin by urinary isolates [28,29].

In this study, a high incidence of MDR *E. coli* was observed in 91.0% of the isolates, similar results were reported by another study [30]. Our finding of ESBL producing *E. coli* was 40.50%. It is high compared to that reported by Hussain et al. [31]. However similar findings were reported by other studies [32-34]. Following the standard published protocol [35], we confirmed 31.4% of *E. coli* isolates as AmpC producers. We found 22.3% of *E. coli* isolates as co-producer of both ESBL and AmpC β-lactamase which differs with Sageerabanoo et al. [36] who reported (35.8%) of *E. coli* isolates producing both ESBL and AmpC β-lactamase.

In our study, MIC E-test results showed similar susceptibility patterns as shown by the disc diffusion technique and indicated no reduced susceptibility to fosfomycin. Several studies also reported low resistance rates to fosfomycin by uropathogenic *E. coli* [37,38]. A recent study from Korea by Seo et al. reported 100% susceptibility to fosfomycin in *E. coli* isolated

from community acquired UTI [39]. In addition, other reports also state that fosfomycin is a promising therapeutic option for *E. coli* [40,41]. Fosfomycin is found in a high concentration for a longer period of time in voided urine, and it is effective for the prevention of biofilm formation. Its tolerability and safety are also excellent [42, 43]. In our study, resistance rates to fosfomycin did not differ significantly between MDR, ESBL producers and AmpC producers *E. coli.* Our results are supported by a similar study from Korea by Seo MR et al. that reported fosfomycin resistance did not differ significantly between ESBL producers and non-producers *E. coli*.

Several investigators have recommended fosfomycin a best choice for the treatment of UTI caused by *E. coli* [42,43]. However, plasmid-mediated resistant is reported by CTX-M producing *Escherichia coli* clinical isolates [44], therefore, a regular monitoring of susceptibility pattern to fosfomycin is required.

Since ampicillin and nalidixic acids are no longer recommended for the treatment of UTI, however, we have included in this study, because they simply indicate the extent of antibiotic resistance among the locally detected isolates. Although, imipenem, amikacin, pipracillin-tazobactum and cefoperazone-sulbactum showed the highest susceptibility after fosfomycin; however, they are only available for parenteral use, therefore, not practically applicable for outpatient clinic setting. All other antibiotics which are included in the table 2 can be recommended only depending on laboratory results if they are found susceptible.

In this study, we performed an E-test for the determination of MIC of fosfomycin against *E.coli* isolates. The E-test is very simple and it greatly reduces the time for MIC testing, several reports have validated sensitivity and specificity of E-test [45-47]. E-test has an advantage over agar dilution method which can be easily applied to obtain MIC values of any antibiotics in a routine clinical laboratory setting.

5. CONCLUSION

In this study, *E. coli* clinical isolates revealed high level of resistance to commonly prescribed antibiotics. In addition, a high level of MDR, ESBL and AmpC beta-lactamase production were observed. Fosfomycin showed the highest efficacy against *E. coli* clinical isolates *in vitro*. Fosfomycin can be the best choice for the empirical treatment of UTI caused by *E. coli.* This study revealed a very low effectiveness of quinolones, cephalosporins and cotrimoxazole; therefore, these antibiotics cannot be used for empirical treatment of UTI in Nepal.

ETHICAL CONSIDERATION

In this study, we used already isolated bacteria from routine cultures. We did not include any personal information from the patients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Flores Meireles A, Walker J, Caparon M, Hultgren S. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. Nature Rev Microbiol. 2015;13:269–284.
2. Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi, Arbab-Soleimani N, Khamesipour F. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. Antimicrob Resist Infect Control. 2016;5: 11.
3. Kalpana Gupta Thomas M. Hooton Kurt G. Naber Bjorn Wullt Richard Colgan Loren G. Miller Gregory J. Moran Lindsay E. Nicolle Raul Raz Anthony J. Schaeffer. 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. Clinical Infectious Diseases. 2011;52:e103–e120.
4. Sanchez GV, Master RN, Karlowsky JA, Bordon JM. In vitro antimicrobial resistance of urinary *Escherichia coli* isolates among U.S. outpatients from 2000 to 2010.

Antimicrob Agensts Chemother. 2012;56: 2181–2183.

5. Demir T, Buyukguclu T. Evaluation of the *in vitro* activity of fosfomycin tromethamine against gram-negative bacterial strains recovered from community- and hospital-acquired urinary tract infections in Turkey. Int J Infect Dis. 2013;17:966–970.
6. Pourakbari B, Ferdosian F, Mahmoudi S, Teymuri M, Sabouni F, Heydari H, Ashtiani MTH, Mamishi S. Increase resistant rates and ESBL production between *E. coli* isolates causing urinary tract infection in young patients from Iran. Braz J Microbiol. 2012;43:766–769.
7. Baral P, Neupane S, Marasini B, Ghimire K, Lekhak B, Shrestha B. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. BMC Res Notes. 2012;5:38.
8. Michalopoulos AS, Livaditis IG, Gougoutas V. The revival of fosfomycin. Int J Infect Dis. 2011;15:e732–e739.
9. De Cueto M, Hernandez JR, Lopez-Cerero L, Morillo C, Pascual A. In vitro activity of fosfomycin against extended-spectrum-β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: Comparison of susceptibility testing procedures. J Antimicrob Chemother. 2006;50:368–370.
10. Maraki S, Samonis G, Rafailidis PI, Vouloumanou EK, Mavromanolakis E, Falagas ME. Susceptibility of urinary tract bacteria to fosfomycin. Antimicrob Agents Chemother. 2009;53:4508-4510.
11. Linsenmeyer K, Strymish J, Weir S, Berg G, Brecher S, Gupta K. Activity of fosfomycin against extended-spectrum-β-lactamase-producing uropathogens in patients in the community and hospitalized patients. Antimicrob Agents Chemother. 2016;60:1134–1136.
12. Vandepitte J, et al. Basic laboratory procedures in clinical bacteriology. WHO manual, 2nd ed; 2003.
ISBN 92 4 154545 3.
13. Clinical and laboratory Standards Institute (CLSI): Performance standard for antimicrobial susceptibility testing. Wayne, PA: USA: CLSI: M100-S25; 2016.
14. 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. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18:268–281.
15. Acharya A, Gautam R, Subedee L. Uropathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal. Nepal Med Col J. 2011;13:30-33.
16. Rijal A, Ghimire G, Gautam K, Barakoti A. Antibiotic susceptibility of organisms causing urinary tract infection in patients presenting to a teaching hospital. J Nepal Health Res Counc. 2012;10:24–27.
17. Chander A, Shrestha C. Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* urinary isolates in a tertiary care hospital in Kathmandu, Nepal. BMC Res Notes. 2013;6:487.
18. Maharjan MN, Mandal KP, Sharma KV. Comparative study among the bacterial causes of urinary tract infection in diabetic and non-diabetic patients visiting Alka Hospital, Lalitpur. Ann Clin Med Microbio. 2015;1:1006.
19. Mandal J, Srinivas AN, Buddhapriya D and Subhash CP. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant *Escherichia coli*. Indian J Med Res. 2012;136:842–849.
20. Gupta V, Rani H, Singla N, Kaistha N, Chander J. Determination of extended-spectrum β-lactamases and AmpC production in uropathogenic isolates of *Escherichia coli* and susceptibility to fosfomycin. J Lab Physicians. 2013;5:90–93.
21. Dogru A, Karatoka B, Ergen P, Sen Aydm O, Tukenmez Tigen E. Extended-Spectrum β-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2013;14:933–951.
22. Tice AD. Short-course therapy of acute cystitis: A brief review of therapeutic strategies. J Antimicrob Chemother. 1999; 43:85–93.
23. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Clin Infect Dis. 1999;29:745–758.
24. Kashef N, Esmaeeli David G, Shahbazi S. Antimicrobial susceptibility patterns of

community-acquired uropathogens in Tehran, Iran. J Infect Dev Ctries. 2010;4: 202-206.

25. Shigemura K, Tanaka K, Okada H, Nakano Y, Kinoshita S, Gotoh A, Arakawa S, Fujisawa M. Pathogen occurrence and antimicrobial susceptibility of urinary tract infection cases during a 20-Year Period. (1983- 2002) at a single institution in Japan. Jpn J Infect Dis. 2005;58:303-308.
26. Sultan A, Rizvi M, Khan F, Sami H, Shukla I, Khan H. Increasing antimicrobial resistance among uropathogens: Is fosfomycin the answer? Urol Ann. 2015;7: 26.
27. Habte TM, Dube S, Ismail N, Hoosen AA. Hospital and community isolates of uropathogens at a tertiary hospital in South Africa. S Afr Med J. 2009;99:584-587.
28. Bindayna K, Senok A, Jamsheer A. Extended spectrum beta-lactamase-producing enterobacteriaceae in Bahrain: Prevalence and antibiotic sensitivity pattern. J Infect Public Health. 2009;2: 129–135.
29. Khanfar HS, Bindayna KM, Senok AC, Botta GA. Extended spectrum beta-lactamases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: Trends in the hospital and community settings. J Infect Dev Ctries. 2009;3:295–299.
30. Jadhav S, Hussain A, Devi S, Kumar A, Parveen S, Gandham N, Wieler LH, Ewers C, Ahmed N. Virulence characteristics and genetic affinities of multiple drug resistant uropathogenic *Escherichia coli* from a semi urban locality in India. PLoS One. 2011;6: e18063.
31. Hussain A, Ewers C, Nandanwar N, Guenther S, Jadhav S, Wieler LH and Ahmed N. Multiresistant uropathogenic *Escherichia coli* from a region in india where urinary tract infections are endemic: Genotypic and phenotypic characteristics of sequence type 131 isolates of the CTX-M-15 extended-spectrum-β- lactamase-producing lineage. Antimicrob Agents Chemother. 2012;56:6358–6365.
32. Ali I, Rafaque Z, Ahmed S, Malik S, Dasti JI. Prevalence of multi-drug resistant uropathogenic *Escherichia coli* in potohar region of Pakistan. Asian Pac J Trop Biomed. 2016;6:60–66.
33. Schwaber MJ, Navon-Venezia S, Schwartz D, Carmeli Y. High levels of antimicrobial coresistance among extended-spectrum-beta- lactamase-producing enterobacteriaceae. Antimicrob Agents Chemother. 2005;49:2137–2139.
34. Morosini MI, Garcia-Castillo M, Coque TM, Valverde A, Novias A, Loza E, Baquero F, Canton R. Antibiotic coresistance in extended- spectrum-β-lactamase producing enterobacteriaceae and in vivo activity of tigecycline. Antimicrob Agents Chemother. 2006;50:2695-2699.
35. Peter-Getzlaff S, Polsfuss S, Poledica M, Hombach M, Giger, J, Bottger EC, Zbinden R, Bloemberg GV. Detection of AmpC beta-lactamase in *Escherichia coli*: Comparison of three phenotypic confirmation assays and genetic analysis. J Clin Microbiol. 2011;49:2924–2932.
36. Sageerabanoo S, Malini A, Magaiyarkarasi T, Hemalatha G. Phenotypic detection of extended spectrum β-lactamase and Amp-C β-lactamase producing clinical isolates in a tertiary care hospital: A preliminary study. J Nat Sc Biol Med. 2015;6:383–387.
37. Marchese A, Gualco L, Debbia EA, Schito GC, Schito AM. In vitro activity of fosfomycin against gram-negative urinary pathogens and the biological cost of fosfomycin resistance. Int J Antimicrob Agents. 2003;22:53–59.
38. Tharavichitkul P, Khantawa, Bousoung V. Activity of fosfomycin against extended-spectrum β-lactamase producing *Klebsiella pnumoniae* and *Escherichia coli* in Mahoney Nakorn Chiang Mai hospital. J Infect Dis Antimicrob Agents. 2005;22: 125- 126.
39. Seo MR, Kim SJ, Kim Y, Kim J, Choi TY, Kang JO, Wie SH, Ki M, Cho YK, Lim SK, Lee JS, Kwon KT, Lee H, Cheong HJ, Park DW, Ryu SY, Chung MH, Pai H. Susceptibility of *Escherichia coli* from community-acquired urinary tract infection to fosfomycin, nitrofurantoin, and temocillin in Korea. J Korean Med Sci. 2014;29: 1178–1181.
40. Liu HY, Lin HC, Lin YC, Yu SH, Wu WH, Lee YJ. Antimicrobial susceptibilities of urinary extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* to fosfomycin and nitrofurantoin in a teaching hospital in Taiwan. J Microbiol Immunol Infect. 2011; 44:364–368.
41. Sabharwal ER, Sharma R. Fosfomycin: An alternative therapy for the treatment of UTI amidst escalating antimicrobial resistance. J Clin Diagn Res. 2015;9:6-9.

42. Schito GC. Why fosfomycin trometamol as first line therapy for uncomplicated UTI? Int J Antimicrob Agents. 2003;22:79–83.
43. Neuner EA, Sekeres J, Hall GS, Van Duin D. Experience with fosfomycin for treatment of urinary tract infections due to multidrug-resistant organisms. Antimicrob Agents Chemother. 2012;56:5744–5748.
44. Wachino J, Yamane K, Suzuki S, Kimura K, Arakawa Y. Prevalence of fosfomycin resistance among CTX-M producing *Escherichia coli* clinical isolates in Japan and identification of novel plasmid-mediated fosfomycin-modifying enzymes. Antimicrob Agents Chemother. 2010;54: 3061–3064.
45. Sushma Nachnani, Amalia Scuteri, Michael G. Newman, Alex B. Avanessian, Stacy L. Lomeli. E-Test: A new technique for antimicrobial susceptibility testing for periodontal microorganisms. J Periodontol. 1992;63(7):576-83.
46. Petra Luber, Edda Bartelt, Elke Genschow, Jutta Wagner, Helmut Hahn. Comparison of broth microdilution, E Test, and Agar Dilution. J. Clin. Microbiol. 2003;41: 1062–1068.
47. Abdalhamid B, Hassan H, Itbaileh A, Shorman M. Characterization of carbapenem-resistant *Acinetobacter baumannii* clinical isolates in a tertiary care hospital in Saudi Arabia. New Microbiol. 2014;37(1):65-73.

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