AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

69 (2022) 1, 46–55

DOI:
10.1556/030.2022.01630

RESEARCH ARTICLE

Detection of AmpC and ESBL-producing *Enterobacterales* isolated from urinary tract infections in Tunisia

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Received: October 13, 2021 • Accepted: January 3, 2022
Published online: January 17, 2022

ABSTRACT

Urinary tract infections (UTIs) are the most frequent human infections in community and hospitals. This study aimed to determine the distribution of bacterial uropathogens among urinary tract infections diagnosed within the regional hospital Houcine Bouzaiene (Gafsa, South West Tunisia) during a survey of 54 days from the 8th of November to the 31st of December 2017. *Enterobacterales* strains were tested for antimicrobial resistance by disk diffusion method and extended-spectrum β-lactamase (ESBL) production was tested by double-disc synergy test. Strains were further subjected to a molecular assessment of ESBL and AmpC β-lactamase production by PCR.

Overall, 173 bacterial isolates were studied, out of which 91.3% were *Enterobacterales*. *Escherichia coli* was the dominant pathogen, followed by *Klebsiella pneumoniae*. High to moderate resistance rates were observed, ranging from 66% to 90.7% for penicillins, from 6.7% to 18.6% for cephalosporins and from 16.2% to 25.4% for fluoroquinolones. *Enterobacterales* with decreased susceptibility to third-generation cephalosporins (3rd GC) carried several resistance genes: *bla*CTX-M group 1 and group 9, and ACC and FOX AmpC β-lactamase genes. Overall, ESBLs and AmpC β-lactamases were detected in 57% and 14% of the 3rd GC-resistant isolates, respectively.

This study proved the high potential of *K. pneumaniae* species to develop resistance against commonly used antibiotics. Thus, rigorous monitoring of the antibiotic resistance of clinical pathogens have to be implemented in Tunisia. Our results are very relevant to evaluate efficiency of the Tunisian therapeutic strategies against UTIs and adapt them to the emerging problem of antimicrobial resistance.

KEYWORDS

urinary tract infection, UTI, antimicrobial resistance, third-generation cephalosporin, ESBLs, AmpC

INTRODUCTION

Antimicrobial resistance is defined as a resistance to an antimicrobial agent to which the microorganism was originally susceptible. Antimicrobial resistance has become a serious threat to public health since medicines are becoming less effective. Drug-resistant pathogens cause 700,000 deaths every year [1–3]. A particular concern is addressed to the developing countries where the control of bacterial resistance is limited and antibiotics are being

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purchased without prescription to treat community-acquired infections [4, 5]. In fact, the misuse of antimicrobials is the leading cause of the development of drug-resistant organisms [1–3, 6–8]. During the last decades, a significant increase in bacterial resistance to β-lactams was noticed, particularly for third-generation cephalosporins (3rd GC) which are expensive broad-spectrum antibiotics, used as last line medicines [2, 6]. In Gram-negative bacteria, the main mechanism of this resistance is the production of β-lactamases, such as the extended-spectrum β-lactamases ESBLs [9–12]. However, AmpC β-lactamases, which are less common than the ESBLs, have higher stability and are broader in spectrum [9, 11].

In Tunisia, few studies investigated antimicrobial susceptibility of uropathogenic bacteria and some of them evaluated the evolution of antimicrobial resistance over the years [13–17]. However, information about the southern regions is very limited [18, 19]. Moreover, the studies mentioned above are observational and did not investigate the genetic determinants encoding antimicrobial resistance.

Thus, the present work was conducted to study *Enterobacterales* uropathogens diagnosed in a regional hospital in south Tunisia for their antimicrobial susceptibilities and the presence of genes encoding resistance to beta-lactams through the screening of ESBL and AmpC β-lactamases.

MATERIALS AND METHODS

Study localization and concerned population

The study involved all urine samples sent to the microbiological laboratory of the regional hospital Houcine Bouzaiene (Gafsa, South-West Tunisia) during the period from the 8th of November to the 31st of December 2017. Urine samples were gathered from outpatients addressed by community structures and hospitalized patients (inpatients) from eight departments (urology, intensive care unit, cardiology, medical oncology, pediatrics, gynaecology-obstertrics, women's department, men's department). In women's and men's departments, women and men suffering from other diseases are hospitalised.

Bacteriological study

Urinary tract infections were detected through a cyto-bacteriological analysis in which the cytological test examines leukocyturia and the presence of erythrocytes, cylinders and crystals. Only urine cultures with one germ were considered.

Antimicrobial susceptibility testing

Susceptibility tests were determined with the Kirby Bauer disc diffusion tests and interpretations followed the EUCAST guidelines (European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters, Version 2.0). For each isolate, the number of the tested antibiotics depends on their availability in the laboratory. ESBL production was examined by the double-disc synergy test [14].

Genomic DNA extraction

Bacterial-DNA extraction was performed by boiling several pure colonies in 400 µl of sterile water for 10 min. The DNA was recovered in the supernatant.

Detection of β-lactamases encoding genes

Multiplex PCR reactions were used to detect the presence of ESBLs and AmpC genes. The blaCTX-M group 1, group 2, group 9 and group 8/25 were determined using specific primers [20]. The latter set of genes covers all known $bla_{CTX\text{-}M}$ subtypes. The GES, PER and VEB minor ESBL-types and the KPC, IMP and VIM genes encoding β-lactamases conferring resistance to carbapenems were also studied [20]. Six family genes specific to AmpC (ACC, FOX, MOX, DHA, CIT and EBC) were amplified via two multiplex PCR reactions [20].

Molecular relatedness study of ESBL-Producing strains

Genetic relatedness of ESBL-producing isolates expressing *in vitro* ESBL activity was analyzed using the pulsed-field gel electrophoresis (PFGE) adapted from Ribot and collaborators protocol [21]. The agarose blocks holding the genomic DNA previously incubated with the XbaI enzyme, were loaded within the agarose gel (Gibco BRL, Paisley, Ecosse) along with a lambda ladder (lambda DNA cI857 ind 1 Sam7, GelSyringe™, UK) (1.5% m/v in 0.5 X TBE buffer). Then, a transverse alternative field electrophoresis was applied as follows: 200 V, 2 s of initial switch time, 20 s of final switch time, and 18–24 h of migration at 14 °C (CHEF-DR® II; Biorad, USA). After migration, the gel was stained at 1 µg ml^{-1} ethidium bromide before visualization.

The PFGE patterns were analyzed (Bionumerics version 6.5; Applied Maths, Belgium) and clustered according to the similarity criterion determined with the Dice coefficient of similarity. Hierarchical clustering was performed based on the UPMGA clustering algorithm. To analyze the genetic relatedness, a cut off line at 85% was considered [22, 23].

Statistical analysis

The statistical analysis was performed with the SPSS program v. 13.0 (SPSS, IBM, Armonk, NY) using the Chi-squared or Fisher's exact tests to compare percentages. Statistical significance was considered for a P-value < 0.05.

RESULTS

Bacteriological profile

During the 54 days of this survey, 1220 urine samples were recovered. These were distributed as follows; 314 from male and 906 from female. Positive cultures were obtained from 195 samples (15.98%). Most of the positive urine samples

Table 1. Distribution of *Enterobacterales* isolated from urine during 54 days at the hospital Houcine Bouzaiene, Gafsa, Tunisia

Enterobacterales	External Outpatients department	Women's department	Pediatrics	Urology	Gynaecology-obstetrics	Oncology	Total
E. coli	99	2	3	0	2	0	106
K. pneumoniae	28	1	1	1	0	0	31
K. oxytoca	2	0	0	0	0	0	2
P. mirabilis	4	0	0	0	1	0	5
Enterobacter spp.	4	0	0	0	0	0	4
E. aerogenes	2	0	0	0	0	0	2
P. rettgeri	1	0	0	0	0	0	1
C. diversus	6	0	0	0	0	1	7
Total	146	3	3	1	3	1	158

came from female patients (87.2%; 170/195) (sex ratio = 0.15). Contamination frequencies of 18.76% (170/906) and 7.96% (25/314) were observed for female and male patients, respectively.

When considering the recovered 173 isolates, most of the positive samples (91.32%; 158/173) came from the outpatients' department. The remaining 15 isolates were from hospitalized patients and were distributed as follows: pediatrics (n = 5); woman Medicine (n = 4); gynaecology-obstetrics (n = 4), urology (n = 1) and medical oncology (n = 1) departments (data not shown).

The uropathogenic isolates were dominantly Gram-negative bacteria (94.21%; 163/173), consisting of *Enterobacterales* (n = 158), *Acinetobacter* spp. (n = 3) and *Pseudomonas* spp. (n = 2) (Data not shown). Within *Enterobacterales*, *Escherichia coli* was the most prevalent species (67.1%; 106/158), followed by *Klebsiella pneumoniae* (19.6%; 31/158), *Citrobacter diversus* (4.4%; 7/158) and *Proteus mirabilis* (3.2%; 5/158). The other enterobacterial species were represented by less than 5 isolates (<3%) (Table 1).

Antimicrobial susceptibility testing

Resistance profiles and frequencies among *Enterobacterales* isolates were analyzed and summarized in Tables 2 and 3. When testing fluoroquinolones, 16.2% and 25.4% of the isolates were non-susceptible to ciprofloxacin and levofloxacin, respectively (Table 2). Gentamycin and amikacin

Table 2. Antimicrobial resistance rates of *Enterobacterales* to fluoroquinolones, aminoglycosides and fosfomycin

	Fluoroquinolones		Aminoglycosides		Others
	Ciprofloxacin CIP	Levofloxacin LEV	Gentamicin GEN	Amikacin AK	Fosfomycin FOS
Species (*N*)			*n/N′* *R (%)*		
E. coli (106)	12/76	11/32	10/86	NT	46/105
	15.8	34.4	11.6	-	43.8
K. pneumoniae (31)	3/16	2/15	2/20	1/9	28/30
	18.5	13.3	10	11.1	93.3
K. oxytoca (2)	NT	1/2	NT	NT	1/2
	-	50	-	-	50
P. mirabilis (5)	0/3	0/2	0/4	NT	2/3
	0	0	0	-	66.7
Enterobacter spp. *(4)*	0/4	NT	0/4	0/1	3/4
	0	-	0	0	75
E. aerogenes (2)	NT	0/2	1/1	NT	2/2
	-				
P. rettgeri (1)	0/1	NT	0/1	0/1	0/1
		-	0	0	0
C. diversus (7)	2/5	0/2	2/6	0/4	4/6
	40	0	33.3	0	66.7
Total (158)	17/105	14/55	15/122	1/15	86/153
	16.2	25.4	12.3	6.7	56.2

N total number of the isolated strains.
N′ Number of strains tested for antimicrobial resistance.
n Number of resistant strains.
R (%); Resistance percentages; calculated by dividing the number of resistant isolates on the number of the tested isolates; (n/N′) × 100. When calculating resistance percentages, the intermediate category was considered susceptible.
NT not tested.

Table 3. Resistance of *Enterobacterales* to β-lactam antimicrobial agents

	Penicillins				Cephalosporins						Monobactams	Carbapenems	
	Amoxicillin AX	Ticarcillin TIC	AMC	TZP	Cephalexin CL	Cefotaxime CTX	Ceftazidime CAZ	Ceftriaxone CRO	Cefexime CFM	Cefoxitin FOX	Aztreonam ATM	Ertapenem ETP	Imipenem IPM
Species (*N*)							$\frac{n/N'}{R\ (\%)}$						
E. coli (106)	85/97	44/75	27/103	0/46	9/44	0/16	15/76	8/32	20/106	3/43	3/39	1/40	0/75
	87.6	58.7	26.2	0	20.4	0	19.7	25	18.9	7	7.7	2.5	0
K. pneumoniae (31)	26/26	15/15	5/30	1/16	1/16	2/5	4/15	1/16	4/31	1/10	3/10	0/16	0/15
	100	100	16.7	6.2	6.2	40	26.7	6.2	12.9	10	30	0	0
K. oxytoca (2)	NT	NT	1/2	0/2	0/2	NT	NT	0/2	0/2	NT	NT	0/2	NT
	-	-	50	0	0	-	-	0	0	-	-	0	-
P. mirabilis (5)	4/5	3/3	1/5	0/3	0/3	NT	0/3	0/3	0/5	0/1	0/1	0/3	0/3
	80	100	20	0	0	-	0	0	0	0	0	0	0
Enterobacter spp. *(4)*	4/4	1/4	3/4	0/1	NT	0/1	0/2	NT	0/4	0/1	0/1	NT	0/4
	100	25	75	0	-	0	0	-	0	0	0	-	0
E. aerogenes (2)	1/1	NT	2/2	0/2	2/2	NT	NT	1/2	1/2	NT	NT	0/2	NT
	100	-	100	0	100	-	-	50	50	-	-	0	-
P. rettgeri (1)	1/1	0/1	0/1	NT	NT	NT	0/1	NT	0/1	0/1	0/1	NT	0/1
	100	0	0	-	-	-	0	-	0	0	0	-	0
C. diversus (7)	6/6	5/5	2/7	0/2	0/2	0/3	0/5	0/2	0/6	0/4	0/4	0/2	0/5
	100	100	28.6	0	0	0	0	0	0	0	0	0	0
Total (158)	127/140	68/103	41/154	1/72	12/69	2/25	19/102	10/57	25/157	4/60	6/56	1/65	0/103
	90.7	66	26.6	1.4	17.4	8	18.6	17.5	15.9	6.7	10.7	1.5	0

AMC: Amoxicillin/Clavulanic acid; TZP: piperacillin+tazobactam.
N total number of the isolated strains; N' Number of strains tested for antimicrobial resisatnce
n Number of resistant strains.
R (%); Resistance percentages; calculated by dividing the number of resistant isolates on the number of the tested isolates; $(n/N') \times 100$.
When calculating resistance percentages, the intermediate category was considered susceptible.
NT not tested.

(aminoglycosides) were inactive in 12.3% and 6.7% of the tested germs, respectively. Fosfomycin showed low activity with a resistance rate of 56.2% (Table 2).

Antimicrobial susceptibility tests for β-lactams showed that 90.7% and 66% of the tested *Enterobacterales* were not susceptible to amoxicillin and ticarcillin, respectively. The association of amoxicillin and clavulanic acid (AMC) was inactive in 26.6% of the tested strains (Table 2). Piperacillin+tazobactam, ertapenem and imipenem were inactive in 1.4%, 1.5% and 0% of the isolates, respectively. Aztreonam (monobactam) also showed good activity as 10.7% of the tested strain were resistant (Table 2).

When testing cephalosporins, the observed resistance rates were 17.4%, 18.6%, 15.9%, 17.5%, 8% and 6.7% against cephalexin (1st GC), ceftazidime, cefexime, ceftriaxone, cefotaxime and cefoxitin (3rd GC), respectively (Table 3).

Overall, 28 isolates were non-susceptible to 3rd GC. These isolates are distributed as follows: *E. coli* (22/28; 78.6%); *K. pneumaniae* (5/28; 17.8%) and *E. aerogenes* (1/28; 3.6%) (Table 4). Most of these isolates (24 isolates) came from the outpatients' department (community-acquired infections), while the remaining four isolates were recovered from patients hospitalized in pediatrics, women, urology and gynaecology-obstetrics departments (Table 4).

The ESBL production was detected phenotypically in 4.4% of the *Enterobacterales* isolates (7/158) using the double-disk synergy test. Five out of them were identified as *E. coli* strains (5/7; 71.4%), followed by *K. penumoiae* and *E. aerogenes;* each was represented by one strain (Table 4).

Molecular study of the third generation cephalosporin-resistant GNB

The third GC-resistant *Enterobacterales* (n = 28) were examined by PCR for the presence of ESBL or/and AmpC β-lactamase genes. Amplifications revealed that 15 isolates harbored the *bla*CTX-M-group1. One isolate (*E. coli*) harbored the CTX-M-group 9 and all strains lacked the CTX-M groups 2, 8 and 25 (Table 5). Furthermore, four strains carried the ACC and the FOX genes and both genes were detected in a single *K. pneumoniae* isolate showing *in vitro* ESBL production. The latter strain also co-harbored *K. pneumoniae* carbapenemase encoding gene (bla_{KPC}) (Table 5).

Genetic relatedness study of the ESBL producers

The genetic relatedness of *Enterobacterales* strains expressing *in vitro* ESBL activity was studied (Fig. 1). The genomic DNA was digested using the XbaI enzyme and migrated with PFGE. Since *K. pneumoniae* and *E. aerogenes* species were presented each by a single isolate, they were included with the PFGE analysis of *E. coli* strains. *E. coli* isolates (n = 5) exhibited a distinct PFGE type for each strain (5 pulsotypes) when considering that 85% of similarity defines a genetic relationship (Fig. 1).

DISCUSSION

Urinary tract infections commonly affect community and hospitals, particularly in developing countries. Their treatment is becoming more complicated with the increasing antimicrobial resistance of *Enterobacterales*, leading to higher morbidity and mortality. Bacterial agents responsible for UTIs diagnosed in the regional hospital of Houcine Bouzaiene (South Tunisia) from 8th of November to the 31st of December 2017 were studied. Bacteria isolated from positive urine cultures were identified and *Enterobacterales* species were further examined for antimicrobial susceptibilities. Among the received urine samples, 16% yielded positive cultures with a single bacterium. A similar percentage was reported in our previous study [18] and a lower rate (13%) ($P < 0.05$) was reported by Smaoui and collaborators [19] from community-acquired UTIs in Sfax (South Tunisia). Moreover, a higher contamination frequency ($P < 0.05$) was observed among females. Similarly, most of the previous studies reported that the majority of UTIs occur for female patients [14, 18, 24–27]. This is probably due to their shorter urethra and to its proximity to the anus [28]. Similar to several previous studies from Tunisia and elsewhere [5, 10, 18, 24–25, 29–33], UTIs were dominantly caused by *Enterobacterales* bacteria with *E. coli* being the most prevalent species, followed by *K. pneumoniae*.

Table 4. Third generation cephalosporin-resistant *Enterobacterales* distribution among community and hospital acquired infections

	E. coli		*K. pneumoniae*		*E.aerogenes*		*Total*	
3rd GC-resistant isolates	22		5		1		28	
Origin	CA	HA	CA	HA	CA	HA	CA	HA
	20	2 Women department. Gynaecology-obstetrics.	3	2 Urology. Pediatrics.	1	0	24	4
ESBL producers by the double disc test	3	2	1	0	1	0	5	2
CTX-M enzyme producers	11	2	1	1 (Pediatrics)	1	0	13	3
AmpC enzyme producers	2	0	1	0	1	0	4	0

CA community aquired (outpatients).
HA hospital aquired (inpatients).

Table 5. Distribution of resistance genes encoding ESBL and carbapenemase genes among 3rd generation cephalosporins-resistant *Enterobacterales*

Strains designation	*Enterobacterales* species	Phenotypic ESBL production	CTX-M group 1	CTX-M group 2	CTX-M group 9	CTX-M group 8/25	Minor ESBLs genes[1]	Plasmid-mediated AmpC beta-lactamases[2]	Carbapenemase genes[3]
1	*E. coli*	+	+	-	-	-	-	-	-
21	*E. coli*	+	+	-	-	-	-	-	-
22	*E. coli*	+	+	-	-	-	-	-	-
37	*E. coli*[a]	+	+	-	-	-	-	-	-
103	*E. coli*[b]	+	+	-	-	-	-	-	-
14	*E. coli*	-	+	-	-	-	-	-	-
65	*E. coli*	-	+	-	-	-	-	-	-
129	*E. coli*	-	+	-	-	-	-	-	-
98	*E. coli*	-	+	-	-	-	-	-	-
99	*E. coli*	-	+	-	-	-	-	-	-
142	*E. coli*	-	+	-	-	-	-	-	-
123	*E. coli*	-	+	-	-	-	-	ACC	-
120	*E. coli*	-	-	-	+	-	-	-	-
32	*E. coli*	-	-	-	-	-	-	-	-
45	*E. coli*	-	-	-	-	-	-	-	-
61	*E. coli*	-	-	-	-	-	-	-	-
63	*E. coli*	-	-	-	-	-	-	-	-
72	*E. coli*	-	-	-	-	-	-	-	-
131	*E. coli*	-	-	-	-	-	-	-	-
133	*E. coli*	-	-	-	-	-	-	FOX	-
151	*E. coli*	-	-	-	-	-	-	-	-
163	*E. coli*	-	-	-	-	-	-	-	-
5	*K. pneumoniae*	+	+	-	-	-	-	ACC, FOX	KPC
164	*K. pneumoniae*[c]	-	+	-	-	-	-	-	-
165	*K. pneumoniae*[d]	-	-	-	-	-	-	-	-
153	*K. pneumoniae*	-	-	-	-	-	-	-	-
124	*K. pneumoniae*	-	-	-	-	-	-	-	-
20	*E. aerogenes*	+	+	-	-	-	-	ACC	-

The hospital acquired bacteria; a: women's department; b: Gynaecology-obstetrics; c: pediatrics; d: urology.

[1] Minor ESBLs genes; GES, PER and VEB genes.

[2] Plasmid-mediated AmpC beta-lactamases; ACC, FOX, MOX, DHA, CIT and EBC genes.

[3] Carbapenemases; KPC, IMP and VIM genes.

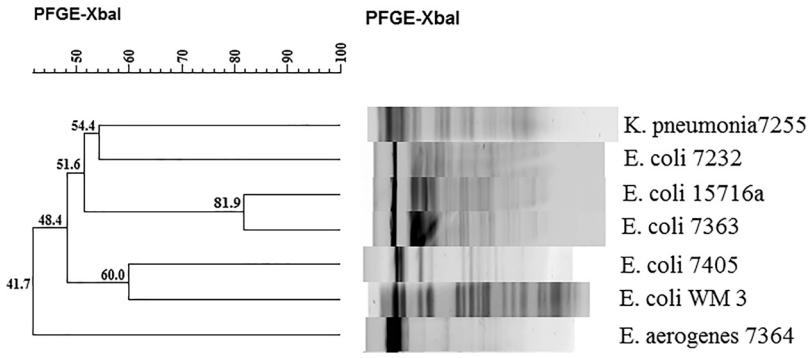

Fig. 1. PFGE dendrogram of ESBL-producing isolates detected by the phenotypic double-disc synergy test

In our study, given the prevalence of *Enterobacterales*, we considered these isolates to be the most interesting for studying antimicrobial resistance. In our earlier study [18], antimicrobial susceptibilities among UTI-bacteria collected from January 2015 to June 2016 were also studied. In both studies, high rates of non-susceptibilities for penicillins were reported, while activities of carbapenems, monobactams and amikacin were very high, just as it was revealed in the literature [26, 33, 34]. In our study, the resistance rates were significantly higher ($P < 0.05$) in *K. pneumoniae* than in *E. coli* isolates against fosfomycin, a broad-spectrum antibacterial agent typically used for UTIs. Additionally, the resistance rates reported in our study for fosfomycin are much higher compared to those found in the literature [27, 35–37] and those of other Tunisian studies [14, 19] where these rates do not exceed 2.2%. This observation may be attributed to the intensive use of this drug following the recommendation of the Tunisian guidelines for the treatment of community-acquired UTIs [38]. Otherwise, according to our study, the decreased susceptibility of UTI isolates to this antibiotic requires the update of these guidelines. Actually, the clinical failure of such routine drugs makes the choice of an effective treatment a complicated procedure [18, 29, 39].

When considering cephalosporins, the overall resistance rates ranged from 6.7% to 18.6%. In Tunisia, some previous studies reported that the resistance rates to 3rd GC in UTI-*E. coli* did not exceed 9% [14, 19]. In our study, these rates were significantly higher ($P < 0.05$) in *K. pneumoniae* than in *E. coli* isolates against cefotaxime. The reported higher resistance rates to all antibiotics classes of *K. pneumoniae* compared to *E. coli*, was previously observed by Navon-Venezia and collaborators [40]. Moreover, our study revealed higher resistance rates ($P < 0.05$) for *K. pneumoniae* against ceftazidime and first-generation cephalosporins compared to our previous study [18]. *K. pneumoniae* rapidly acquires resistance determinants against most of the last-line antibiotics, which is particularly problematic in community-acquired infections. Thus, it is of great importance to monitor 3rd GC prescription and track the spread of resistant pathogens in the community, particularly the multidrug resistant *K. pneumoniae* isolates.

Almost all the Tunisian research studying the antimicrobial resistance of UTI bacteria was observational. Thus, it is important to study the genetic determinants encoding antimicrobial resistances against the most potent antimicrobial agents. In the present paper, uropathogenic bacteria were subjected to a molecular assessment of resistance genes. The presence of ESBL β-lactamases implicated in the non-susceptibility to 3rd GC was examined within resistant *Enterobacterales*. The CTX-M- group 1 was found in 53.6% (15/28) of the enterobacterial isolates non-susceptible to 3rd GC, while the CTX-M-group 9 was detected in one isolate (1/28; 3.6%). The remaining groups were absent. In fact, the CTX-M-group 1 was previously reported as the most prevalent CTX group within bacteria causing UTIs in Tunisia and elsewhere, particularly for *E. coli* and *K. pneumoniae* species [10, 41–46]. Our results are also in line with previous studies reporting the dominance of *bla*CTX-M-group 1 and the low prevalence of CTX-M-group 9 genes among the five groups of CTX-M genes [46, 47]. ESBL production is increasingly being reported as the major mechanism for 3rd GC non-susceptibility. Their spread is a challenging issue because these organisms display additional resistances to many other classes of antibiotics, which complicates therapy of infections.

Actually, when an isolate shows resistance to 3rd GC, the AmpC β-lactamase production should also be suspected. These enzymes are the second most common β-lactamases conferring resistance to 3rd GC [9, 48]. However, AmpC-producing isolates are broader in spectrum (confer resistance to penicillins, cephalosporins, 3rd GC, cephamycins, and monobactams) and are able to develop resistance to more powerful antibiotics such as carbapenems [11, 48, 49], which considerably limits the treatment options. Additionally, their phenotypic identification is not well optimized and the co-existence of the AmpC type and other types of β-lactamases may lead to confusing results, which causes their under-estimation [11]. Thus, the differentiation between ESBL and AmpC β-lactamases by genotypic assessment is critical [11, 50]. In our study, 14.3% of the entrobacterial strains with decreased susceptibility to 3rd GC (4/28), carried an *ampC* gene. Only the ACC and FOX genes were detected, with the ACC-type being the most represented (Table 3). In anterior studies from Tunisia and elsewhere, FOX and ACC genes were not detected in UTI infections [50–56]. To the best of the authors' knowledge, this is the first report on the presence of FOX and ACC *ampC* genes in UTI-bacteria in Tunisia. Interestingly, Tunisian studies

proved that AmpC/ESBL producing bacteria could be found in food, vegetables and wastewater and thus, could be transmitted to humans through foodborne infections [57–59].

It is worth noting that one *K. pneumoniae* strain co-harbored the ACC and FOX *ampC* determinants. The finding that multiple *ampC* genes were simultaneously detected within a *K. pneumoniae* species was similarly observed in Tunisia and elsewhere [26, 52, 54, 56]. The emergence of AmpC-like cephalosporinases in *K. pneumoniae* is due to the notable ability of this species to integrate β-lactamase genes [40].

Interestingly, the *K. penumoniae* strain co-harboring two *ampC* genes along with the CTX-M-group 1 also showed the presence of the KPC gene (for *Klebsiella pneumoniae* carbapenemase) (Table 5). This carbapenemase, which is active against all beta-lactams, is the most prevalent carbapenemase in *K. pneumoniae* in the world [40]. However, this enzyme is still uncommon in Tunisia [51, 60]. This is the first report of a KPC-producing *K. pneumoniae* from UTI in Tunisia. Considering all of these observations, *K. pneumoniae* has become a particular concern since it presents a growing threat to public health [11].

Finally, the genetic relatedness of strains expressing *in vitro* ESBL activity was studied with a PFGE analysis after XbaI restriction. The dendrogram showed that *E. coli* strains were unrelated when considering that 85% is the used threshold to confirm a clonal relationship (Fig. 1). Accordingly, we can conclude that the spread of ESBLs results from the dissemination of resistance genes rather than the spread of a specific clone. Similar interpretations were reported for clinical and uropathogenic ESBL-producing *E. coli* [61, 62]. The rapid spread of resistance genes is a major problem suggesting the implementation of surveillance programs that are not available in a developing country like Tunisia.

CONCLUSION

E. coli and *K. pneumoniae* species were dominantly isolated from UTI infections recovered from the community (Tunisian South-West). Moderate to high rates of antimicrobial resistance were detected for *Enterobacterales* against diverse classes of antimicrobial agents. The molecular analysis of the β-lactamases-producing *Enterobacterales* revealed the presence of CTX-M-group 1, the ACC/FOX *ampC* and the bla_{KPC} genes. A single multi-drug *K. pneumoniae* strain isolated from a community-acquired UTI co-harbored all these genes, representing a real threat given its spread in the community. The expansion of third-generation cephalosporins resistance among common *Enterobacterales* species causing urinary infections makes therapeutic options very limited. Therefore, restrictions should be put on antibiotic prescriptions, particularly in community structures.

DECLARATIONS

Competing interests: The authors declare that they have no competing interests.

Funding: Authors are funded by the Ministry of higher education and scientific research and the ministry of health of Tunisia.

ACKNOWLEDGEMENTS

The authors would like to thank Mr Abdelmajid DAMMAK for his proofreading and correction of the English of the paper.

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