ORIGINAL ARTICLES

Prevalence of CTX-M beta-Lactamases in *Escherichia coli* from community-acquired urinary tract infections and associated risk factors among women in Cameroon

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ABSTRACT

Background: In recent years, a worldwide dissemination of CTX-M beta-lactamase-type in Escherichia coli strains isolates from community-acquired urinary tract infections (CA-UTI) has been observed. However little is known on the prevalence and risk factors of this global threat in developing countries.
Objective: The aim of this study was to study the prevalence and risk factors for CA-UTI in Yaoundé, Cameroon.
Methods: Eighty six patients with urinary *E.coli* infection recruited from 10 health structures in the town of Yaoundé, Cameroon. After taking the first urine, faeces were collected from the patients for the study of the intestinal flora. The sample collection of faeces was done on a selected gel of *enterobacteria* resistant to third generation of cephalosporin. The molecular typing of extended-spectrum β-lactamase (ESBL) was carried out.
Results: Eighty-six strains of *E. coli* from 86 patients were included. We found that 39 (45.3%) strains produced an extended-spectrum beta-lactamase. Among risk factors, previous use of antibiotic and the dry season were associated with the presence of an ESBL-producing strain in the urine. All ESBL were identified as CTX-M. The production of CTX-M was found to be significantly associated with resistance to fluoroquinolones, aminoglycosides and to the association of trimethoprim-sulfamethazole.
Conclusions: The prevalence of CTX-M ESBL in Yaoundé, Cameroon, provides new evidence on the global dissemination of CTX-M and the extent of this phenomenon in developing countries.

Key Words: Urinary tract infection, Community, *Escherichia coli* ESBL, CTX-M, Cameroon.

1. INTRODUCTION

Urinary tract infections (UTIs) are a major public health problem in terms of morbidity and financial cost.[1] There are currently 150 million UTIs per year worldwide and this remains a major health problem in developing countries.[2] These infections are mostly caused by bacteria and acquired in community.[3] Women are mostly affected.[4] *Escherichia coli* is responsible for 75%-90% of all urinary tract infections in community patients. Many studies of antimicrobial susceptibility of *E. coli* strains in urinary commu-

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nity patients were performed in industrialized countries.[3] The global spreading of CTX-M extended-spectrum beta-lactamase (ESBL) in these strains *E.coli* was observed and is been described as pandemic.[5] There is paucity of reports on ESBL in *E. coli* strains in community-acquired UTIs in developing countries.[2,6–8] Few studies have been done on ESBL however they only looked at intestinal carriage.[9–11] The prevalence of CTX-M in *E. coli* strains responsible for community-acquired UTI in Cameroon or Central Africa has not been documented. The aim of our study was to determine the prevalence of bla CTX-M-type in *E. coli* strains in community-acquired urinary tract infections in women as well as its risk factors in Cameroon.

2. METHODS

From May 2011 to April 2012, we recruited all women who had UTI in 10 healthcare facilities (3 public hospitals and 7 laboratories in Yaoundé, Cameroon). Only those who had signed a consent form, filled and questionnaire on clinical data (history of UTIs, antibiotic use in the three preceding months, hospitalization in the last three months and the presence of burn) and demographical data and submitted a sample of stool were included. 86 smples from women with biological signs of *E. coli* UTI (leukocyturia > 10^5 leukocytes per ml of urine) and *E. coli* bacteriuria (> 10^5 UFC per ml of urine) were isolated in Cytine Lactose Electrolyte Deficient and identified by the API 20 E gallery (bioMérieux, Marcy l'Etoile, France). *E. coli* strains were stored in 1 ml of brain heart broth, supplemented with 10% glycerol and frozen at -80°C until shipped together with the stool samples to the Bacteriology Laboratory of the Bichat-Claude Bernard Hospital, Paris, France were analyses were done.

2.1 Antimicrobial drug susceptibility and ESBL confirmatory testing

E. coli urinary strains were reseeded on Trypticase Soja Agar (TSA) incubated at 37°C for 18 to 24 hours, and a re-identification was made using mass spectrometry, MALDI-TOF (Brucker, Bremen, Germany). ESBL phenotype was identified using *E. coli* urinary isolates by the Mueller-Hinton agar disc diffusion method as recommended by the Antibiogram Committee of the French Society for Microbiology (Comité d'antibiogramme de la société française de Microbiologie). Susceptibility to amoxicillin tircacillin, amoxicillin + clavulanic acid, cefotaxime, cefoxitin, ceftazidime, cefepime, ertapenem, nalidixic acid, ofloxacin, ciprofloxacin. gentamicin kanamycin, amikacin, fosfomycin, sulfamthoxazole-trimethoprim were determined using the disk-diffusion method (Bio-Rad, Marnes-la-Coquette, France). Results were interpreted according to the recommendations from the French Society for Microbiology (Comité d'antibiogramme de la société française de Microbiologie).

2.2 Fecal antibiotic activity

Exposition to antibiotics at the time of fecal sampling was defined by the detection of fecal antibiotic activity. Fecal antibiotic activity was detected using a simple microbiological assay, performed as described elsewhere.[12]

2.3 PCR amplification

This was done as decribed previously by Ruppé et al.[2] except that we used 1 colony and 1.7% agar gel on Sybergreen (see Table 1).

Table 1. Primers used to study CTX-M β-lactamases in *Escherichia coli*, Cameroun, 2011-2012

Gene detected	Primer name	Primer sequence (5' → 3')	Reference
^{bla}*TEM*	C	TCG GGG AAA TGT GCG CG	[13]
	D	TGC TTA ATC AGT GAG GCA CC	
^{bla}*SHV*	OS-5	TTA TCT CCC TGT TAG CCA CC	[14]
	OS-6	GAT TTG CTG ATT TCG CTC GG	
^{bla}*CTX-M* group 1	M13U	GGT TAA AAA ATC ACT GCG TC	[13]
	M13L	TTG GTG ACG ATT TTA GCC GC	

2.4 Statistical analysis

We used for analysis the R software, version 3.1.0. Risk factors were identified using univariate analysis with the Chi^2 or Fisher exact tests for qualitative variables of ANOVA for quantitative variables. All variables in the univariate analysis with a *P*-value less than .20 were introduced into a multivariate logistic model, using the backward strategy and testing some possible interactions. Some variables such as: the presence of antibiotics in stool and hospitalization in the past three months were systematically maintained in the model. Statistical differences with a *p*-value less than .05 were considered significant.

2.5 Ethics considerations

The Cameroon National Ethical Committee issued an ethical clearance (approval No. 207/CNE/SE/2011). After the main

investigator had explained and read the project, all patients signed a consent form.

3. Results

3.1 Population characteristics

We recruited a total of 86 patients in 3 government hospitals and seven laboratories. The median age was 33 years (IQR 22.75). Twelve patients (14%) had a history of urinary tract infection. The use of antibiotics at the time of sampling was found in about one third patients and 29.1% in the three months prior to inclusion. 12% of patients were hospitalized in the three months before the inclusion, and 11% of the patients had undergone surgery.

Eighty six *E. coli* strains were isolated. Among these, 74.4% of patients lived in the Center region, 11.6% were from the East, 10.5% from the West, 2.3% from the Littoral and 1.2% from the North West regions respectively.

3.2 Prevalence of antimicrobial drug resistance in *E. coli* strains

Almost half of the strains 39/86 (45.35%) were producing ESBL CTX-M. These strains were co-resistant to quinolones (94.9% to nalidixic acid), to fluoroquinolones (82.1% to ciprofloxacin), to aminoglycosides (97.4% to gentamicin, 87.2% to kanamycin and 30.8% to amikacin), 100% to trimethoprim-sulfamethazole and 7.7% to fosfomycin. No resistance to ertapenem and cefoxitin was observed.

3.3 Beta-lactamase characterization

All thirty nine strains of *E. coli* with an ESBL phenotype were analyzed. All ESBL producing *E. coli* strains were CTX-M of group 1. No strain of *E.coli* had TEM or SHV.

3.4 Risk factors associated with ESBL *E. coli* urinary tract infection

The mean age of patients with ESBL producing *E. coli* infection was 39.8 years against 36.7 years for patients with non-ESBL producing *E. coli* strain infection.

The univariate analysis (see Table 2) showed that factors associated with the occurrence of urinary tract infections were the dry season, the presence of a burn and presence of antibiotic in stool. Age of patients, previous hospitalizations for cases such as diabetes and kidney failure were not significantly associated with the occurrence of ESBL urinary tract infections.

The multivariate analysis as presented in Table 3 showed that the dry season (OR = 17.13, 95% CI [3.1 to 95.0]), the occurrence of a burn (OR = 5.4, 95% CI [1.6 to 18.1]) and the absence of urinary recurrence (OR = 7.7, 95% CI [1.4 to 43.1]) were independently associated with ESBL *E. coli* urinary tract infections. With a p value at the limit of significance, patients who have consumed antibiotics in the last three months were more likely to be infected by ESBL *E. coli* strains (OR = 4.4, 95% CI [1.1-16.5]).

Table 2. Univariate analysis of associated risk factors

	Presence of ESBL		OR.95.CI.	*P*
	Yes	No		
Total	39 (45.4)	47 (54.6)		
Season of the year				
Rainy	2 (5.1)	22 (46.8)	Ref	
Dry	37 (94.9)	25 (53.2)	16.28 (3.51, 75.48)	< .001
Patient's home region				.382
Centre	30 (76.9)	34 (72.3)	Ref	
East	4 (10.3)	6 (12.8)	0.76 (0.19, 2.94)	.686
Littoral	0 (0)	2 (4.3)	0 (0, Inf)	.992
Northwest	0 (0)	1 (2.1)	0 (0, Inf)	.995
West	5 (12.8)	4 (8.5)	1.42 (0.35, 5.76)	.627
Age				
median(IQR)	37 (26,49.5)	32 (23,46.5)	1.0076 (0.9867, 1.029)	.479
Hospital training/Laboratory				
Public	19 (48.7)	27 (57.4)	Ref	
Private	20 (51.3)	20 (42.6)	1.42 (0.61, 3.34)	.42
Burn				
Yes	22 (56.4)	14 (29.8)	Ref	
No	17 (43.6)	33 (70.2)	0.33 (0.13, 0.8)	.014
Lumbar pain				
Yes	9 (23.1)	14 (29.8)	Ref	
No	30 (76.9)	33 (70.2)	1.41 (0.53, 3.74)	.485
Antibiotics at the time of sampling				.948
Yes	12 (30.8)	13 (27.7)	Ref	
No	14 (35.9)	18 (38.3)	0.84 (0.29, 2.41)	.749
Do not know	13 (33.3)	16 (34)	0.88 (0.3, 2.57)	.816
History of urinary tract infections				
Yes	3 (7.7)	8 (17)	Ref	
No	36 (92.3)	39 (83)	2.46 (0.61, 10)	.208
Recurrences of urinary tract infections				
Yes	3 (7.7)	9 (19.1)	Ref	
No	36 (92.3)	38 (80.9)	2.84 (0.71, 11.34)	.139
Risk factors for urinary tract infections				
Yes	23 (59)	23 (48.9)	Ref	
No	16 (41)	24 (51.1)	0.67 (0.28, 1.57)	0.354
Urological history				
Yes	0 (0)	2 (4.3)	Ref	
No	39 (100)	45 (95.7)	13564512.33 (0, Inf)	0.992

(Table 2 continued on page 54.)

Table 2. (continued.)

	Presence of ESBL		OR.95.CI.	*P*
Pregnancy				
Yes	13 (33.3)	13 (27.7)	Ref	
No	26 (66.7)	34 (72.3)	0.76 (0.3, 1.92) 0.569	
Diabetes				
Yes	5 (12.8)	9 (19.1)	Ref	
No	34 (87.2)	38 (80.9)	1.61 (0.49, 5.28)	.431
Kidney infection				
Yes	5 (12.8)	6 (12.8)	Ref	
No	34 (87.2)	41 (87.2)	0.9951 (0.2792, 3.5467)	.994
Hospitalisation in the past three months				
Yes	5 (12.8)	5 (10.6)	Ref	
No	34 (87.2)	42 (89.4)	0.81 (0.22, 3.03)	.754
Use of antibiotics in the past three months				.08
Yes	15 (38.5)	8 (17)	Ref	
No	20 (51.3)	33 (70.2)	0.32 (0.12, 0.9)	.03
Do not know	4 (10.3)	6 (12.8)	0.36 (0.08, 1.64)	.185
Invasive procedure in the past three months				
Yes	5 (12.8)	4 (8.5)	Ref	
No	34 (87.2)	43 (91.5)	0.63 (0.16, 2.54)	.518
Presence of ATB in stool				
Yes	24 (61.5)	19 (40.4)	Ref	
No	15 (38.5)	28 (59.6)	0.42 (0.18, 1.01)	.053

Table 3. Multivariate analysis

Variables	OR.95.CI.	*P*-value
Season of the year		
Rainy	Ref	
Dry	17.13 (3.09, 94.99)	.001
Burn		
No	Ref	
Yes	5.45 (1.64, 18.08)	.006
Recurrences of urinary tract infections		
Yes	Ref	
No	7.73 (1.39, 43.08)	.02
Use of antibiotics in the past three months		.072
No	Ref	
Yes	4.36 (1.15, 16.49)	.03
Do not know	1.35 (0.22, 8.21)	.742
Presence of ATB in stool		
No	Ref	
Yes	2.46 (0.81, 7.53)	.114

4. DISCUSSION

Our study is the first study on *E. coli* producing CTX -M in UTI among women in Cameroon. Although the number of acquired strains may be considered to be limited (n = 86), their level of antibiotic resistance is quite high regarding their community origin. As such, 39 strains of *E. coli* of community origin had an ESBL CTX-M.

Our study was conducted on the same target population and followed a similar protocol as those used in Senegal. The average prevalence found in this country was 6.5%.[15] Meanwhile the prevalence found in Cameroon was 45.3%. This result is higher than the Senegal study carried out 10 years ago.

In Cameroon, all *E. coli* strains isolated CTX-M are co-resistant to other antibiotics families such as aminoglycosides, fluoroquinolones, trimethoprimsulfamethazole. A study in Dakar revealed low resistance rates of Enterobacteriaceae to tromethanolfosfomycin, a form of fosfomycin characterized by a high oral bioavailability.[16] Findings obtained in that study corroborates our results. Fosfomycin may be recommended as a reliable empirical treatment for simple urinary tract infections because of its ease in use (one dose), good tolerance and effectiveness.[17]

The high prevalence of antibiotic resistance in the community would also be associated to the poor hygiene in Cameroon. Since it is densely populated. During the dry season access to drinking water is a major problem. Water certainly promotes the circulation of Enterobacteriaceae strains (be they resistant or not).[18–25] Moreover, access to drinking water is not properly insured in many parts of the city. As such, Enterobacteriaceae from stools can easily affect many people through contaminated water. Many households get water from unhealthy streams used as a dumping ground by residents. A study carried out by De Boeck in Kinshasa (Central Africa) revealed the presence of ESBL producing Enterobacteriaceae in drinking water[26] and another study revealed the presence of *E. coli* in rivers and wells.[21] In a village community in South Guyana, it was also proven that even in the absence of a strong antibiotic pressure, promiscuity promoted the circulation of resistant strains and thus increased the prevalence of carrying them.[27] The uncontrolled consumption of antibiotics may promote the selection of *E. coli* strains producing ESBL, which usually results in the empirical prescription for self-medication, poor adherence of patients to antibiotic therapy, the quality of antibiotics (generic under dosed) and the questionable preservation (street drugs). The prescription of antibiotics is in most cases dispensed without microbiological data, and is therefore based on clinical and also on the patient's ability to pay his/her antibiotics. This also applies to medical tests, which are non-refundable, and this is because Cameroon has not implemented the social security system.

In addition, most of these strains were co-resistant to fluoroquinolones, aminoglycosides and trimethoprim-sulfamethazole, thus drastically reducing therapeutic options in the treatment of such infections.

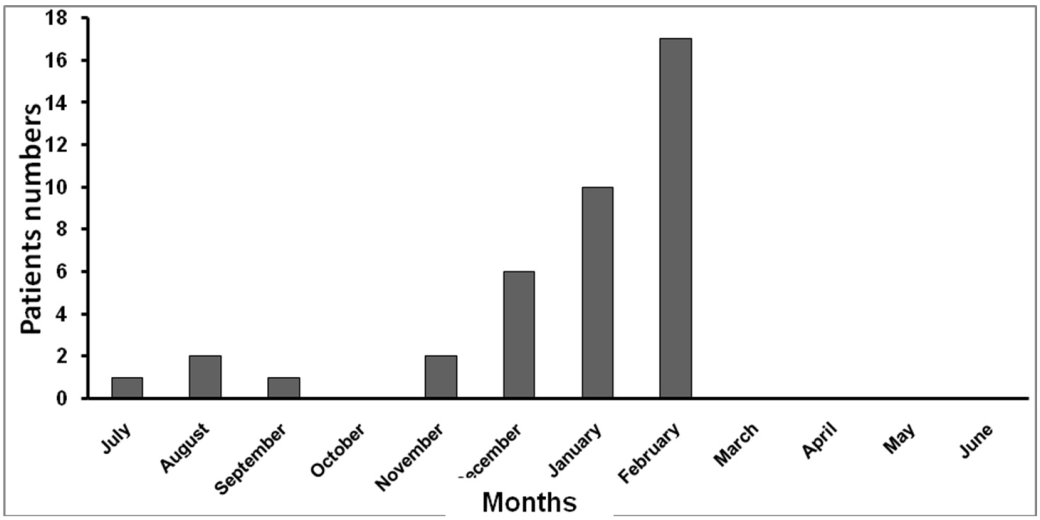

Figure 1. Number of ESBL *E. coli* cases according to the month of collection

5. Conclusion

The findings of this study show that the global dissemination of CTX-M-type ESBL affects particularly Cameroon and its capital city, since nearly half of the community strains of *E. coli* accounting for urinary tract infections had this type of ESBL. Furthermore, these strains were in most cases co-resistant to other antibiotics widely used in *E. coli* infection treatments.

The situation of CTX-M in Cameroon he brings new facts to explain the current global pandemic. Hygiene and the normal use of antibiotics must be in the centre for the fight against the spread of such multidrug-resistant strains.

We believe it is right time authorities in Africa in general and Cameroon in particular should be concerned by providing health facilities structures to carry out complete bacteriological analysis, harmonize antibiogram procedures in order to detect bacteria producing extended spectrum beta-lactamases and to stop the use of antibiotics such as amoxicillin, ciprofloxacin and cotrimozaxole in empirical treatment since there's a strong resistance of Enterobacteriaceae to these antibiotics. Fosfomycin may be recommended as a reliable empirical treatment for urinary tract infections, unfortunately this antibiotic is not yet available in Cameroon.

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Conflicts of Interest Disclosure

Authors declare that they have no competing interests.

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