Original Article

In Vitro Efficacy of Six Alternative Antibiotics against Multidrug Resistant *Escherichia Coli* and *Klebsiella Pneumoniae* from Urinary Tract Infections

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Abstract

Introduction: Increasing resistance in *Escherichia coli* and *Klebsiella pneumoniae* to firstline antibiotics makes therapeutic options for urinary tract infections (UTIs) challenging. This study investigated the in vitro efficacies of 6 antibiotics against multidrug resistant (MDR) uropathogens. Materials and Methods: Minimum inhibitory concentrations to ceftibuten, cefpodoxime, fosfomycin, mecillinam, temocillin, and trimethoprim were determined against 155 MDR-isolates of *E. coli* and *K. pneumoniae*. The presence of extended-spectrum beta-lactamases (ESBL) and plasmid-borne AmpC enzymes was determined by phenotypic testing with genotyping performed by multiplex polymerase chain reaction. Results: Temocillin demonstrated highest susceptibility rates for both *E. coli* (95%) and *K. pneumoniae* (95%) when breakpoints for uncomplicated UTIs were applied; however, temocillin susceptibility was substantially lower when "systemic infection" breakpoints were used. Fosfomycin demonstrated the best in vitro efficacy of the orally available agents, with 78% and 69% of *E. coli* and *K. pneumoniae* isolates susceptible, respectively. The next most effective antibiotics were ceftibuten (45%) and mecillinam (32%). ESBL and *ampC* genes were present in 47 (30%) and 59 (38%) isolates. Conclusion: This study demonstrated few oral therapeutic options for MDR-uropathogens, with fosfomycin demonstrating the best in vitro activity.

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Key words: Extended-spectrum beta-lactamases, Fosfomycin, Temocillin, Ceftibuten

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections worldwide. Enteric Gram-negative organisms account for more than 90% of UTIs, of which *Escherichia coli* remains the predominant uropathogen. UTIs also remain as one of the most common indications for antimicrobial prescription.[1] Current guidelines recommend nitrofurantoin and trimethoprim-sulfamethoxazole as firstline antimicrobials for treatment of acute uncomplicated cystitis, with fluoroquinolones and β-lactams listed as alternative options.[1] However, resistance among uropathogens against these prescribed antibiotics is increasing worldwide. Rising antibiotic resistance poses a serious public health threat and therapeutic challenge. As initial treatment of acute uncomplicated cystitis is typically empirical, likelihood of clinical failure overshadows the benefits of a specific empirical drug therapy as population resistance towards the drug increases.[1] This limits therapeutic options and delays appropriate therapy. Reduced susceptibility to oral antibiotics also results in an increased use of broad-spectrum, intravenous antibiotic therapy for treatment of uncomplicated UTIs. Additional hospitalisation increases both individual and national demands for scarce healthcare resources, which emphasises the need to search for alternative agents in the treatment of multidrug resistant (MDR) UTIs. Regional data suggests that antibiotic resistance in uropathogens to recommended firstline antibiotics is prevalent in the Asia Pacific region,[2] while local data from Singapore indicates that resistance to quinolones, trimethoprim-sulfamethoxazole and cephalothin exceeds

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30%.[3] The worldwide spread of broad-spectrum beta-lactamases such as extended-spectrum beta-lactamases (ESBLs) and plasmid-borne AmpC enzymes represent an additional threat to therapeutic options for UTIs.

Alternative and less commonly used oral antibiotics for the treatment of UTIs include third generation cephalosporins, pivmecillinam (in European countries) and fosfomycin; while temocillin has been suggested as a potential intravenous therapeutic option instead of carbapenems or third-generation cephalosporins. Trimethoprim has been used in the United Kingdom as firstline therapy for UTIs, as the absence of the sulphonamide component may reduce the risk of adverse reactions. However, these alternative antibiotics are not commonly used in Singapore and there is no data on local susceptibility. This study was performed to evaluate the in vitro efficacy of these 6 antibiotics uncommonly used in Singapore against MDR uropathogens.

Materials and Methods

This single centre retrospective study consisted of *Escherichia coli* (n=81) and *Klebsiella pneumoniae* (n=74) clinical urinary isolates collected from 2005 to 2013, with routine disc susceptibility testing performed according to guidelines from the Clinical Laboratory Standards Institute (CLSI).[4] Test isolates were selected based solely on the presence of an MDR-phenotype, defined as "resistance to 3 or more antimicrobial classes".[5] Study isolates were nearly all resistant to amoxicillin-clavulanate (87%), ciprofloxacin (97%), cephalexin (95%), cefuroxime (96%) and trimethoprim-sulfamethoxazole (87%), and predominantly resistant to ceftriaxone (52%) and nitrofurantoin (55%). Phenotypic screening for ESBL was performed by the disk approximation method and confirmed by supplemental double-disk testing. Phenotypic screening for AmpC beta-lactamases was performed on cefoxitin-resistant isolates, using a previously described method.[6]

Susceptibility testing to fosfomycin was performed by the reference agar dilution method, as broth dilution methods are not recommended by the CLSI. Doubling dilutions of fosfomycin (testing range, 0.5-256 mg/L) were prepared in Mueller Hinton agar (Becton Dickinson) supplemented with 25 mg/L glucose-6-phosphate (Sigma-Aldrich). Bacterial isolates were inoculated using an inoculum replicator which delivered 104 CFU/10 µl. Susceptibility testing for mecillinam (testing range, 0.06-64 mg/L), ceftibuten (0.06-64 mg/L) and trimethoprim (0.5-32 mg/L) was performed by microbroth dilution according to current CLSI guidelines.[4] Serial dilutions of each antibiotic (Sigma-Aldrich, Singapore) in solution were inoculated into 96-well microtitre trays which were stored at -70°C until required. On the day of testing, a 0.5 McFarland suspension of each isolate was inoculated and trays incubated at 35°C for 18 hours in an ambient air condition. Susceptibility testing for temocillin and cefpodoxime was performed by Etest® (bioMérieux, France), according to manufacturer's guidelines, as the test compounds were not commercially available for preparation of broth dilution. Etest® strips were applied on Mueller Hinton agar inoculated with bacterial suspensions and inhibition endpoints interpreted following 18 hours of incubation at 35°C. Concurrent quality control testing for all test methods was performed according to standard CLSI guidelines. Antibiotic susceptibilities for cefpodoxime, ceftibuten, fosfomycin, mecillinam and trimethoprim were interpreted using current breakpoints from the CLSI.[4] For isolates with detected ESBL or AmpC enzymes, cephalosporin susceptibilities were interpreted as resistant regardless of the tested minimum inhibitory concentration (MIC). In the absence of formal breakpoints for temocillin, susceptibilities were interpreted using MIC breakpoints from the British Society for Antimicrobial Chemotherapy (BSAC),[7] which defines Enterobacteriaceae as susceptible if the MIC is ≤8 mg/L in systemic infections, or ≤32 mg/L in UTIs.

Multiplex polymerase chain reaction (PCR) was performed to characterise *ampC* and ESBL genes. Colonies were emulsified in 100 µl of sterile water, and 5 µl of the suspension was used for PCR. Testing for *bla*SHV, *bla*TEM and *bla*CTX-M and plasmid-borne *ampC* genes was performed by methods previously described.[8,9]

Results

When considering the study isolates as a whole, temocillin and fosfomycin demonstrated the best in vitro efficacy. Temocillin susceptibility was dependent on the breakpoints applied, with 147 (94.9%) isolates susceptible when applying "urinary infection" breakpoints compared with 55 (35.5%) isolates susceptible when "systemic infection" breakpoints were applied. The in vitro efficacy of fosfomycin was lower, with 114 (73.5%) isolates tested as susceptible. Susceptibilities for the 4 other tested antibiotics were less than 50%, with highest resistance to trimethoprim. Of note, resistance to mecillinam was high with only 49 (31.6%) study isolates susceptible.

There were species-specific differences in susceptibility to the tested antibiotics. Most *E. coli* isolates were susceptible to temocillin (n = 77, 95%) based on "urinary infection" breakpoints, but susceptibility was much lower (n = 36, 44.4%) when using "systemic infection" breakpoints (Table 1). The other antibiotics with relatively good in vitro activity against *E. coli* were fosfomycin (n = 63, 77.8%) and ceftibuten (n = 51, 63%), with lower susceptibility to cefpodoxime (n = 38, 46.9%) and mecillinam (n = 40, 49.4%). As seen for *E. coli*, temocillin susceptibilities in *K. pneumoniae* varied depending on the breakpoint chosen, with

Table 1. In Vitro Activities of Tested Antibiotics against *Escherichia coli*

Antibiotic	ESBL/ AmpC*	S (%)	I (%)	MIC 90	MIC Range	Minimum Inhibitory Concentration (mg/L)										
						≤0.25	0.5	1	2	4	8	16	32	64	128	≥256
Temocillin†	+	53 (93%)	n/a	32	4 – 48					3 (5%)	8 (14%)	19 (33%)	23 (40%)	4 (7%)		
	-	17 (100%)	n/a	16	2 – 16				1 (6%)	1 (6%)	6 (35%)	9 (53%)				
Mecillinam	+	6 (11%)	2 (4%)	128	0.25 – 128	1 (2%)		1 (2%)	3 (5%)		3 (5%)	2 (4%)	3 (5%)	9 (16%)	37 (65%)	
	-	3 (18%)	1 (6%)	128	1 – 128			3 (18%)				1 (6%)	1 (6%)		12 (71%)	
Cefpodoxime	+	2 (4%)	0 (%)	>256	0.38 – 512		1 (2%)	1 (2%)			1 (2%)	1 (2%)	1 (2%)	1 (2%)	3 (5%)	1 (2%)
	-	16 (94%)	1 (6%)	2	0.125 – 4	2 (12%)	3 (18%)	5 (29%)	5 (29%)	1 (6%)						
Ceftibuten	+	2 (4%)	4 (7%)	128	0.5 – 128		2 (4%)					4 (7%)	7 (12%)	16 (28%)	28 (49%)	
	-	17 (100%)	0 (%)	1	0.125 – 8	4 (24%)	6 (35%)	1 (6%)			1 (6%)					
Trimethoprim	+	5 (9%)	n/a	64	2 – 64				2 (4%)	3 (5%)		2 (4%)	2 (4%)	48 (84%)		
	-	4 (24%)	n/a	64	0.5 – 64		1 (6%)	1 (6%)	1 (6%)	1 (6%)				13 (76%)		
Fosfomycin	+	38 (67%)	11 (19%)	256	0.5 – 256		1 (2%)		2 (4%)		1 (2%)	4 (7%)	11 (19%)	19 (33%)	11 (19%)	8 (14%)
	-	13 (76%)	1 (6%)	256	2 – 256				1 (6%)			3 (18%)	4 (24%)	5 (29%)	1 (6%)	3 (18%)

I: Intermediate susceptibility; MIC: Minimum inhibitory concentration; S: Susceptible

*+ = present, - = absent.

†Urinary infection breakpoints applied for temocillin.

Table 2. In Vitro Activities of Tested Antibiotics against *Klebsiella pneumoniae*

Antibiotic	ESBL/ AmpC*	S (%)	I (%)	MIC 90	MIC Range	Minimum Inhibitory Concentration (mg/L)										
						≤0.25	0.5	1	2	4	8	16	32	64	128	≥256
Temocillin†	+	53 (93%)	n/a	32	4 – 48					3 (5%)	8 (14%)	19 (33%)	23 (40%)	4 (7%)		
	-	17 (100%)	n/a	16	2 – 16				1 (6%)	1 (6%)	6 (35%)	9 (53%)				
Mecillinam	+	6 (11%)	2 (4%)	128	0.25 – 128	1 (2%)		1 (2%)	3 (5%)		1 (2%)	2 (4%)	3 (5%)	9 (16%)	37 (65%)	
	-	3 (18%)	1 (6%)	128	1 – 128			3 (18%)				1 (6%)	1 (6%)		12 (71%)	
Cefpodoxime	+	2 (4%)	0 (%)	>256	0.38 – 512		1 (2%)	1 (2%)			1 (2%)	1 (2%)	1 (2%)	1 (2%)	3 (5%)	1 (2%)
	-	16 (94%)	1 (6%)	2	0.125 – 4	2 (12%)	3 (18%)	5 (29%)	5 (29%)	1 (6%)						
Ceftibuten	+	2 (4%)	4 (7%)	128	0.5 – 128		2 (4%)					4 (7%)	7 (12%)	16 (28%)	28 (49%)	
	-	17 (100%)	0 (%)	1	0.125 – 8	4 (24%)	6 (35%)	1 (6%)			1 (6%)					
Trimethoprim	+	5 (9%)	n/a	64	2 – 64				2 (4%)	3 (5%)		2 (4%)	2 (4%)	48 (84%)		
	-	4 (24%)	n/a	64	0.5 – 64		1 (6%)	1 (6%)	1 (6%)	1 (6%)				13 (76%)		
Fosfomycin	+	38 (67%)	11 (19%)	256	0.5 – 256		1 (2%)		2 (4%)		1 (2%)	4 (7%)	11 (19%)	19 (33%)	11 (19%)	8 (14%)
	-	13 (76%)	1 (6%)	256	2 – 256				1 (6%)			3 (18%)	4 (24%)	5 (29%)	1 (6%)	3 (18%)

I: Intermediate susceptibility; MIC: Minimum inhibitory concentration; S: Susceptible

*+ = present, - = absent.

†Urinary infection breakpoints applied for temocillin.

94.6% (n = 70) susceptible when using "urinary infection" breakpoints, compared with 25.7% (n = 19) susceptible when using "systemic infection" breakpoints. *K. pneumoniae* isolates were also mostly susceptible to fosfomycin (n = 51, 68.9%), with significantly lower susceptibility to ceftibuten (25.7%), cefpodoxime (24.3%), mecillinam (12.2%) (Table 2). Susceptibility to trimethoprim was equally low for both *E. coli* (n = 64, 13.6%) and *K. pneumoniae* (n = 20, 12.2%).

Fosfomycin MIC testing showed that *E. coli* had very different MIC distributions compared to *K. pneumoniae* (Fig. 1), with the current fosfomycin breakpoint bisecting the normal MIC distribution for *Klebsiella* species. In our study population, current fosfomycin breakpoints would have an increased tendency for intermediate susceptibility results when testing *K. pneumoniae* against this antibiotic. The MIC50 value for temocillin was 12 mg/L for *E. coli* and 16 mg/L for *K. pneumoniae*, which straddles the 8 mg/L and 32 mg/L breakpoints for urinary and systemic infections, respectively. This distribution of temocillin MIC values explains the wide variation of susceptibility depending on whether a "urinary" or "systemic infection" breakpoint was applied.

ESBLs were present in 15 (18.5%) *E. coli* and 32 (43.2%) *K. pneumoniae* isolates. The predominant ESBL genes present were CTX-M, which were detected in 87% of all ESBL-positive isolates of *E. coli* and *K. pneumoniae*. Plasmid-borne *AmpC* genes were present in 20 (24.7%) *E. coli* and 39 (52.7%) *K. pneumoniae* isolates, with CIT-like genes predominantly found in *E. coli* and DHA-like genes predominantly found in *K. pneumoniae*. As expected, MIC values for cephalosporins were significantly elevated for both *E. coli* (Table 1) and *K. pneumoniae* in the presence of either ESBL or AmpC enzymes (Table 2). The presence of either ESBL or AmpC beta-lactamases did not significantly affect overall susceptibilities to fosfomycin, mecillinam or temocillin ($P > 0.05$) (Tables 1 and 2).

Discussion

In this study of MDR *E. coli* and *K. pneumoniae* urinary

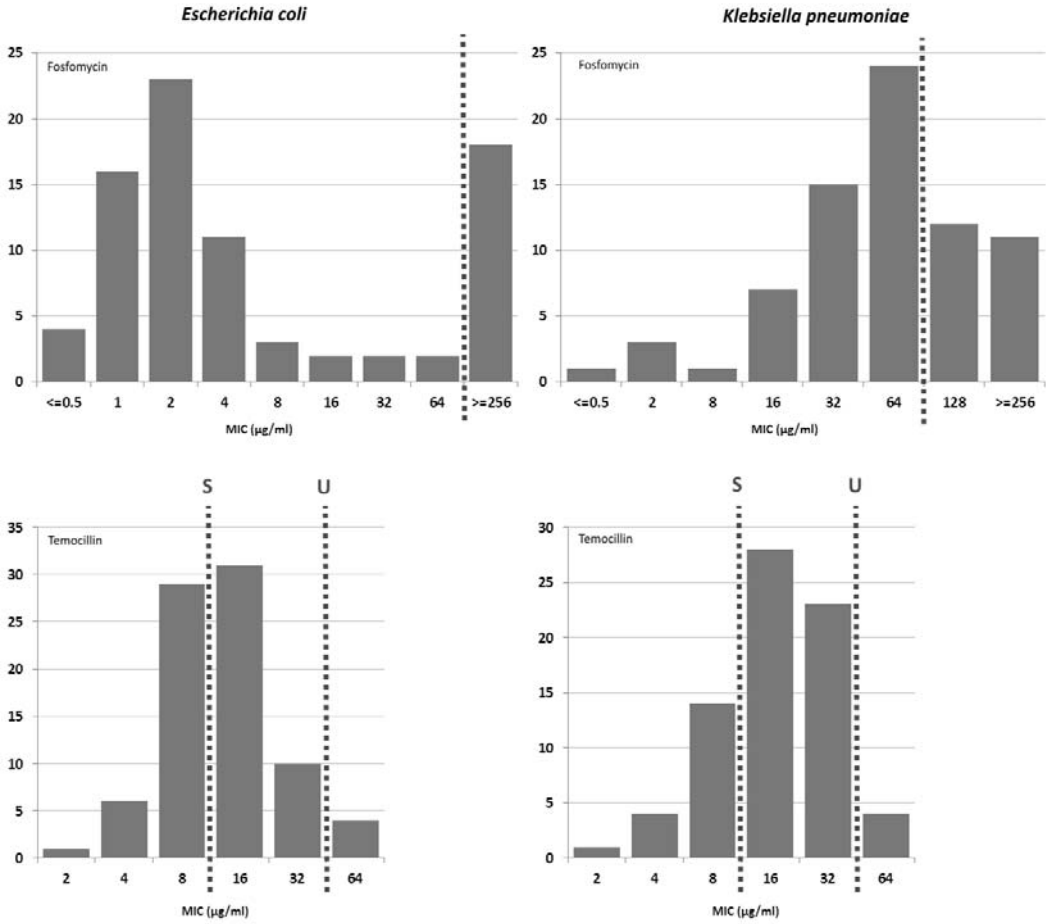

Fig. 1. Charts showing the MIC distributions for fosfomycin and temocillin. Dotted lines show susceptibility breakpoints for fosfomycin (S ≤64, R ≥256) and temocillin (U = "urinary infection" breakpoints, S ≤32; S = "systemic infection" breakpoints S ≤8).

isolates, the oral antibiotic with the best in vitro activity was fosfomycin with susceptibility rates of 77.8% in *E. coli* and 68.9% in *K. pneumoniae*. Temocillin also demonstrated high (>94%) in vitro susceptibility for both *E. coli* and *K. pneumoniae* when the lower "urinary infection" breakpoints were applied. The breakpoints for temocillin fall in the middle of the MIC distributions for *K. pneumoniae* (and to a lesser extent, *E. coli*), which resulted in significantly lower susceptibility rates when "systemic infection" breakpoints were applied. This effect also reduces the reproducibility of susceptibility testing for temocillin against these organisms. Similar temocillin MICs have been reported in other studies on ESBL-producing *E. coli* isolates.[10] In the Singapore context, empirical use of temocillin for MDR-resistant *E. coli* and *Klebsiella* spp. would only be appropriate for the treatment of uncomplicated UTIs, with the added caveat that temocillin is only available for administration via the intravenous route. Both fosfomycin and temocillin remained equally effective in MDR-isolates carrying ESBL or AmpC beta-lactamases.

Ceftibuten is an oral cephalosporin, with enhanced stability against beta-lactamase activity in Enterobacteriaceae. In the absence of either AmpC or ESBL enzymes, 98.5% of urinary isolates were susceptible to ceftibuten, which made this the best alternative antibiotic in strains lacking extended-spectrum cephalosporinases. In contrast, only 46.9% and 24.3% isolates of *E. coli* and *K. pneumoniae* were susceptible to cefpodoxime, with 75.8% susceptibility in isolates without AmpC or ESBL enzymes. A few isolates with ESBL or AmpC enzymes demonstrated in vitro susceptibility to ceftibuten and cefpodoxime, with MIC values in the susceptible range. The clinical extrapolation of these in vitro results to clinical outcomes remains uncertain, but conventional guidance suggests the use of non-cephalosporin agents in ESBL or AmpC-producing strains.

In comparison to other surveys, less than a third of tested isolates were susceptible to mecillinam, even in the absence of ESBL or AmpC enzymes. These results were unexpected because neither temocillin nor pivmecillinam are available in Singapore, and because studies in other geographic regions have shown more promising results. However, in vivo data demonstrates there are multiple genetic pathways that may give rise to mecillinam resistance,[11] and there is one other study from Southeast Asia that similarly reports lower rates of susceptibility to this antibiotic.[12] Additional epidemiological studies would be recommended to adequately assess the extent of mecillinam resistance in this geographic region.

There is a paucity of clinical data for the use of these alternative antibiotics for the treatment of UTIs. The most substantial body of data exists for fosfomycin, both for the treatment of uncomplicated UTI and other UTIs with MDR-organisms,[13] while a study from the United Kingdom reported good clinical and microbiological efficacy for temocillin, provided an optimal dosing regime was used.[14] Several studies reported that ceftibuten demonstrated comparative efficacy to other standard antimicrobials for treatment of lower UTIs, with a few studies also reporting good results for complicated UTIs.[15,16] Pivmecillinam, the oral form of mecillinam, has been widely administered for treatment of acute cystitis in Scandinavian countries for over 20 years, with reports of good safety profile, efficacy and negligible rate of resistance.[17] Despite optimism for mecillinam to be an ideal treatment choice for MDR-pathogens, reports of higher treatment failure rates associated with ESBL-producing Enterobacteriaceae may limit the role of this antibiotic.[18]

Conclusion

This study of alternative antibiotics for MDR-pathogens associated with UTIs has demonstrated few oral therapeutic options. Fosfomycin remains the option with best in vitro activity, while ceftibuten remains an option for Enterobacteriaceae without ESBL and AmpC enzymes. Temocillin may also be considered if an intravenously administered antibiotic is an option, but temocillin susceptibility should be confirmed when treating systemic infections caused by complicated UTI.

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REFERENCES

1. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. 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 2011;52:e103-20.
2. Hsueh PR, Hoban DJ, Carmeli Y, Chen SY, Desikan S, Alejandria M, et al. Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region. J Infect 2011;63:114-23.
3. Bahadin J, Teo SSH, Mathew S. Aetiology of community-acquired urinary tract infection and antimicrobial susceptibility patterns of uropathogens isolated. Singapore Med J 2011;52:415-20.
4. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-third Informational Supplement. CLSI document M100-S23. Wayne, PA: Clinical Laboratory Standards Institute; 2013.

5. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-81.
6. Tan TY, Ng LS, He J, Koh TH, Hsu LY. Evaluation of screening methods to detect plasmid-mediated AmpC in Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis. Antimicrob Agents Chemother 2009;53:146-9.
7. BSAC. BSAC Methods for Antimicrobial Susceptibility Testing. Version 12 May 2013: British Society for Antimicrobial Chemotherapy;2013. Available at: http://bsac.org.uk/wp-content/uploads/2012/02/Version-12-Apr-2013_final.pdf. Accessed on 27 July 2014.
8. Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. APMIS 2007;115:1400-8.
9. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 2002;40:2153-62.
10. Rodriguez-Villalobos H, Malaviolle V, Frankard J, de Mendonça R, Nonhoff C, Struelens MJ. In vitro activity of temocillin against extended spectrum beta-lactamase-producing Escherichia coli. J Antimicrob Chemother 2006;57:771-4.
11. Thulin E, Sundqvist M, Andersson DI. Amdinocillin (Mecillinam) resistance mutations in clinical isolates and laboratory-selected mutants of Escherichia coli. Antimicrob Agents Chemother 2015;59:1718-27.
12. Moore CE, Sona S, Poda S, Putchhat H, Kumar V, Sopheary S, et al. Antimicrobial susceptibility of uropathogens isolated from Cambodian children. Paediatr Int Child Health. 2015:2046905515Y0000000008.
13. Falagas ME, Vouloumanou EK, Togias AG, Karadima M, Kapaskelis AM, Rafailidis PI, et al. Fosfomycin versus other antibiotics for the treatment of cystitis: a meta-analysis of randomized controlled trials. J Antimicrob Chemother 2010;65:1862-77.
14. Balakrishnan I, Awad-El-Kariem FM, Aali A, Kumari P, Mulla R, Tan B, et al. Temocillin use in England: clinical and microbiological efficacies in infections caused by extended-spectrum and/or derepressed AmpC β-lactamase-producing Enterobacteriaceae. J Antimicrob Chemother 2011;66:2628-31.
15. Ho MW, Wang FD, Fung CP, Liu CY. Comparative study of ceftibuten and cefixime in the treatment of complicated urinary tract infections. J Microbiol Immunol Infect 2001;34:185-9.
16. Neuhaus TJ, Berger C, Buechner K, Parvex P, Bischoff G, Goetschel P, et al. Randomised trial of oral versus sequential intravenous/oral cephalosporins in children with pyelonephritis. Eur J Pediatr 2008;167:1037-47.
17. Bjerrum L, Gahrn-Hansen B, Grinsted P. Pivmecillinam versus sulfamethizole for short-term treatment of uncomplicated acute cystitis in general practice: a randomized controlled trial. Scand J Prim Health Care 2009;27:6-11.
18. Soraas A, Sundsfjord A, Jorgensen SB, Liestol K, Jenum PA. High rate of per oral mecillinam treatment failure in community-acquired urinary tract infections caused by ESBL-producing Escherichia coli. PLoS One 2014;9:e85889.