IN VITRO ANTIMICROBIAL RESISTANCE OF URINARY *ESCHERICHIA COLI* ISOLATES FROM OUTPATIENTS COLLECTED IN A LABORATORY DURING TWO YEARS, 2015 - 2017

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ABSTRACT

Objectives. This study describes the antimicrobial susceptibility profiles of *Escherichia coli* strains originated from urine specimens collected from outpatients between 2015 and 2017, with the goal to improve the laboratory-based information regarding the current situation of antibiotic resistance of urinary tract infection (UTI) - associated *E. coli* strains circulating in the local population.

Methods. A collection of 543 *E. coli* strains was retrospectively analysed with respect to resistance to representatives of clinically important classes of antibiotics (penicillins/penicillin with beta-lactamase inhibitors, extended-spectrum cephalosporins, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, nitrofurans, and phosphonic acid derivatives).

Results. More than half of the study strains (366/543 strains) proved to be resistant to at least one of the antibiotics tested. Based on their patterns of resistance, 158 strains qualified as multi-drug resistant and 71 strains were extended spectrum beta-lactamases (ESBL) producers. Susceptibility was highest for fosfomycin (99%) followed by nitrofurantoin (97%), gentamicin (88%), third-generation cephalosporins (87%), amoxicillin with clavulanic acid (71%), fluoroquinolones (68%), trimethoprim-sulfamethoxazole (60%) and lowest for ampicillin (38%).

Conclusions. Accordingly, whereas fosfomycin and nitrofurantoin represent effective therapeutic options, trimethoprim-sulfamethoxazole and ampicillin, even in combination with beta-lactamase inhibitor, should not be recommended empirically for UTI treatment. Also, given the concerning findings that the local rate of resistance to fluoroquinolones is high and ESBL-producing strains are beginning to limit the use of extended-spectrum cephalosporins, surveillance studies to monitor *in vitro* susceptibilities for these antibiotics are particularly important for identifying further changes among urinary tract *E. coli* isolates and guide the empiric treatment of infections of locals in community.

Keywords: *Escherichia coli*, *in vitro* antimicrobial susceptibility, community urinary tract infections, ESBL producers.

REZUMAT

Obiective. Studiul de față descrie profilurile de sensibilitate antimicrobiană ale unui set de tulpini de *Escherichia coli,* provenite din probe de urină colectate de la pacienți din comunitate, pe parcursul a doi ani, între 2015-2017. Acest studiu a avut drept scop îmbunătățirea informației de laborator referitoare la situația actuală a rezistenței la antibiotice a tulpinilor de *E. coli* asociate infecțiilor de tract urinar (ITU), care circulă în rândul populației autohtone.

Metode. O colecție de 543 tulpini de *E. coli* a fost analizată retrospectiv privind rezistența la antibiotice aparținând celor mai importante clase de antimicrobiene (peniciline/peniciline cu inhibitori de betalactamaze, cefalosporine cu spectru extins, aminoglicozide, fluoroquinolone, trimetoprim-sulfametoxazole, nitrofurani și derivați ai acidului fosfonic).

Rezultate. Mai mult de jumătate dintre tulpinile analizate (366/543, 67%) s-au dovedit a fi rezistente la cel puțin unul dintre antibioticele testate. Pe baza profilurilor de rezistență, 158 de tulpini au fost clasificate ca multirezistente și 71 de tulpini ca producătoare de betalactamaze cu spectru extins (BLSE). Sensibilitatea cea mai mare a fost pentru fosfomicină (99%), urmată de nitrofurantoin (97%), gentamicină (88%), cefalosporine de generația a treia (87%), amoxicilina cu acid clavulanic (71%), fluoroquinolone (68%), trimetoprim-sulfametoxazole (60%), iar cea mai scăzută pentru ampicilină (38%).

Concluzii. Drept urmare, în timp ce fosfomicina și nitrofurantoinul reprezintă opțiuni terapeutice eficiente, trimetoprim-sulfametoxazole și ampicilina, chiar în combinație cu inhibitori de betalactamaze, nu pot fi recomandate în tratamentul empiric al ITU. În același timp, ținând cont de rezultatele îngrijorătoare privind rata ridicată de rezistență la fluoroquinolone și că tulpinile producătoare de BLSE încep să limiteze utilizarea cefalosporinelor cu spectru extins, studiile de monitorizare a sensibilității *in vitro* la aceste antibiotice sunt deosebit de importante pentru identificarea viitoarelor schimbări ale izolatelor urinare de *E. coli* și totodată pentru orientarea tratamentului empiric al infecțiilor urinare authtone din comunitate.

Cuvinte-cheie: *Escherichia coli*, susceptibilitate antimicrobiană *in vitro*, infecții de tract urinar comunitare, producători de BLSE.

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MATERIALS AND METHODS

Bacterial collection

The study was conducted by the Bacterial Enteric Infections Laboratory in Cantacuzino Institute that received during two years, from May 2015 to May 2017, a total of 1927 urine samples for UTI laboratory confirmation. All the *E. coli* strains isolated from these urine specimens were included in the study and patients data were anonymised. The inclusion criteria was a pure culture of *E. coli* with a significant growth of $\geq 10^5$ colony forming units (CFU)/ml urine, indicating a significant bacteriuria for UTI laboratory-based confirmation. Quantitative urine culture protocol used 1 µl of urine, spread onto 5% sheep blood and MacConkey agars and incubated aerobically at 37 °C overnight. Microbial identification was performed by API 20E test and/or the VITEK 2 system using GN identification cards.

Antibiotic susceptibility testing

The *E. coli* strains were tested against several antimicrobial classes, including penicillins, penicillin with beta-lactamase inhibitor, extended-spectrum cephalosporins (ESC), aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, nitrofurans and phosphonic acid derivatives. The susceptibility of antibiotics was performed by the Kirby-Bauer disk diffusion method as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [7] and the following antimicrobial agents were tested: ampicillin (AMP, 10 µg), amoxicillin with clavulanic acid (AMC, 20-10 µg), ceftazidime/cefotaxime (CAZ, 10 µg/ CTX, 5 µg), gentamicin (CN, 10 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), levofloxacin (LEV, 5 µg), nitrofurantoin (F, 100 µg), trimethoprim with sulfamethoxazole (SXT, 1,25/23,75 µg) and fosfomycin with trometamol as phosphonic acid derivate (FOT, 200 µg). In this study, intermediate susceptibility was analysed as resistance. Multidrug-resistant (MDR) isolates were those resistant to at least 1 representative of ≥ 3 antimicrobial classes.

In this study, the ESBL producers were phenotypically confirmed using Etest ESBL strips commercially available (cefotaxime +/- clavulanic acid; ceftazidime +/- clavulanic acid) (AB BIODISK) in accordance with the manufacturer's instructions. A commercially available assay (KPC, MBL and OXA-48 confirm kit) (Rosco Diagnostics) was also used as a confirmation test for the AmpC producers.

RESULTS

Overall, of the total 1927 urine specimens investigated during the two years, 543 (28%) yielded an *E. coli* positive culture. Of these UTI-associated *E. coli*, 366 strains displayed resistance against at least one of the antibiotics tested to several classes of antibiotics and 177 strains proved to be still susceptible. Fosfomycin-trometamol and nitrofurantoin were the most active antibiotics with an overall prevalence of 1% and 3% of resistant strains, respectively. Low resistance rates were also found for gentamicin (12%) while ampicillin, trimethoprim with sulfamethoxazole, fluoroquinolones, and amoxicillin/clavulanic acid were the least active antibiotics. Specifically, 62%, 40%, 32%, and 29% of the strains were resistant against these antibiotics. Concerning resistance to the extended-spectrum cephalosporins tested, 71 of the *E. coli* strains (13%) expressed this phenotype and were identified as ESBL producers. Of note, none of these strains displayed AmpC activity.

In accordance with the resistance expressed against the tested antibiotic combination, a total of 45 resistance profiles (antibiotypes) were identified (Table 1). Twelve of them were represented by ≥10 strains. While 20% of the *E. coli* strains displayed resistance to only one of the antibiotics tested which was mostly ampicillin (53 strains) or the combination trimethoprim with sulfamethoxazole (14 strains), 43% were resistant to antibiotics representing at least three different classes, which qualified them as multidrug resistant strains. Moreover, among the MDR category there were 67 ESBL producing-strains.

DISCUSSION

In this study we analysed the occurrence of antibiotic resistance in *E. coli* urinary strains recovered in the last two years from community patients and gathered laboratory-based evidence that the empiric antibiotic treatment of the autochthonous UTI cases should be carefully reviewed because of the high rates of resistance to commonly used antibiotics

observed. Thus, we found that almost half of the *E. coli* strains collected in the last two years became MDR and as already noticed in different regions of the world [8-11], not only the levels of *E. coli* resistance to aminopenicillins and trimethoprim with sulfamethoxazole are such that clinicians have reason to remain reluctant to use these drugs, but also the use of otherwise potent antimicrobials such as fluoroquinolones and extended-spectrum cephalosporins is becoming quite risky.

In a recent study that addressed the antibiotic resistance of urinary pathogens isolated from Romanian elderly patients with prostatic disease in which *E. coli* was the predominant species, high rates of resistance

Table 1. **Antibiotypes identified among the 543 *E. coli* strains investigated in this study**

Resistance phenotypes	Number of *E. coli* strains
AMP	53
AMP+SXT	40
AMP+AMC	35
AMP+SXT+FQ	29
AMP+AMC+SXT	29
AMP+AMC+SXT+FQ	21
AMP+CAZ/CTX+SXT+FQ	15
AMP+AMC+CAZ/CTX+CN+SXT+FQ	14
SXT	14
AMP+FQ	12
AMP+AMC+CN+SXT+FQ	12
AMP+AMC+CAZ/CTX+SXT+FQ	10
AMP+CN+FQ+SXT	8
AMP+CAZ/CTX+FQ	8
FQ	6
AMP+AMC+CAZ/CTX+CN+FQ	6
FQ+SXT	4
AMP+AMC+FQ	4
AMP+CN+SXT	4
AMP+AMC+CN+FQ	4
AMP+AMC+CAZ/CTX	3
AMP+AMC+CAZ/CTX+CN+FQ	3
AMP+CAZ/CTX+CN+FQ+SXT	3
F	2
AMP+CN	2
F+SXT	2
AMP+SXT+F	2
AMP+AMC+FQ+F	2
AMP+AMC+FQ+SXT+F	2
AMP+AMC+CAZ/CTX+FQ+SXT+F+FOT	2
AMP+CAZ/CTX	1
AMP+F	1
AMP+CN+FQ	1
CN+FQ+SXT	1
AMP+CN+CAZ/CTX+FQ	1
AMP+AMC+FQ+FOT	1
AMP+AMC+CN+SXT	1
AMP+AMC+CAZ/CTX+CN+SXT	1
AMP+AMC +CN+FQ+SXT+F	1
AMP+AMC+CAZ/CTX+FQ+SXT+F	1
AMP+AMC+CN+FQ+SXT+FOT	1
AMP+AMC+CAZ/CTX+CN+FQ+FOT	1
AMP+AMC+CN+FQ+F+FOT	1
AMP+AMC+CAZ/CTX+CN+FQ+SXT+F	1
AMP+AMC+CAZ/CTX+CN+FQ+SXT+F+FOT	1

Legend: AMP, ampicillin; AMC, amoxicillin with clavulanic acid; CAZ, ceftazidime; CTX, cefotaxime; CN, gentamicin; F, nitrofurantoin; FOT, fosfomycin-trometamol; FQ, fluoroquinolone; SXT, trimethoprim-sulfamethoxazole

to fluoroquinolones and sulphonamides were also reported [12].

Regarding the effectiveness of third generation cephalosporins as first-line agents for UTI treatment, it seems that it began to be compromised by the spread of ESBL-producing *E. coli* strains. The results of our current studies are in concordance with other reports about the rise of such *E. coli* strains in Europe [13] and they were not a surprise as in one of our previous studies that focused on the characteristics of the fluoroquinolone-resistant *E. coli*, we found that more than half of the strains displayed an ESBL phenotype which was explained by the presence of the $bla_{CTX\text{-}M}$ genes [14]. Already recognized as pandemic [15], the CTX-M beta-lactamases may most probably be expressed by the urinary strains currently reported but this aspect remains to be demonstrated by further investigations. Meanwhile, another important finding of our study was the high rate of sensitivity of the Romanian strains to fosfomycin and nitrofurantoin, two drugs which seem to be still quite effective on *E. coli* and are recommended for treatment of uncomplicated cystitis in premenopausal women and uncomplicated pyelonephritis by the European Association of Urology [16].

Our study has several limitations such as its retrospective nature and reliance on a preexisting culture collection for analysis which is definitely not representative for the *E. coli* population from community. Nonetheless, due to the scarcity of Romanian published information about the antibiotic resistance temporal trends of *E. coli* affecting people in the community, we consider that data on the prevalence of resistant members provide a useful reference for disease management decisions.

In conclusion, according to the results of our study, whereas fosfomycin and nitrofurantoin have preserved their overall *in vitro* efficacy and represent effective therapeutic options, trimethoprim-sulfamethoxazole and ampicillin even in combination with beta-lactamase inhibitor should not be recommended for empiric treatment of UTI in locals. At the same time, given the concerning findings that the local rate of resistance to fluoroquinolones is high and ESBL-producing strains are beginning to limit the use of extended-spectrum cephalosporins, surveillance studies to monitor *in vitro* susceptibilities for these antibiotics are particularly important for identifying further changes among urinary tract *E. coli* isolates and guide the empiric treatment of infections of locals in community.

Conflict of interests: None to declare.

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