Infect Dis Ther (2015) 4:425–432
DOI 10.1007/s40121-015-0089-3

ORIGINAL RESEARCH

# In Vitro Activity of Oral Cephalosporins (Cefprozil and Cefixime) Against Ciprofloxacin-Resistant Enterobacteriaceae from Community-Acquired Urinary-Tract Infections

Aikaterini Pistiki · Thomas Tsaganos · Irene Galani · Evangelos J. Giamarellos-Bourboulis

To view enhanced content go to www.infectiousdiseases-open.com
Received: August 5, 2015 / Published online: September 21, 2015


## ABSTRACT

***Introduction***: The global emergence of pathogens of urinary-tract infections resistant to ciprofloxacin or producing extended-spectrum β-lactamases (ESBL) led us to investigate the activity of older antimicrobials such as cefprozil and cefixime against a recent broad collection of urine enterobacteria from 2012 and 2013.

***Methods***: Minimum inhibitory concentrations and minimum bactericidal concentrations of cefprozil, cefixime and ciprofloxacin were determined against 293 *Escherichia coli* (40 ESBL producers), 54 *Klebsiella pneumoniae* (10 ESBL producers) and 53 *Proteus mirabilis* isolates.

***Results***: Cefprozil was more active than ciprofloxacin against non-ESBL-producing *E. coli* (93.7% vs 80.2%, $p < 0.0001$); this was not the case for cefixime (85.7% vs 80.2%, $p$: 0.125). Overall, cefprozil and cefixime inhibited 80–90% of ciprofloxacin-resistant isolates of all studied species. However, they were active against less than 20% of ESBL-producing isolates.

***Conclusion***: Results suggest that cefprozil and cefixime remain a good therapeutic alternative against urine enterobacteria particularly in case of ciprofloxacin-resistant pathogens. Their activity against ESBL-producing pathogens is limited.

**Keywords:** Cefprozil; Cefixime; Ciprofloxacin; *Escherichia coli*; Urinary tract

## INTRODUCTION

Urinary-tract infections (UTIs) are the second most common cause of community-acquired infections, with *Escherichia coli* being the most common causative pathogen [1]. The great majority of UTIs are easily manageable, although some patients experience frequent relapses [2]. Development of resistance is a common characteristic of uropathogenic microorganisms in the case of relapse [3].

**Electronic supplementary material** The online version of this article (doi:10.1007/s40121-015-0089-3) contains supplementary material, which is available to authorized users.

A. Pistiki · T. Tsaganos · I. Galani · E. J. Giamarellos-Bourboulis (✉)
4th Department of Internal Medicine, University of Athens, Medical School, Athens, Greece
e-mail: egiamarel@med.uoa.gr

Cefprozil and cefixime are antimicrobials introduced in the market more than two decades ago. Cefprozil is a cephem antimicrobial and cefixime is an orally available third-generation cephalosporin. Since enterobacteriaceae belong to their antimicrobial spectrum, both these antimicrobials are suggested for the management of UTIs [4, 5]. For many years, cefixime was considered an ideal alternative for patients with acute pyelonephritis switching from intravenous to oral therapy [5]. The application of both these agents in the therapeutic armamentarium against UTIs has been abandoned over the years, whereas their activity on urinary pathogens is not reported in studies published over the last 5 years. The emerging resistance of uropathogenic enterobacteriaceae to commonly prescribed antimicrobials [3] led us to conduct the current study to investigate the activity of cefprozil and cefixime against enterobacteriaceae pathogens from patients with community-acquired UTIs.

## METHODS

This was a multicenter study that was conducted during the period November 2012 until April 2013 among patients admitted for urine culture into 10 different microbiology laboratories in Greece. The study was approved by the Ethics Committees of the Prefectures that the labs refer to. Inclusion criteria were: (a) written informed consent; (b) female gender; (c) age $\geq$18 years; (d) at least two of the following symptoms of acute cystitis, i.e., micturition, pain at urination and increased frequency of urination; (e) one Gram-negative isolate grown at quantity $\geq 10^5$ cfu/ml from midstream urine culture; (f) uncomplicated cystitis; and (g) community-acquired UTI. The episode of cystitis was considered uncomplicated for non-pregnant immunocompetent women without signs of urinary obstruction on ultrasound, without any abnormal findings on gynecologic evaluation and without any urinary catheter. Each patient could be enrolled once in the study. For the UTI to be considered community acquired, the patients should not have any contact with health care systems for more than 90 days (i.e., no hospitalization, no residence in long-term care facilities, no out-patient drug infusions and no patients under chronic hemodilution).

All isolates were transported onto slant agar into the central lab of the 4th Department of Internal Medicine at ATTIKON University Hospital. After re-culture onto MacConkey agar (BBL Becton–Dickinson, Cockeysville, MD, USA), susceptibilities to ampicillin, amoxicillin/clavulanate, cefoxitin, cefotaxime, ceftazidime, cefepime, gentamycin, amikacin, fosfomycin and trimethoprim/sulfamethoxazole were determined by the disk diffusion method using commercially available antimicrobial-impregnated disks (Oxoid Ltd, London, UK). Susceptibilities were interpreted by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [6]. Extended-spectrum β-lactamases (ESBL) production was tested with the double disk approximation test [7].

All ESBL-producing isolates, all non-*E. coli* isolates and almost half of the non-ESBL-producing *E. coli* isolates were selected for further study of susceptibilities to cefprozil and cefixime. This was done by selecting half of the non-ESBL-producing *E. coli* coming from each laboratory. Minimum inhibitory concentrations (MIC) to cefprozil, cefixime and ciprofloxacin were determined by a microdilution technique at a 0.1 ml final

volume using one $5 \times 10^5$ cfu/ml log-phase inoculum. Commercially available antibiotic powders were purchased (Sigma Co, St. Louis, USA). MIC was considered the lower antimicrobial concentration inhibiting visible bacterial growth after 18 h of incubation at 35 °C. The EUCAST MIC susceptibility breakpoints were used [6]. Minimum bactericidal concentrations (MBC) were determined after plating the content of clear wells onto MacConkey agar performing three times 1:10 serial dilutions. MBC was considered as the lowest concentration which resulted in the killing of 99.9% of the plated inoculum. American Tissue Cell Collection isolate *E. coli* 25,922 was used as a reference strain.

MIC50 and MBC50 were determined as the MIC and MBC respective values that inhibited/killed 50% of isolates; MIC90 and MCB90 were determined as the respective values inhibiting/killing 90% of isolates. Susceptibilities were compared by the Fischer exact test (SPSS Statistics version 22.0, IBM, NY, USA). Any value of $p$ below 0.05 was considered statistically significant.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed consent was obtained from all patients for being included in the study.

## RESULTS

A total of 747 isolates were collected belonging to the species of *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Fig. 1). Overall prevalence of ESBL production was 6.7%. *E. coli* was the most common pathogen isolated from 85.7% of patients. Among *E. coli* isolates, 93.8% were non-ESBL producers and 6.2% were ESBL producers. Among *K. pneumoniae* isolates, 81.8% were non-ESBL producers and 18.2% were ESBL producers. None of the *P. mirabilis* isolates were ESBL producers.

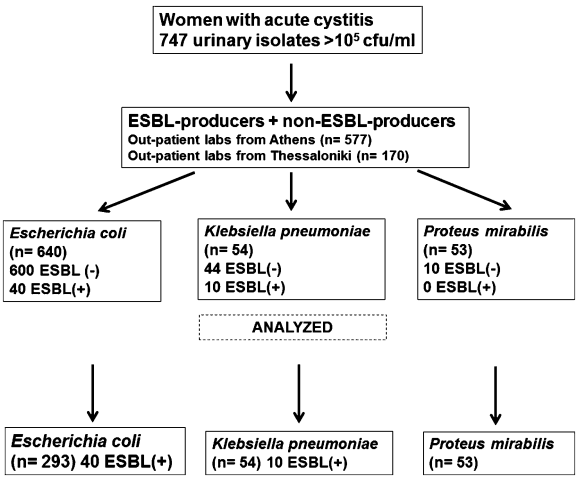


**Fig. 1** Flowchart of the selection of isolates for the study. *ESBL* extended-spectrum β-lactamase

Against non-ESBL-producing *E. coli,* cefprozil was more active than ciprofloxacin (93.7% vs 80.2% of isolates inhibited, $p < 0.0001$). This was not the case for cefixime (85.7% vs 80.2% of isolates inhibited, $p$ 0.125) (Table 1).

Although the activity of cefprozil and cefixime was limited against ESBL-producing isolates, they were active against isolates resistant to ciprofloxacin (Table 2).

## DISCUSSION

Current results suggest that in an era of emerging antimicrobial resistance, cefprozil and cefixime retain good activity against enterobacteria from patients with community-acquired UTI. However, both antimicrobials are not active against isolates that produce ESBL. Both tested drugs are available for oral administration. Cefixime was studied instead of other oral third-generation

**Table 1** Susceptibility patterns of 400 urinary pathogens to cefprozil, cefixime and ciprofloxacin in relation to their resistance phenotype

| | MIC (μg/ml) | | | | MBC (μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ | % inhibited | Range | $MBC_{50}$ | $MBC_{90}$ | % killed |
| *E. coli* | | | | | | | | |
| Cefprozil (16)[a] | | | | | | | | |
| ESBL (−) ($n = 253$) | ≤0.125 to >512 | 2 | 16 | 93.7 | ≤0.125 to >512 | 2 | 16 | 93.7 |
| ESBL (+) ($n = 40$) | 1 to >512 | >512 | >512 | 17.5 | 1 to 512 | >512 | >512 | 15.0 |
| Cefixime (1)[a] | | | | | | | | |
| ESBL (−) ($n = 253$) | ≤0.125 to >512 | 0.50 | 2 | 85.7 | ≤0.125 to 512 | 0.50 | 2 | 84.2 |
| ESBL (+) ($n = 40$) | ≤0.125 to >512 | 128 | >512 | 17.5 | ≤0.125 to 512 | 128 | >512 | 17.9 |
| Ciprofloxacin (1)[a] | | | | | | | | |
| ESBL (−) ($n = 253$) | ≤0.015 to >32 | 0.03 | >32 | 80.2 | ≤0.015 to 64 | 0.03 | >64 | 78.7 |
| ESBL (+) ($n = 40$) | ≤0.015 to >32 | 8 | >32 | 37.5 | ≤0.015 to 256 | >64 | >64 | 27.5 |
| *K. pneumoniae* | | | | | | | | |
| Cefprozil (16)[a] | | | | | | | | |
| ESBL (−) ($n = 44$) | ≤0.125 to >512 | 1 | 16 | 93.2 | ≤0.125 to >512 | 1 | 16 | 90.9 |
| ESBL (+) ($n = 10$) | 1 to >512 | >512 | >512 | 10.0 | 4 to 512 | >512 | >512 | 10.0 |
| Cefixime (1)[a] | | | | | | | | |
| ESBL (−) ($n = 44$) | ≤0.125 to >512 | 0.25 | 1 | 95.5 | ≤0.125 to 512 | 0.25 | 1 | 95.5 |
| ESBL (+) ($n = 10$) | ≤0.125 to >512 | >512 | >512 | 10.0 | ≤0.125 to 512 | >512 | >512 | 10.0 |
| Ciprofloxacin (1)[a] | | | | | | | | |
| ESBL (−) ($n = 44$) | ≤0.015 to >32 | 0.03 | >32 | 77.3 | ≤0.015 to 64 | 0.03 | >32 | 77.3 |
| ESBL (+) ($n = 10$) | ≤0.015 to >32 | 0.50 | >32 | 50.0 | ≤0.015 to 64 | 64 | 64 | 30.0 |
| *P. mirabilis* | | | | | | | | |
| Cefprozil (16)[a] | | | | | | | | |
| ESBL (−) ($n = 53$) | 0.50 to 128 | 2 | 16 | 90.6 | 0.50 to 128 | 4 | 32 | 88.7 |
| Cefixime (1)[a] | | | | | | | | |
| ESBL (−) ($n = 53$) | ≤0.125 to 4 | ≤0.125 | 1 | 100.0 | ≤0.125 to 4 | ≤0.125 | 1 | 100.0 |

**Table 1** continued

| | MIC (μg/ml) | | | | MBC (μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ | % inhibited | Range | $MBC_{50}$ | $MBC_{90}$ | % killed |
| Ciprofloxacin (1)[a] | | | | | | | | |
| ESBL (−) ($n = 53$) | ≤0.015 to >32 | 0.06 | 8 | 79.2 | ≤0.015 to 64 | 0.06 | 8 | 77.4 |

*ESBL* extended-spectrum β-lactamase, *MBC* minimum bactericidal concentration, *MIC* minimum inhibitory concentration
[a] Susceptibility concentration breakpoint

cephalosporins because it is the only drug of this class still in the market both in Greece and in many other European countries. The plasma half-life of cefprozil is 1.3 h and its bioavailability is 100%; it should be administered at a 500-mg twice-daily oral dose for 5–7 days for the treatment of UTIs. The plasma half-life of cefprozil is 4 h and its bioavailability is 50%; it should be administered at a 400-mg once-daily oral dose for 5 days for the treatment of UTIs [8].

Current guidelines suggest oral quinolones as the first-line treatment for community-acquired UTIs [9]. However, resistance to quinolones has emerged and mounts close to 20% as reported in a large survey of 499 *E. coli* isolates from Germany [10]. This increase in resistance of *E. coli* urine isolates to ciprofloxacin seems to be a worldwide phenomenon [2]. A retrospective analysis of the yearly susceptibility trends of 1107 community *E. coli* urine isolates in the Adelaide and Meath Hospital, that is a national tertiary reference center for urology in Ireland, indicated resistance rate of 10.6%. This amounts to 17.8% for hospital-acquired isolates and to 28.6% for isolates coming from patients hospitalized in urology departments [2]. However, the resistance rates to ciprofloxacin of 723 community-acquired urine *E. coli* isolates from inpatients enrolled in the SMART program of antimicrobial surveillance in USA during the years 2009–2011 was 22.4%. This was 7.4% for the 167 community-acquired urine isolates of *K. pneumoniae* [11]. The main driver for acquisition of ciprofloxacin resistance in the community is consumption of ciprofloxacin the last 3 months associated with odds ratio 4.20 [3]. In our study, cefprozil inhibited *E. coli* at a rate significantly greater than ciprofloxacin; both cefprozil and cefixime retained good activity against ciprofloxacin-resistant isolates. As a consequence, they remain a good therapeutic alternative either for empirical treatment, in the case of high suspicion for ciprofloxacin-resistant pathogens, or when one ciprofloxacin-resistant pathogen is isolated from urine.

One huge emerging problem in the management of community-acquired UTIs is enterobacteria pathogens that are β-lactam-resistant through the production of ESBL. Current publications suggest that the prevalence of these isolates ranges between 2 and 6% in *E. coli* and it is higher in *K. pneumoniae* [10, 12, 13]. These rates are in accordance with the 6.7% prevalence reported in the present study. Female gender, recurrent UTIs and presence of comorbidities are the most common risk factors for the acquisition of these isolates [12, 13]. Our findings suggest that both cefprozil and cefixime have poor activity against these isolates and that they cannot be suggested for management. Their empirical use should not be considered when there is

**Table 2** Comparative susceptibility of 13 antimicrobials against urinary-tract pathogens in relation to resistance phenotype

| | *E. coli* (%) | | | *K. pneumoniae* (%) | | | *P. mirabilis* (%) | |
|---|---|---|---|---|---|---|---|---|
| | **Ciprofloxacin susceptible/ ESBL (−) (*n* = 203)** | **Ciprofloxacin resistant (*n* = 50)** | **ESBL (+) (*n* = 40)** | **Ciprofloxacin susceptible/ ESBL (−) (*n* = 34)** | **Ciprofloxacin resistant (*n* = 10)** | **ESBL (+) (*n* = 10)** | **Ciprofloxacin susceptible/ ESBL (−) (*n* = 42)** | **Ciprofloxacin resistant (*n* = 11)** |
| Ampicillin | 48.3 | 26.0 | 0 | 5.9 | 0 | 0 | 66.7 | 27.3 |
| Amoxycillin/clavulanate | 94.6 | 92.0 | 70.0 | 100.0 | 80.0 | 30.0 | 97.6 | 100.0 |
| Cefoxitin | 98.5 | 100.0 | 90.0 | 100.0 | 80.0 | 30.0 | 100.0 | 100.0 |
| Cefprozil | 94.6 | 90.0 | 17.5 | 100.0 | 70.0 | 10.0 | 92.9 | 81.8 |
| Cefotaxime | 97.5 | 94.0 | 22.5 | 100.0 | 80.0 | 10.0 | 100.0 | 90.9 |
| Ceftazidime | 98.5 | 96.0 | 52.5 | 100.0 | 80.0 | 20.0 | 100.0 | 100.0 |
| Cefixime | 87.2 | 78.0 | 17.5 | 100.0 | 80.0 | 10.0 | 88.1 | 100.0 |
| Cefepime | 99.5 | 98.0 | 67.5 | 100.0 | 80.0 | 10.0 | 100.0 | 100.0 |
| Ciprofloxacin | 100.0 | 0 | 37.5 | 100.0 | 0 | 50.0 | 100.0 | 0 |
| Gentamicin | 98.5 | 74.0 | 70.0 | 100.0 | 80.0 | 70.0 | 97.6 | 54.5 |
| Amikacin | 100.0 | 96.0 | 87.5 | 100.0 | 60.0 | 60.0 | 100.0 | 100.0 |
| Fosfomycin | 100.0 | 100.0 | 92.5 | 97.1 | 100.0 | 100.0 | 95.2 | 90.9 |
| Trimethoprim/sulfamethoxazole | 95.4 | 34.0 | 45.0 | 100.0 | 40.0 | 30.0 | 76.2 | 18.2 |

*ESBL* extended-spectrum β-lactamase

increased clinical suspicion for UTIs by ESBL-producing pathogens.

The current study did not focus on epidemiological information of patients with UTIs caused by ESBL-producing isolates and ciprofloxacin-resistant isolates. This is because a previous large-scale epidemiological study in Greece has clearly shown the impact of previous antimicrobial consumption in the last 3 months as a risk factor for the emergence of these resistant isolates [3]. Major emphasis was given on the use of cefprozil and cefixime as alternative treatments for UTIs.

## CONCLUSION

The results of the present study indicate that cefprozil and cefixime retain good activity against urine pathogens. According to our findings, they can be empirically used for management. Their use is particularly encouraged in the following cases: (a) UTIs with documented or high suspicion for implication of ciprofloxacin-resistant pathogens; and (b) UTIs by pathogens cross-resistant to other antimicrobials provided that they do not produce ESBL.

## ACKNOWLEDGMENTS

The study was funded by a kind donation from the Hellenic Institute for the Study of Sepsis. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published.

***Disclosures.*** Evangelos Giamarellos-Bourboulis has received honoraria for advising AbbVie USA, Astellas Greece SA, Biotest AG Germany, BRAHMS ThermoFisher GmbH Germany and by the Medicines Company USA. He has received research funding from AbbVie Greece SA, Biotest AG Germany, BRAHMS ThermoFisher GmbH Germany, Sanofi Greece. He is funded by the FP7 Program HemoSpec.

Aikaterini Pistiki, Thomas Tsaganos and Irene Galani have nothing to disclose.

***Compliance with ethics guidelines.*** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2013. Informed consent was obtained from all patients for being included in the study.



## REFERENCES

1. Alexiou Z, Mouktaroudi M, Koratzanis G, Papadopoulos A, Kavatha D, Kanellakopoulou K, et al. The significance of compliance for the success of antimicrobial prophylaxis in recurrent lower urinary tract infections: the Greek experience. Int J Antimicrob Agents. 2007;30(1):40–3.

2. Cullen IM, Manecksha RP, McCullagh E, Ahamd S, O'Kelly F, Flynn RJ, et al. The changing pattern of antimicrobial resistance within 42033 *Escherichia coli* isolates from nosocomial, community and

urology patient-specific urinary tract infections, Dublin, 1999–2009. BJU Int. 2012;109(8):1198–206.

3. Katsarolis I, Poulakou G, Athanasia S, Kourea-Kremastinou J, Lambri N, Karaiskos E, et al. Acute uncomplicated cystitis: from surveillance data to a rationale for empirical treatment. Int J Antimicrob Agents. 2010;35(1):62–7.

4. Wiseman LR, Benfield P. Cefprozil. A review of its antibacterial activity, pharmacokinetic properties, and therapeutic potential. Drugs. 1993;45(2):295–317.

5. Hamilton-Miller JM. Overview of cefixime use in community-acquired infections. Clin Microbiol Infect. 2000;6(Suppl 3):79–81.

6. European Committee on Antimicrobial Susceptibility Testing: Clinical Breakpoints 2014 http://www.eucast.org/clinical_breakpoints. Accessed Dec 2014.

7. Stürenburg E, Mack D. Extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. J Infect. 2003;47(4):273–95.

8. Thompson EM, Shaughnessy AF. Oral cephalosporins: newer agents and their place in therapy. Am Fam Physician. 1994;50(2):401–5.

9. 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(5):e103–20.

10. Kresken M, Pfeifer Y, Hafner D, Wresch R, Körber-Irrgang B. Occurrence of multidrug resistance to oral antibiotics among *Escherichia coli* urine isolates from outpatient departments in Germany: extended-spectrum β-lactamases and the role of fosfomycin. Int J Antimicrob Agents. 2014;44(4):295–300.

11. Bouchillon SK, Badal RE, Hoban DJ, Hawser SP. Antimicrobial susceptibility of inpatient urinary tract isolates from Gram-negative bacilli in the Unites States: results from the study for monitoring antimicrobial resistance trends (SMART) program: 2009–2011. Clin Ther. 2013;35(6):872–7.

12. Meier S, Webere R, Zbinden R, Ruef C, Hasse B. Extended-spectrum β-lactamase-producing Gram-negative pathogens in community-acquired urinary tract infections: an increasing challenge for antimicrobial therapy. Infection. 2011;39(4):333–40.

13. Briongos-Figuero LS, Gómez-Traveso T, Bachiller-Luque P, Domínguez-Gil González M, Gómez-Nieto A, et al. Epidemiology, risk factors and comorbidity for urinary tract infections caused by extended-spectrum beta-lactamase (ESBL)-producing enterobacteria. Int J Clin Pract. 2012;66(9):891–6.