Revival of older antibiotics for the therapy of urinary tract infections: old, but gold Part 1: Antimicrobial susceptibility of extended-spectrum β-lactamase-producing and AmpC β-lactamase-producing *Escherichia coli* isolates

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The most prevalent causative agent of urinary tract infections (UTIs) is uropathogenic *Escherichia coli*, corresponding to 50–90% of uncomplicated, around 30–70% of nosocomial UTIs. There has been renewed interest toward the clinical value of older, nonβ-lactam antibiotics (including fosfomycin, nitrofurantoin, trimethoprim/sulfamethoxazole) used for the therapy UTIs caused by drug resistant bacteria, including AmpC-producing or an extended-spectrum β-lactamases (ESBL)-producing Gram-negative strains. The aim of our study was to determine the resistance levels of AmpC-producing or ESBL-producing *E. coli* strains, against the relevant ancillary antibiotics that may be used in the treatment of UTIs. Isolates were collected from the time period between 1 January 2013 and 31 December 2017 from patients with uncomplicated and complicated UTIs treated at the Albert Szent-Györgyi Clinical Center (Szeged, Hungary). Antibiotic susceptibility testing was carried out using the Kirby-Bauer method. Out of the 10 837 isolates, $n = 2010$ (18.5%; 402 ± 43 isolates/year) *E. coli* isolates were either AmpC-producers or ESBL-producers, whereas $n = 1398$ (12.8%; 280 ± 12 isolates/year) produced the two groups of β-lactamases simultaneously. The highest levels of coresistance overall was seen for ciprofloxacin (68.2%), followed by trimethoprim-sulfamethoxazole (58.6%), whereas resistance levels were lower in regards to gentamicin (39.0%), fosfomycin (20.3%) and considerably lower for nitrofurantoin (11.1%). Our analysis of urine-specific AmpC-producing or ESBL-producing *E. coli* isolates is a useful addition to the literature, as clinicians may rely on this data for empiric antibiotic selection for UTI.

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Introduction

Urinary tract infections (UTIs) are one of the most prevalent infections in human medicine, accounting for 10–20% of all infections in the community-setting and 25–50% of hospital-acquired infections [1,2]. The economic losses due to UTIs (healthcare-related costs, antibiotic-therapy, workplance absenteeism) are estimated to be around 5 billion US$, as UTIs are responsible for 10 million general practitioner (GP) visits, 1.5 million emergency room visits and 300 000 hospital admissions in the United States alone [3]. The common symptoms associated with UTIs includeurinary urgency, dysuria, suprapubic pain, feeling of incomplete bladder emptying and visible blood in urine [4]. UTIs have been described as an important infections in both sexes and in all age groups (infants, children, adults and the elderly); nonetheless, uncomplicated UTIs are most common

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between women over 18 years of age, with around two-thirds of women in the ages of 20–40 years experiencing a UTI at least once during their lifetime; in addition, recurrent UTIs (rUTIs) in adult women is present in 20–30% of cases, within 3–4 months of the initial infection [5]. rUTIs are also frequently associated with psychiatric symptoms, such as reduced social activity, guilt (due to inability to perform various everyday tasks), anxiety (e.g. associated with incontinence in the elderly) and depression, which also major contributing factors to the decreased quality of life associated with these infections [6,7].

The most common causes of UTIs are the members of the Enterobacterales order; these bacteria are abundantly present in the gut microbiota within the anatomical vicinity of the genito-urinary tract [1,2]. The most prevalent uropathogen is the uropathogenic *Escherichia coli*, corresponding to 50–90% of uncomplicated, community-associated UTIs and around 30–70% of nosocomial UTIs [8]. In addition to *E. coli*, other Gram-negative bacteria, such as *Klebsiella* spp., members of the CES (*Citrobacter-Enterobacter-Serratia*) group and the PPS (*Proteus-Providencia-Morganella*) group, whereas among Gram-positive bacteria, *Enterococcus* spp. and *Staphylococcus saprophyticus* are also relevant uropathogens, especially in older patients with an inserted urinary catheter [9–11]. The therapy of UTIs is mainly empirical, based on the signs or symptoms of infections. The therapy of UTIs is an increasingly complex challenge for GPs and urologists; recent reports have shown a marked increase in the resistance levels and the number of multidrug resistant (MDR) isolates causing UTIs. MDR in *E. coli* has also been shown, due to the excessive, imprudent use of antibiotics [12]. *E. coli* has also been included in the group of so-called ESKAPE bacteria, representing bacterial pathogens, in which the developments of resistance were the most concerning [13].

The recommended drugs for the treatment of uncomplicated UTIs include nitrofurantoin, fosfomycin, trimethoprim/sulfamethoxazole and mecillinam (if local resistance levels do not exceed 20% per pathogen); if drug hypersenstivity needs to be considered, then other therapeutic options, such as β-lactam antibiotics, fluoroquinolones and aminoglycosides should be primarily used [14,15]. β-lactam-antibiotics (namely penicillins, cephalosporins and carbapenems) are considered the backbone of the therapy of bacterial infections, due to their broad spectrum and advantageous pharmacokinetic properties [16]. In addition, in several vulnerable patient populations (children, pregnant women, patients with liver/kidney failure), there drugs are the first-choice agents, due to the teratogenicity or debilitating side effect-profile of the alternate drugs [17]. Resistance to β-lactam is mainly due to the presence of degrading enzymes called β-lactamases: from a clinical standpoint of UTIs, AmpC-type β-lactamases and extended-spectrum β-lactamases (ESBLs) are the most prevalent, resulting in therapeutic failures [16,17]. AmpC-type β-lactamases are mostly chromosomally encoded, capable of hydrolyzing penicillin-derivatives and cephalosporins (including third generation cephalosporins and aztreonam, but not fourth generation cephalosporins), whereas ESBLs are plasmid-encoded enzymes, capable of hydrolyzing penicillin-derivatives and cephalosporins (including third and fourth generation cephalosporins) [18,19]. In UTIs with an AmpC-producing or an ESBL-producing *E. coli* isolate, carbapenems essentially remain the only safe drug choice, whereas other 'last resort' agents (e.g. tigecycline, colistin, ceftazidime-avibactam) are rarely used, due to their price, pharmacokinetic profile or side effects. In addition, isolates with plasmid-borne ESBLs most frequently carry resistance determinants to other antibiotic classes as well [18,19].

There has been renewed interest towards the clinical value of older, nonβ-lactam antibiotics (including fosfomycin, nitrofurantoin, trimethoprim/sulfamethoxazole) used for the therapy UTIs caused by drug resistant bacteria, including AmpC-producing or an ESBL-producing Gram-negative strains [20]. In fact the effectiveness of fosfomycin was evaluated *in vitro* and in a clinical setting, both for oral and parenteral administration [21]. Clinicians are encouraged to use these UTI-specific antibiotics to treat their patients to spare β-lactams, however, reliable susceptibility data is needed to ascertain the safety and adequacy of the drug choices [22]. The increase in the prevance of drug resistance in UTI-causing pathogens has been reported in Hungary, however, susceptibility-data for some antibiotics is scarce. Therefore, the aim of our study was to determine the resistance levels of AmpC-producing or ESBL-producing *E. coli* strains isolated from UTIs, against the relevant ancillary antibiotics that may be used in the treatment of these infections, with a special focus on the Southern Region of Hungary.

Materials and methods

Study design

The current study was carried out by collecting AmpC and/or ESBL-positive *E. coli* isolates from the time period between 1 January 2013 and 31 December 2017 from patients with uncomplicated and complicated UTIs treated at the Albert Szent-Györgyi Clinical Center, a 1800-bed tertiary-care medical center, which annually serves more than 400 000 patients in Szeged, Hungary [9]. Samples with clinically significant colony counts for the abovementioned bacteria [$10^5 <$ colony forming units (CFU)/ml]; however, this was subject to interpretation, based on the information provided on the request forms for microbiological analysis and relevant international guidelines were included in the study. Only the first

isolate per patient was included in the study, however, isolates with different antibiotic-susceptibility patterns were considered as different individual isolates. In addition, limited patient data (age and sex of the affected patients) was also collected, during the electronic search in the records of the laboratory information system for urine samples containing AmpC and/or ESBL-positive *E. coli*.

Sample size determination

The sample size of AmpC and/or ESBL-positive *E. coli* isolates required for the study from each year of the study was determined using the formula by the methodology described by Thrusfield (1), where n was the calculated sample size, z the desired level of confidence (1.96), i the standard sampling error (5%), and p the estimated prevalence set at 30% [23,24]. The minimum required sample size required ($n = 249$) was increased by 20% for added contingency, thus the required sample size of 300/year was determined.

$$n = \frac{z^2 p(1-p)}{i^2} \quad (1)$$

Identification of isolates

An amount of 10 μl each uncentrifuged urine sample was cultured on UriSelect chromogenic agar plates (Bio-Rad, Berkeley, California, USA) with a calibrated loop, according to the manufacturer's instructions. The plates were incubated at 37 °C for 24–48 h, aerobically. If *E. coli* was detected in significant colony counts, the plates were passed on for further processing. Bacterial identification was carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The methodology of sample preparation for MALDI-TOF MS measurements was described previously [25]. Mass spectrometry measurements were performed by the Microflex LT MALDI Biotyper (Bruker Daltonics, Bremen, Germany) in positive linear mode across the m/z range of 2–20 kDa; for each spectrum, 240 laser shots at 60 Hz in groups of 40 shots per sampling area were collected. The MALDI Biotyper RTC 3.1 software (Bruker Daltonics) and the MALDI Biotyper Library 3.1 were used for spectrum analysis.

Antimicrobial susceptibility testing, resistotyping

Susceptibility testing was performed for antibiotics relevant in the treatment of UTIs caused by AmpC and/or ESBL-positive *E. coli*, using the Kirby–Bauer disk diffusion method (Liofilchem, Abruzzo, Italy) on Mueller–Hinton agar plates. Verification of discrepant results was performed using the VITEK 2 Compact ID/AST (bioMérieux, Marcy-l'Étoile, France) automated system. The following antibiotics were included in the study: ertapenem (10 μg), ciprofloxacin (5 μg), gentamicin (10 μg), fosfomycin (200 μg), nitrofurantoin (100 μg) and trimethoprim-sulfamethoxazole (23.75/1.25 μg). The interpretation of the results was based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, available at the time of isolation. During data analysis, intermediately-susceptible results were grouped with and reported as resistant. Classification of isolates as MDR and extensively drug resistant (XDR) was based on the criteria of Magiorakos *et al.* [26]. Resistotypes from the respective susceptibility-results were defined based on criteria described previously [27]. *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Detection of AmpC and extended-spectrum β-lactamases-production

Screening for β-lactamase production was performed on the basis of the recommendations of EUCAST. For the purposes of this study, AmpC and ESBL-producing strains were not further differentiated and their molecular characterization was not performed.

Screening of *E. coli* isolates for AmpC β-lactamase production was carried out using cefoxitin disks (10 μg): isolates with a zone of inhibition of 18 mm or less was considered as a potential AmpC-producer [16]. ESBL-production was suspected, if the isolates showed resistance toward third-generation cephalosporins (either cefotaxime, ceftriaxone, ceftazidime) during routine susceptibility testing. ESBL-detection was carried out using the AmpC-ESBL Detection Set (MAST Diagnostica GmbH, Reinfeld, Germany) and VITEK 2 Compact ID/AST (bioMérieux), according to the manufacturer's instructions [10].

Statistical analysis

Descriptive statistical analysis (including means or medians with ranges and percentages to characterize data) was performed using Microsoft Excel 2013 (Microsoft Corp., Redmond, Washington, USA).

Ethical considerations

The study was deemed exempt from ethics review by the Institutional Review Board, and informed consent was not required as data anonymity was maintained.

Results

Demographic characteristics, isolation frequency

During the 5-year study period (1 January 2013–31 December 2017), the Institute of Clinical Microbiology received 19 387 urine samples (originating from inpatient departments and outpatient clinics, respectively) that turned out to be positive for a significant urinary pathogen. Out of the positive urine samples *E. coli*

Table 1. Isolation frequency and resistance levels associated with AmpC-producing or extended-spectrum β-lactamases-producing *Escherichia coli* from urinary tract infections (2013–2017).

	AmpC-producing or ESBL-producing isolates	AmpC-producing and ESBL-coproducing isolates	Ertapenem	Ciprofloxacin	Gentamicin	Fosfomycin	Nitrofurantoin	Trimethoprim-sulfarmethoxazole
2013	364	261 (71.7%)	0 (0.0%)	276 (75.8%)	167 (45.8%)	66 (18.1%)	36 (9.9%)	215 (59.2%)
2014	389	284 (73.0%)	0 (0.0%)	333 (85.7%)	130 (33.3%)	75 (19.2%)	46 (11.8%)	222 (57.1%)
2015	476	287 (60.3%)	1 (0.1%)	279 (58.5%)	182 (38.2%)	114 (23.9%)	56 (12.4%)	268 (56.2%)
2016	394	291 (73.8%)	1 (0.1%)	289 (73.4%)	150 (38.1%)	66 (16.7%)	45 (11.4%)	227 (57.8%)
2017	387	275 (71.0%)	1 (0.1%)	195 (70.9%)	155 (40.0%)	87 (22.1%)	40 (10.3%)	245 (63.2%)
Overall	2010	1398 (69.6%)	3 (0.1%)	1372 (68.2%)	784 (39.0%)	408 (20.3%)	223 (11.1%)	1177 (58.6%)

ESBL, extended-spectrum β-lactamases.

represented the majority ($55.9 \pm 6.5\%$; $n = 10\,837$) of relevant urinary isolates.

The median age of affected patients by *E. coli* UTIs overall, was 61 years (range: 0.3–96), with a female-to-male ratio of 3.02, whereas for the patients affected by AmpC-producers or ESBL-producers, this was 74 years (range: 24–97), with a female-to-male ratio of 2.21, respectively.

Resistance levels of AmpC-producing and extended-spectrum β-lactamases-producing *Escherichia coli*

Out of the 10 837 isolates, $n = 2010$ (18.5%; 402 ± 43 isolates/year) *E. coli* isolates were either AmpC-producers or ESBL-producers (thus, fulfilling our requirements for minimum sample size), whereas $n = 1398$ (12.8%; 280 ± 12 isolates/year) produced the two groups of β-lactamases simultaneously. The complete epidemiology and resistance levels of the relevant isolates is presented in Table 1. Among AmpC-producing or ESBL-producing *E. coli* isolates, the highest levels of coresistance overall was seen for ciprofloxacin (68.2%), followed by trimethoprim-sulfamethoxazole (58.6%), whereas resistance levels were lower in regards to gentamicin (39.0%), fosfomycin (20.3%) and considerably lower for nitrofurantoin (11.1%). Lowest levels of resistance were seen for ertapenem (only 3 isolates), which was not surprising, as the prevalence of carbapenem-resistant isolates is low in this geographical region.

The distribution of isolates into various resistotypes is shown in Table 2.; Type 0 represents *E. coli* isolates AmpC-producers or ESBL-producers, but are otherwise fully-susceptible to the ancillary urinary antibiotics tested (31.7%), Type I (I-A, I-B and I-C) includes isolates resistant to either ciprofloxacin, trimethoprim-sulfamethoxazole or gentamicin only (24.6%); whereas Type II (II-A, II-B, II-C and II-D) introduces resistance to multiple antibiotics at once, and resistance to nitrofurantoin and fosfosmycin, respectively (41.1%). Type III (2.5%) represents resistance to five, whereas Type IV (0.1%) represents resistance to all individual antibiotics tested. Based on EUCAST Expert Rules, isolates in the Type III category also represent MDR *E. coli* isolates, whereas isolates in the Type IV category should be considered as XDR isolates.

Discussion

The diagnosis and treatment of UTIs are a considerable burden on healthcare systems [1–3]. *E. coli* and other members of the Enterobacterales order are shown to be successful pathogens in the urinary system, as they possess the relevant virulence factors required to successfully survive on and adhere to urinary epithelium. In addition, after successful adherence, they may also cause damage to the tissues, ascent to the upper urinary tract and cause cUTIs [1–3,8]. The following virulence-determinants

Table 2. Distribution of various resistotypes among AmpC-producing or extended-spectrum β-lactamases-producing *Escherichia coli* from urinary tract infections (2013–2017).

Resistotype	Ciprofloxacin	Trimethoprim-sulfamethoxazole	Gentamicin	Fosfomycin	Nitrofurantoin	Ertapenem	Number of isolates
0	S	S	S	S	S	S	638 (31.7%)
I-A	**R**	S	S	S	S	S	333 (14.3%)
I-B	S	**R**	S	S	S	S	138 (5.7%)
I-C	S	S	**R**	S	S	S	112 (4.6%)
II-A	**R**	**R**	S	S	S	S	147 (6.3%)
II-B	**R**	**R**	**R**	S	S	S	264 (11.1%)
II-C	**R**	**R**	S	**R**	S	S	405 (16.5%)
II-D	**R**	**R**	S	S	**R**	S	160 (7.2%)
III	**R**	**R**	**R**	**R**	**R**	S	60 (2.5%)
IV	**R**	**R**	**R**	**R**	**R**	**R**	3 (0.1%)

ESBL, extended-spectrum β-lactamases; R, resistant; S, susceptible.

were determined having principal roles in the pathogenesis of UTIs: lipopolysaccharide, polysaccharide capsule, outer membrane vesicles, iron-uptake (aerobactin) and siderophore receptors, adhesins, Type-1 fimbriae, cytotoxins and urease production [8,10,28]. These virulence factors are also relevant in their survival on abiotic surfaces, like in hospital wards, catheters [2,8,10,28].

Ideally, the effective therapy of bacterial UTIs relies on identification of the causative organism and the selection of an effective antibiotic agent, which is made possible by continuous surveillance of antimicrobial susceptibility patterns of uropathogens in the well defined geographical region [29]. The emergence of β-lactam-resistance depending on the presence of AmpC-based and ESBL-based enzymes in UTIs is a serious public health issue, which is only expected to worsen, due to the slow but growing incidence of carbapenemase-producing strains [30,31]. The overuse of β-lactams in inappropriate indications, or as self-medication with antibiotics, enhances the selection pressure put onto these microorganisms [32,33]. Since the beginning of the 21st century, $bla_{\text{CTX-M}}$-type ESBLs are the most prevalent around the globe, with pronounced genetic diversity among the various subtype of these enzymes; among AmpC-enzymes, bla_{CMY} and bla_{DHA} types are the most prevalent [18,30,34]. Due to their transmissibility, ESBLs are considered a serious public health/infection control issue, especially because these enzymes are encoded on larger-sized plasmids [18,30,34]. As several resistance-determinants are often present in bacteria simultaneously, in addition to β-lactams, other antibiotic-groups may be affected, rapidly resulting in MDR, or even XDR strains, with severely limited therapeutic options left [26,35]. These isolates have been associated with increased morbidity and mortality, particularly in ICUs and in severely immunocompromised patients [36].

Our analysis of urine-specific AmpC-producing or ESBL-producing *E. coli* isolates is a useful addition to the literature, as clinicians may rely on this data for empiric antibiotic selection for UTI. Our study has highlighted that *E. coli* is the most frequently occurring pathogen in UTIs in our tertiary-care teaching hospital, and that almost one in every fifth isolated *E. coli* expressed either AmpC-type or ESBL-type β-lactamases, or both of them. In addition, our report has shown that more, than 40% of these isolates were resistant to at least two nonβ-lactam antibiotics, relevant in the therapy of UTIs. Resistance to the preferred UTI-antibiotics was variable; whereas resistance levels to nitrofurantoin slightly went above 10%, fosfomycin was above 20% and resistance to trimethoprim/sulfamethoxazole was almost 60%. Thus, among these agents, the empiric use of nitrofurantoin may be considered well treated, even in the case of isolates carrying AmpC-type or ESBL-type β-lactamases, whereas for fosfomycin and trimethoprim/sulfamethoxazole, susceptibility-testing is recommended (according to the guidelines of the Infectious Diseases Society of America) [1,2]. Fosfomycin-resistance was shown to be around 3–50% in Enterobacterales in various literature sources, however, the resistance levels are usually lowest in *E. coli*, and steadily increasing in CES pathogens and *Klebsiella* spp. [9–12,21,37]. Fosfomycin and nitrofurantoin both have unique chemical structures and mechanisms of action, therefore, these is no need to expect cross-resistance with other antibacterial drugs [9–12,21,38]. The utility of trimethoprim/sulfamethoxazole in the therapy of ESBL-positive and carbapenemase-positive Gram-negative isolates has been reported previously, both in UTIs and invasive infections [39]; nevertheless, this was not highlighted in our study.

In conclusion, the aim of our study was produce reliable data on the utility of various older or ancillary antibiotics for the treatment of UTIs with a special focus on the Southern Region of Hungary, if the causative agent has been identified as a AmpC-producing or ESBL-producing *E. coli* isolate. As UTIs are among the most common reasons for seeking medical attention in the community and the introduction of antibiotics in nosocomial settings, knowledge of resistance-trends is of utmost importance, as the emergence of MDR strains of uropathogens, results in therapeutic difficultures and clinical failure [40].

Some limitations of this study must be acknowledged: first, the study was carried out for *E. coli* only, and this is not representative for all urine isolates belonging to the Enterobacterales order; second, data was not collected on the inpatient/outpatient status and the presence of a urinary catheter in affected patients; third, molecular characterization of the genetic background of resistance in the individual isolates was not performed; fourth, selection bias must be taken into consideration.

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Ethical considerations: The study was deemed exempt from ethics review by the Institutional Review Board, and informed consent was not required as data anonymity was maintained.

Conflicts of interest

The authors declare no conflict of interest, monetary or otherwise. The authors alone are responsible for the content and writing of this article.

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