

Antimicrobial Susceptibility Patterns of *Escherichia coli* among Tunisian Outpatients with Community-Acquired Urinary Tract Infection (2012–2018)

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Key Words

Antimicrobial susceptibility • *Escherichia coli* •
Community-acquired urinary tract infection • Epidemiology

Abstract

Introduction: Community-acquired urinary tract infection is one of the most common reasons for consultation in everyday practice; it represents a major source of antibiotic consumption. *Escherichia coli* (*E. coli*) is the main pathogen incriminated. **Objective:** The aim of this study was to evaluate antimicrobial susceptibility patterns of community-acquired uropathogenic *E. coli* throughout a 7-year period. **Methodology:** All strains of *E. coli* isolated from urine samples between January 1st 2012 and December 31st 2018 were included. Presence of $\geq 10^3$ CFU/ml in urine culture media was considered as significant for urinary tract infection. The identification of *E. coli* strains was realized using standard laboratory techniques. Antibiotic susceptibility testing was performed using the disk diffusion method according to the CA-SFM/EUCAST criteria. **Results:** A total of 1,335 *E. coli* strains were isolated. Overall susceptibility rates to antimicrobial agents were as follows: ampicillin 39.1%, amoxicillin-clavulanic acid 64.9%, cefotaxime 94.9%, trimethoprim/sulfamethoxazole 67.6%, ciprofloxacin 89.2%, ofloxacin 86.9%, amikacin 98.6%, gentamicin 93.9%, nitrofurantoin 97.6% and fosfomy-

cin 99.3%. All isolates were susceptible to carbapenems. The frequency of extended spectrum beta-lactamases-producing *E. coli* strains was 4.7%. Susceptibility rates of *E. coli* for ampicillin, trimethoprim/sulfamethoxazole and amikacin remained relatively stable over the study period, whereas susceptibility to amoxicillin-clavulanic acid, cefotaxime and fluoroquinolones showed a 2-phase pattern. As for gentamicin, a continuous decrease in susceptibility rates was observed. **Conclusion:** Antimicrobial susceptibility profiles of uropathogenic *E. coli* are constantly changing, due to modifications in the antibiogram interpretation criteria and antibiotic prescription habits. Rigorous surveillance of resistance rate is necessary to determine appropriate empirical treatment and limit the spread of multiresistant strains.

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Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases in clinical practice. It is the second most common bacterial infection managed in primary care, accounting for approximately 8.1 million visits to health care providers each year [1, 2]. UTI affects mostly women, with an estimated two in every three

women experiencing at least one episode of UTI during a lifetime [3, 4]. Different bacteria are implicated; *Escherichia coli* (*E. coli*) remains by far the most common uropathogen, accounting for up to 90% of community-acquired and 50% of hospital-acquired UTIs [5–7].

To treat UTI, an empirical antibiotic treatment is frequently initiated, since antibiotic susceptibility necessitates a minimum of 48 h for testing [8]. However, this strategy of treatment leads to the emergence of resistance to several first-line antimicrobial agents, multidrug resistance and extended spectrum beta-lactamases (ESBLs), which are raising major concern worldwide [7, 9]. To control the increasing prevalence of antibiotic resistance, experts recommend that resistance rates against antibacterial drugs should not exceed 10–20% for starting empirical treatment [10]. This rate is set, by the French society of infectious diseases guidelines, at 20% for uncomplicated cystitis and 10% for acute pyelonephritis, male UTI, cystitis in pregnancy, and other cystitis presentations at risk of complication [11]. For that reason and in order to choose the appropriate antimicrobial empirical treatment, knowledge of region-specific antimicrobial susceptibility patterns that is based on up-to-date epidemiological data is essential [8].

In Tunisia, *E. coli* was isolated in 67.1–73% of urine samples with criteria of UTI [12, 13]. Its antimicrobial susceptibility patterns were not well known; isolates were characterized by high acquired resistance to beta-lactams and trimethoprim/sulfamethoxazole (TMP/SXT) and ESBLs were reported in 1–3% of cases [12, 13]. However, these data were recorded especially from inpatients and may not reflect susceptibilities in community-acquired infections. Therefore, this study was undertaken to evaluate the antimicrobial susceptibility patterns of *E. coli* strains isolated from community-acquired UTIs in our institution.

Materials and Methods

Collection and Identification

It was a retrospective study conducted during the period January 1st 2012 and December 31st 2018. It included all non-redundant uropathogenic *E. coli* strains isolated from urine samples collected from outpatients with UTI. Patients were referred from consultation units of the hospital and the sanitary structures affiliated to it.

There were 10 µL of each sample that were inoculated by calibrated wire loop, onto a chromogenic agar and incubated during 18–20 h at 35 ± 2°C. Bacterial growth was determined and considered positive at ≥ 10³ CFU/ml. Samples showing growth of more than 2 bacterial species were excluded.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of the *E. coli* isolates was determined according to the Antibiogram Committee of the French Society for Microbiology/European Committee on Antimicrobial Susceptibility Testing (CA-SFM/EUCAST) criteria using the disk diffusion method [14]. This involved Mueller-Hinton agar, an inoculum of 0.5 McFarland and an incubation for 20 ± 4 h at 35 ± 2°C.

The following antimicrobial agents were tested: ampicillin (10 µg), amoxicillin/clavulanic acid (AMC) (20 µg for amoxicillin and 10 µg for clavulanic acid), cefotaxime (5 µg), ceftazidime (10 µg), ertapenem (10 µg), imipenem (10 µg), TMP/SXT (1.25–23.75 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), nitrofurantoin (100 µg) and fosfomycin (200 µg). *E. coli* ATCC 25922 was used as a control strain. The breakpoints for susceptibility were in accordance with the CA-SFM/EUCAST guidelines and the results were reported as sensitive, intermediate or resistant. For analysis purposes, isolates categorized as 'intermediate' were included in the 'resistant' category.

ESBL production among the isolates was determined using the disk diffusion method suggested by the CA-SFM/EUCAST. The presence of ESBL was confirmed by a synergy test between AMC and a third or fourth generation cephalosporin.

Statistical Analysis

The present study used SPSS software version 21.0 (IBM Corporation, NY, USA) for the statistical analysis. Comparison of percentages was performed by the Chi-square test. We analyzed susceptibility patterns by using the Spearman's correlation test. A p-value less than 0.05 was considered statistically significant.

Results

A total of 1,335 *E. coli* strains were identified; they were isolated from female patients in 97% of cases (n = 1,294).

Isolates were fully susceptible to all antimicrobials tested in 32.6% of cases (n = 432). There were 261 cases (19.5%) that were resistant to only 1 agent, 352 (26.4%) to 2 agents and 287 (21.5%) to 3 or more agents. Overall, susceptibility rates of *E. coli* were the following: ampicillin 39.1% (n = 522), AMC 64.9% (n = 867), third-generation cephalosporins (cefotaxime, ceftazidime) 94.9% (n = 1,267), carbapenems 100%, gentamicin 93.9% (n = 1,254), amikacin 98.6% (n = 1,316), ofloxacin 86.9% (n = 1,160) and ciprofloxacin 89.2% (n = 1,191). *E. coli* isolates were susceptible to TMP/SXT in 67.6% (732/1,082) of cases and to nitrofurantoin in 97.6% (1,118/1,146) of cases. Only 9 isolates (0.7%) were resistant to fosfomycin.

ESBL phenotype was identified for 4.7% isolates (n = 63); these strains exhibited a significantly higher resistance level to other antimicrobial agents except for fosfomycin and nitrofurantoin (table 1).

Table 1. Susceptibility of ESBL and non-ESBL isolates to antimicrobial agents

Antibiotic	Susceptibility of ESBL strains (n = 63)		Susceptibility of non-ESBL strains (n = 1,272)		p
	n	%	n	%	
Ciprofloxacin	24	38.1	1,167	91.7	< 0.001
Ofloxacin	23	36.5	1,137	89.4	< 0.001
Gentamicin	31	49.2	1,223	96.1	< 0.001
Amikacin	56	88.9	1,264	99.4	< 0.001
TMP/SXT	20/55	36.4	712/1,027	69.3	< 0.001
Fosfomycin	63	100	1,263	99.3	0.640
Nitrofurantoin	53/55	96.4	1,065/1,091	97.6	0.694

Table 2. *E. coli* susceptibility rates by antibiotic and by year

Antibiotic	2012 (n = 224)	2013 (n = 203)	2014 (n = 176)	2015 (n = 191)	2016 (n = 161)	2017 (n = 197)	2018 (n = 183)
Ampicillin, n (%)	97 (43.3)	72 (35.5)	56 (31.8)	69 (36.1)	64 (39.8)	78 (39.6)	86 (47)
Amoxicillin/clavulanic acid, n (%)	161 (71.9)	135 (66.5)	102 (58)	109 (57.1)	95 (59)	131 (66.5)	133 (72.7)
Cefotaxime, n (%)	220 (98.2)	195 (96.1)	162 (92)	175 (91.6)	151 (93.8)	185 (93.9)	178 (97.3)
Imipenem/ertapenem, n (%)	224 (100)	203 (100)	176 (100)	191 (100)	161 (100)	197 (100)	183 (100)
Ofloxacin, n (%)	200 (89.3)	183 (90.1)	141 (80.1)	152 (79.6)	139 (86.3)	176 (89.3)	169 (92.3)
Ciprofloxacin, n (%)	204 (91.1)	191 (94.1)	145 (82.4)	162 (84.8)	145 (90.1)	178 (90.4)	166 (90.7)
Amikacin, n (%)	222 (99.1)	200 (98.5)	172 (97.7)	190 (99.5)	161 (100)	196 (99.5)	179 (97.8)
Gentamicin, n (%)	214 (95.5)	195 (96.1)	168 (95.5)	180 (94.2)	149 (92.5)	181 (91.9)	167 (91.3)
Fosfomycin, n (%)	224 (100)	202 (99.5)	176 (100)	191 (100)	159 (98.8)	195 (99)	179 (97.8)
TMP/SXT, n (%)	133/183 (72.7)	127/189 (67.2)	113 (64.2)	131 (68.6)	106/146 (72.6)	122 (61.9)	not tested
Nitrofurantoin, n (%)	181/181 (100)	201 (99)	171 (97.2)	185 (96.9)	85/87 (97.7)	123/125 (98.4)	172 (94)

Evolution of susceptibility rates of *E. coli* isolates to antibiotics during the study period was represented in table 2.

Susceptibility rates of *E. coli* for ampicillin remained relatively stable, whereas susceptibility to AMC and cefotaxime showed a 2-phase pattern, a significant decrease between 2012 and 2015 ($r_s = -0.964$; $p = 0.036$ for AMC and $r_s = -0.961$, $p = 0.039$ for cefotaxime), followed by a gradual increase.

Susceptibility rates of *E. coli* to fluoroquinolones were also characterized by a 2-phase pattern: a decrease in susceptibility with a resistance rate that doubled between 2012 and 2014 then a significant increase ($r_s = 0.963$; $p = 0.008$ for ofloxacin and $r_s = 0.917$; $p = 0.028$ for ciprofloxacin). As for gentamicin, a continuous and significant decrease in susceptibility rates was recorded ($r_s = -0.937$; $p = 0.002$).

Figure 1 represents the variation of the rate of ESBLs during the study period; a peak of enzyme production was observed in 2015 (fig. 1).

Discussion

This observational study conducted during 7 years showed that there was a high resistance to antibiotics commonly used to treat UTI caused by *E. coli* in Tunisia. Obtained results are very important to evaluate the effectiveness of the therapeutic strategy used for UTIs in the country and to adapt it to current epidemiological data. The most common antibiotic resistance rate was observed for ampicillin and AMC. These results are consistent with those reported throughout the world, showing that ampicillin was the least active antimicrobial agent against *E. coli*; with resistance rates ranging between 50 and 75% [10, 15–18]. However, these rates vary widely between regions; they can rise up to 89% in developing countries while in European countries, they were estimated to be between 21 and 34% [6, 19]. These high levels of *E. coli* resistance to ampicillin may be a consequence of frequent and inappropriate use of this antibiotic in empirical therapy. That's why, ampicillin is

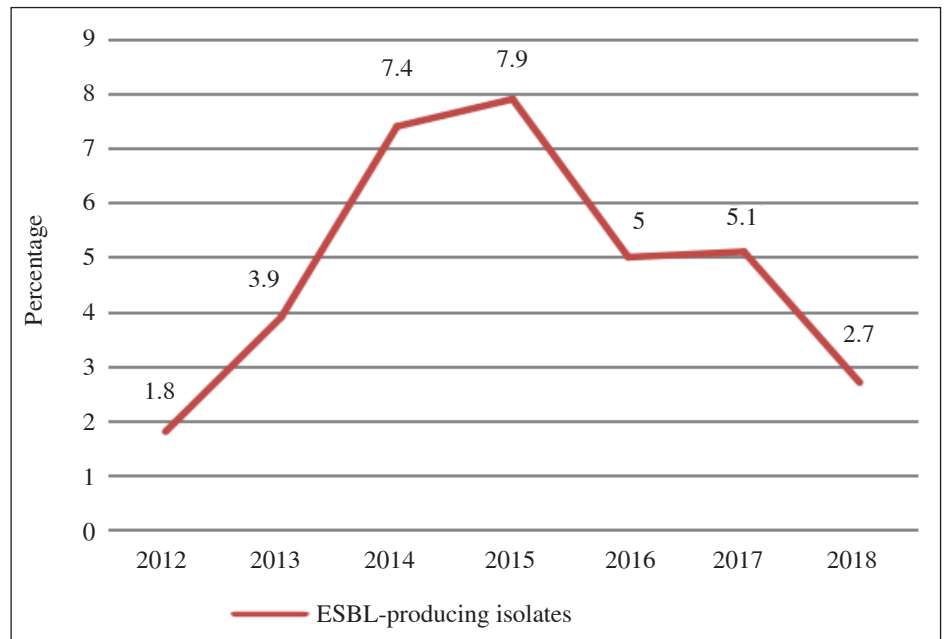


Fig. 1. Number of ESBL-producing *E. coli* per year.

no longer recommended for empirical treatment of UTIs [10]. As for AMC, the acquisition of resistance to this antibiotic is also a worldwide phenomenon reported with variable rates, estimated at 18.9% in Portugal, 43% in Morocco and 64.9% in Iran [4, 6, 20]. Activity of AMC against uropathogen *E. coli* evolved in a 2-phase pattern during the study period with an increase since 2015; the change in interpretation criteria might be behind this evolution. In fact, during this year, the CA-SFM/EUCAST has increased breakpoints for AMC. Using new breakpoints for cystitis has lowered resistance to AMC from 40 to 10% [11].

Fluoroquinolones have been largely prescribed in the treatment of UTI, particularly in the empirical treatment of uncomplicated acute cystitis in women [20]. As reported previously in Tunisia, more than 85% of isolates were susceptible to ciprofloxacin and ofloxacin [15]. In European countries, susceptibility rates ranged between 0.5 and 7.6% [19], while in Turkey, 50% of *E. coli* isolates were resistant to ciprofloxacin [10]. Resistance to fluoroquinolones has become a growing concern worldwide [11]. Increased bacterial resistance is the consequence of increased consumption with a well-documented excess risk of resistance in subjects exposed to fluoroquinolones within the previous 6 months [11, 20]. Susceptibility to fluoroquinolones evolved in a 2-phase

pattern. These findings can be attributed to a decrease in fluoroquinolone prescription by physicians following the implementation of new guidelines [16]. The increase in fluoroquinolone resistance has been highlighted by the French Infectious Diseases Society guidelines in 2017, which recommended the use of fluoroquinolones for the treatment of cystitis only after antibiotic susceptibility testing and not on an empirical basis [11].

As reported previously, gentamicin and amikacin continued to be active against uropathogenic *E. coli* [4, 15, 17]. Susceptibility rates to gentamicin ranged between 99.7 [19] and 63.4% [21]. Resistance has been attributed to the erroneous use of a single dose of gentamicin for the ambulatory treatment of community-acquired UTIs [10]. For amikacin, susceptibility rates are ranging between 96.1 [4] and 100% [22]. French Infectious Diseases Society guidelines recommend using these molecules in association with intravenous 3rd generation cephalosporin for the treatment of complicated forms of pyelonephritis [11].

Evaluation of susceptibility to TMP/SXT showed a high resistance level, exceeding the agreed threshold of 20%, which eliminates this antibiotic as a first-line treatment for UTIs. Similar results were reported previously [15, 17]; resistance rates to TMP/SXT varied largely between countries, ranging from 19.6% in France [18]

to 70.4% in Ethiopia [1]. Even though the susceptibility rate to TMP/SXT in this study remained consistent over the study period, certain authors recorded an increase in susceptibility, attributed to a decreased use of this antibiotic [10, 16].

In this study, 4.7% of isolates produced ESBLs; an increase of prevalence was observed from 2012 to 2015 followed by a decrease since 2016. The rate reached 2.7% in 2018. Previously, Tunisian prevalence rates of this type of isolates varied from 0.6 to 10.2% [23]. An increase of prevalence was reported from 1.6 to 10.2% in the governorate of Sfax (south of Tunisia) between 2004 and 2015 [23]. An increase in the prevalence of ESBL-producing *E. coli* was also reported in other countries with rates ranging from 2.5 to 24% [10, 15, 16, 20, 24]. It is important to emphasize that in 2016, Tunisian guidelines for the treatment of community-acquired UTIs in adults were revised. It is recommended to use nitrofurantoin, fosfomycin or pivmecillinam for the empirical treatment of cystitis. Beta-lactams should not be used in the first-line treatment but only after susceptibility testing results [23]. The successful implementation of these guidelines may explain the decrease in ESBLs since this date. However, these findings need to be confirmed by studies evaluating the changes in prescription rates of antimicrobial agents. Anyway, it is well known that the use of broad spectrum antibiotics facilitates the emergence of ESBL-producing microorganisms [16]. The emergence of ESBL-producing organisms among community isolates has forced the clinical microbiology laboratories to check for their presence compulsorily. ESBL production is frequently associated with other acquired resistance towards fluoroquinolones, aminoglycosides and TMP/SXT, which are considered to be important treatment alternatives. This situation creates problems in clinical use [10]. However, ESBL-producing isolates are still highly susceptible to carbapenems. These molecules continue to be active against uropathogenic *E. coli*; they should be preserved and used only as a final alternative in case of multidrug resistance [17, 20, 22].

Only few isolates of *E. coli* included in this study were resistant to nitrofurantoin and fosfomycin. These 2 molecules remain active against uropathogenic isolates; that's why they have been repositioned by several guidelines as first-line antibiotics for the management of UTIs instead of TMP/SXT, fluoroquinolones and beta-lactams [2]. For nitrofurantoin, reported susceptibility rates exceeded 80%; it was estimated to be 83.6% in India, 88% in Morocco and 94.1% in Iran [6, 17, 20]. These rates ranged from 96 to 99% in western countries [4, 7, 18]. As for

fosfomycin, a low resistance rate was reported (3.4% in Portugal) [4]. Some studies, even, failed to detect *E. coli* stains resistant to this molecule [15]. In addition to the advantage of a single dose administration, this antibiotic has limited toxicity and side-effects on the microbiota [2]. However, the increased consumption of these 2 molecules since their reintroduction as first-line therapy for uncomplicated UTI may lead to the emergence of resistant strains in upcoming years [25]. In the current study, resistance rates to nitrofurantoin increased from 2012 to 2018. This was also reported in the US, where this rate increased from 0.8 to 1.6% between 2000 and 2010 [26].

Conclusion

In conclusion, the emergence of antimicrobial resistance among uropathogenic *E. coli* to penicillin, fluoroquinolones and TMP/SXT and the rise of ESBL-producing organisms limited the use of these drugs as first-line treatment of UTIs. However, due to high susceptibility, fosfomycin and nitrofurantoin could be considered as appropriate antimicrobials for empirical therapy of UTIs in the country. Nevertheless, regular and continuous monitoring of antibiotic susceptibility of the most common uropathogen, *E. coli*, is necessary in order to optimize empirical treatment and control antimicrobial resistance.

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