CTX-M-TYPE EXTENDED SPECTRUM B-LACTAMASE-PRODUCING ESCHERICHIA COLI RESPONSIBLE FOR COMMUNITY URINARY INFECTIONS IN DOUALA-CAMEROON

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Abstract

Background: *Informations regarding the resistance mechanisms of extended spectrum ß-lactamase (ESBL)-producing Escherichia coli during urinary tract infection in the community are keys to designing efficient therapeutic strategies in Douala, Cameroon. Therefore, we aimed at determining the prevalence and risks factors of CTX-M-type ESBL)-producing E coli infection among patients consulting at Douala Laquintinie hospital.*

Method: *The samples were seeded on EMB medium and added to Cefotaxime. The Enterobacteriaceae strains were identified using an Api20 E gallery followed by an antibiogram carried out for the strains which were identified as Escherichia coli CTX-M to determine those producing ESBL and see their resistance profile to other families of antibiotics. The clinical and biological characteristics of the different participants were studied and analyzed using a statview statistical software.*

Results: *We selected a total of 111 patients and identified 20 strains of Enterobacteriaceae. At the end of the biological analysis, of these 20 strains isolated, Escherichia coli was the most represented species (70%) followed by Klebsiella pneumoniae (20%). 14 strains of Enterobacteriaceae of the genus Escherichia coli of CTX-M type were obtained, among which 2 (14%) producers of Beta Lactamases with Broader Spectrum. These results showed Co resistance to other families of antibiotics including aminoglycosides (20 %) and fluoroquinolones (35 %).* ***Conclusion***. *The results of this preliminary study show that the multi-resistant strains of Escherichia coli present in our region could be linked not only to self-medication (including traditional decoctions), and overuse of antibiotics but also to non-compliance hygiene rules by women.*

Keywords: *Urinary tract infections, Escherichia coli, ESBL, CTX-M, Community.*

BACKGROUND

A urinary tract infection designates the invasion, and the multiplication of microorganisms within the urinary tract accompanied by compatible symptomatology [1], causing pyelonephritis in the upper tract and cystitis, urethritis, and epididymitis in the lower tract [2]. Bacterial infections of the urinary tract are clinically manifested by a variety of signs and symptoms and can be caused by a multitude of microorganisms. Urinary tract infections are among the most common bacterial infections in both community and hospital settings [3] and are second to bacterial infections after respiratory tract infections according to the WHO. They mainly affect women because, in general, women their urethra is much shorter than that of men, and the urinary orifice is closer to the anal opening in women than in men [4]. A study conducted at the Clinics of Université des Montagnes in NDE Cameroun in 2017 showed that women had more urinary tract infections than men with a percentage of 92.7. In 2012, a study carried out in two towns in Cameroon (Buea and Bamenda), showed that the bacteria most isolated in cases of urinary tract infections were Escherichia coli (31.4 %), Klebsiella oxytoca (25.5%), Staphylococcus spp (24.1%) [5]. The resistance pro*le of Escherichia coli to antibiotics has changed a lot in recent years, it has developed resistance to the most common antibiotics such as aminopenicillins (30-45% of resistant strains) and cotrimoxazole (10-15% of resistant strains) [6]. In 2004, in Cotonou, Zoumahoum had shown that 29.1% of the strains isolated in his study were resistant to the association "amoxicillin / clavulanic acid" [7] and in 2016, in Canada, Mentouri et al. found a much higher rate, ie 42.4% resistant to this association [8]; which testifies to the evolution of resistance of Escherichia coli to antibiotics. In Gram-negative bacilli (BGN), one of the most important emerging traits is resistance to β- lactams. ESBLs were derived from TEM and SHV-type enzymes in the 1990s and were distributed mainly within hospital clones of Klebsiella pneumoniae and Enterobacter sp. However, the dissemination of CTX-M within the E.coli species has changed this situation, CTX-Ms now constitute the majority of ESBLs regardless of the region of the world to such an extent that their dissemination is quali*ed as a pandemic. [9]. The emergence of antimicrobial resistance genes over the past two decades poses a particular threat to human health [10]. In 2016, a study carried out in two hospitals in Cameroon (Center University Hospital de Yaoundé and Hôpital Central de Yaoundé) showed that the predominant bacteria in urinary tract infections is Escherichia coli with a rate of 50.9 %, and it was also reported that most isolates had a high level of resistance to ampicillin (50 %), amoxicillin clavulanic acid (23.6 %) and nalidixic acid, beta-lactam resistance showed that E. coli secreting ESBLs called Extended Spectrum Beta Lactamase secreting Enterobacteriaceae (EBLSE) was 22.5% [11]. ESBL-producing CTX-M-producing E. coli infections represent a threat to public health and sometimes lead to treatment failures [12]. It is with this in mind that we initiated this work, setting as aim to determine the prevalence of strains of E. coli producing CTX-M-type ESBLs responsible for community infections in women at Laquintinie Hospital of Douala.

MATERIALS AND METHODS

Study setting and population.

We carried out a cross-sectional and descriptive study between June and November 2020 at the Laquintinie Hospital in Douala located in the Littoral region more precisely in the Akwa district. The study population consisted of women who came for consultation for suspected urinary tract infections. (n=111). Sampling was consecutive and women in labor who gave their free and informed consent were included in the study. A standardized questionnaire was performed for the collection of demographic information on women (age of the mother, marital status, employment status).

Sample collection and bacterial identification

We carried the registration of each patient on a container of sterile urine: name, patient code, examination requested, age, and date of sampling, then we performed an aseptic urine sample. Urine was collected in the middle of the stream and half-filled in a sterile jar. Once the sample was collected, the samples were sent to the medical Bacteriology laboratory in a cooler, accompanied by the duly completed technical sheet. At the Bacteriology laboratory, compliance was checked and the macroscopic appearance was noted, that is, the color and appearance of the sample. Each sample of urine has undertaken a cytobacteriological examination routine comprising: A microscopic examination to note the possibly present elements like leukocytes, red blood cells, and other elements of the urine (epithelial cells, cylinders, crystals…); A bacteriological examination with germs enumeration. The culture medium used was EMB agar supplemented with cefotaxime, a medium favorable for Enterobacteriaceae such as Escherichia coli. The interpretation was done taking into account urinary tract parameters infection: leukocyturia $\geq$ 104 leukocytes/ml urine and bacteremia $\geq$ 105. The identification of the isolated bacteria is based on the use of mini galleries Api 20 E from Bio Mérieux. The study of the sensitivity to antibiotics was carried out by diffusion in agar medium Mueller Hinton and the interpretation was made according to the recommendations of CASFM 2020. The detection of ESBLs was carried out by the synergy between the acid clavulanic of the co-amoxiclav disc (AMC) and ceftriaxone (CRO), ceftazidime (CAZ), aztreonam (ATM), cefepime (FEP) or cefotaxime (CTX), characterized by a double image synergy materializing in *champagne cork* and signs the presence of an ESBL.

Phenotypic Detection of ESBL Producers

The detection of ESBLs was carried out by the synergy between the acid clavulanic of the co-amoxiclav disc (AMC) and ceftriaxone (CRO), ceftazidime (CAZ), aztreonam (ATM), cefepime (FEP) or cefotaxime (CTX), characterized by a double image synergy materializing in *champagne cork* and signs the presence of an ESBL.

Identification of the CT-M phenotype

The bacteria producing CTX-M are those resistant to cefotaxime (MIC> 64μg /mL) and more or less sensitive to ceftazidime (MIC from 2 to 8μg / mL).

Data management and statistical analysis
Extracted data were recorded in Microsoft Excel 2016 spreadsheet and the variables calculated were presented as a percentage. The statistical analysis of all data was done using the STATVIEW Statistical Package version 5 (SAS University Edition, SAS Institute Inc., Cary, USA). The variables studied in this study were the diameters of inhibition for the detection of strains of Escherichia coli ESBL CTX-M; the determination of ESBL CTX-M and coresistant Escherichia coli strains to other families of antibiotics such as fluoroquinolones, aminoglycosides. The prevalence of strains of Escherichia coli producing CTX-M type ESBLs was determined. Data were expressed in frequencies and proportions; the parameters were calculated for a con*dence interval of 95%. The significance level was set at a P-value less than or equal to 0.05. Univariate and multivariate logistic regression analyzes were used to determine the association between variables. Multivariate logistic regression analyzes were used to determine the risk factors for developing an ESBL urinary tract infection type CTX-M in women. The multivariate results were reported using odds ratios (ORs) and a 95% confidence interval (95% CI). The significance level was set at a P-value less than or equal to 0.05.

Ethical considerations
This study was approved by the "Université des Montagnes" Ethics Committee ((AUTHORIZATION N ° 2020/159 / UdM / PR / CIE). A research authorization to collect data and analyze samples from eligible patients was issued by the Laquintinie Hospital in Douala (N ° 03954 / AR / MINSANTE / DHL / CM). Before starting our study, an information letter on the objectives and progress of the investigation was given to each of the participants. All eligible participants had given their free and informed consent and assent with the signature of the participants. The confidentiality of the research results was respected by using a unique identification code for each participant.

RESULTS
Socio-demographic characteristics of the study population
We performed a total of 111 ECBUs of which 20 met the classical criteria for a urinary tract infection, with 18.02% of positivity. Regarding the resistance to antibiotics in the strains of Escherichia coli ESBL CTXM, all were resistant to cefotaxime, 57% resistant to ceftazidime, 42% to ticarcillin, and 43% to amoxicillin, these strains also showed resistance of the order of 24% and 14% against fosfomycin and imipenem. In addition, these strains maintained good sensibility to amikacin (11% resistance).
The most represented age group was that of 26 to 33 years with a frequency of 24.32%. Married women were the most represented with a frequency of 58.6%. The professions of the informal sector were the most represented with a frequency of 56%. The Littoral region was also the most represented with a frequency of 47.8%. (Table 1)

Prevalence of isolated germs and **frequency of resistance of** *Escherichia coli* **strains to β lactamins**
The isolated strains were grouped according to their species. The analysis of 20 bacterial isolates showed a predominance for Escherichia coli with 70% and Klebsiella pneumoniae with 20 %. The least isolated bacterial species were Proteus mirabilis and Enterobacter cloacae with 5% (Table 2). The study of the sensitivity of Escherichia coli strains to βlactamins showed resistance to each ATB. Regarding antibiotic resistance in Escherichia coli ESBL CTX-M strains, all were resistant to cefotaxime, 57% resistant to ceftazidime, 42% to ticarcillin, and 43% to amoxicillin (Table 3).

Frequency of resistance of Escherichia coli strains to other families of antibiotics and frequencies of the ESBL CTXM Escherichia coli strains
The results show that most strains were resistant to other families' antibiotics including aminoglycosides and +uoroquinolones. These strains have also shown resistance in the order of 24% and 14% to fosfomycin and imipenem (Table 4). Furthermore, these strains maintained good sensitivity to amikacin (11% resistance). This makes aminoside a good choice if a combination of antibiotics is needed for treatment.
The ESBL CTXM E. coli strains have been grouped in frequency. After analysis of the 14 Escherichia coli CTXM strains, it emerges that of these 14 strains, 2 strains produce ESBLs, for a frequency of 14% (table 5).

Risk factors for contracting an ESBL-producing E. coli urinary tract infection type CTX-M
To determine the risk factors associated with ESBL-producing E. coli urinary tract infection CTX-M type, the following parameters were analyzed: selfmedication, marital status, and occupation. The parameters were analyzed by multiple logistic regression, and the results obtained show that marital status does not constitute a risk factor for developing a urinary tract infection due to ESBL-producing E. coli CTX-M type (p >0.05). However, self-medication (taking antibiotics) (OR = 9.82; 95% CI= 1.25-76.81; p=0.0295and profession (OR = 19,56; 95% CI= 4,55-84,01; p =<0,0001) are risk factors (Table 5).

DISCUSSION
To contribute to the fight against the emergence of bacterial resistance, this study was carried out to determine the prevalence of E. coli ESBL CTXM strains responsible for community urinary infections among women at Laquintinie Hospital in Douala.
To carry out this study, the strains were isolated from a culture medium added to cefotaxime to testify to the resistance to the antibiotic tested and were subjected to an antibiogram to see the synergistic reactions to determine bacteria produced Broad Spectrum β-Lactamases and to see the resistance of these strains to other families of antibiotics. From the methodology used, the results show that out of 20 strains of Enterobacteriaceae resistant to cefotaxime isolated, 14 (70

%) of them were strains of E. coli, and thanks to the precise arrangement of the antibiotic discs, we were able to identify 2 (14 %) among them that produced ESBLs. These strains were also found to be co-resistant to other families of antibiotics (fluoroquinolones; aminoglycosides and cotrimoxazole (table 4)). These levels of resistance may be linked to the excessive and uncontrolled use of antibiotics [13].

A total of 111 urine samples were recruited from the patients. Of the different germs isolated from the urine of our patients, the most represented species was E. coli with a frequency of 70% followed by Klebsiella pneumoniae at 20% (table 2). This result can be compared to that obtained in a study carried out by Gonsu et *al* in 2016 which revealed that the most represented species were E. coli (50.9 %) followed by Klebsiella pneumoniae (16.4 %) [14]. This difference could be explained by the fact that E. coli is the predominant bacterial species in the human gut microbiota [14]. This result is also close to that obtained in a study carried out by Ebongue et al from 2012 to 2017 in which the most isolated species was Escherichia coli (48.5%) followed by Klebsiella pneumoniae (32.8%). Another carried out by Missac et *al* in 2016, showed that Escherichia coli is the bacterial agent the most incriminated and is therefore considered to be the first responsible for community infections which would be due to the pathophysiology of the urinary tract infection which is generally ascending and with strong colonization of the perineum by Enterobacteriaceae of digestive origin, especially Escherichia coli [18]. Based on a precise arrangement of the discs of antibiotics associated or not with inhibitors recommended by the Antibiogram Committee of the Société Francaise de Microbiologie 2020, and the rigorous measurement of the diameters of inhibition, we have could classify the 14 strains of E. coli CTXM. It emerges from this classification that the distribution of frequencies of the Enlarged Spectrum β-lactamase phenotypes of the CTXM type is n = 2, ie 14% (*gure 4). This rate is higher than that recorded by Lalmadi et al (12.2 %) in 2015 [15] indicating an increase in the frequency of ESBL Escherichia coli CTXM within the community. A higher rate of resistance to β-lactams has been revealed [17]. Indeed, penicillins are sold nowadays with an important frequency and the third-generation cephalosporins are more and more used in human and animal medicine; moreover, they are easily accessible. The co-resistance of these strains was also observed during the performance of the antibiogram. Thus, we have observed high co-resistance to aminoglycosides (20%), fluoroquinolones (35%), and sulfonamides (14%). These results are similar to those obtained by Gonsu et al in 2017, who found 30.9% for fluoroquinolones, 16% for aminoglycosides, and 8.7% for sulfonamides [16] and are also superimposable on those of Ruppé in 2015 which, according to him, most strains are not only resistant to the majority of βlactamins but also many antibiotics from other families such as fluoroquinolones, aminoglycosides and cotrimoxazole [13]. This can be because hygiene is probably not respected by these patients, and auto-medication is usually practiced by these patients; more for anatomical reasons, the germs located at the level of the anus can easily be found at the level of the urinary meatus of the woman and cause urinary infections, this phenomenon is favored by the proximity between the anus and the vulva in women.

The increase in bacterial resistance to broad-spectrum beta-lactamases is alarming, And this resurgence of resistance is linked to several factors. Multivariate logistic regression analyzes have shown that self-medication is the main risk factor in women for developing a urinary tract infection due to Eschérichia coli producing ESBL type CTX-M (<0,0001). Self-medication, the anarchic intake of antibiotics helps to promote the development of bacterial resistance to antibiotics, the final consequence of which is therapeutic failure [19]. These results were also reported by Maria Bitsori et al. in 2009 who reported antibiotic prophylaxis as a major factor in contracting an antibioticresistant urinary tract infection (p<0.0001) [19]. The inappropriate use of antibiotics is a real public health problem as reported by Mohamed E. El Zowalaty et al. in 2016; in whom 63.6% of the study population claims to consume antibiotics without a medical prescription and which also shows a positive association between self-medication and the development of antimicrobial resistance [20].

The results of this preliminary study show that the multi-resistant strains of Escherichia coli present in our region could be linked not only to selfmedication (including traditional decoctions), and overuse of antibiotics but also to non-compliance hygiene rules by women. These results could be exploited to fight against the development of bacterial resistance in Cameroon.

CONCLUSION

Given the above, resistance to antibiotics constitutes a real public health problem because they are the cause of several treatment failures. At the end of this work, out of the 111 samples obtained, 20 (18.02%) strains of enterobacteria were isolated, in particular, 14 (70%) strains of Escherichia coli of these 20 strains; the strains of Escherichia coli were all resistant to cefotaxime, 57% were resistant to Ceftazidime, 42% to Ticarcillin and 43% to Amoxicillin; our results document 14% Escherichia coli producing ESBLs of CTX-M type isolated from the urine of women. Self-medication is a risk factor for contracting a urinary tract infection caused by Escherichia coli Extended Spectrum Beta Lactamase type CTX-M.

Study limitations

Preliminary study reporting the prevalence of Eschérichia coli CTX-M urinary tract infections in women in the community. Need to conduct the study on a larger population size to have more characteristic results of the general population.

Authors' Contributions

Fadel Laure Medjou Foffou conducted experimental design, data interpretation, and manuscript writing. Romaric De Manfouo Tuono conducted experimental design and interpretation of data and made a major contribution to writing the manuscript. Simon Ngamli Fewou contributed to data interpretation, statistical analysis, and manuscript writing. All authors read and approved the final manuscript.

Ethical statement

This study was approved by the "Université des Montagnes" Ethics Committee ((AUTHORIZATION N ° 2020/159 / UdM / PR / CIE). A research authorization to collect data and analyze samples from eligible patients was issued by the Laquintinie Hospital in Douala (N ° 03954 / AR / MINSANTE / DHL / CM).

Financial disclosure

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data availability statement

The data of the article are available from the authors.

Tables

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Table 1: Socio-demographic characteristics of the study population

Age intervals	Effective (n)	Frequency (%)
[20-35[	51	45.94
[35-50[	29	26.13
[50-65[	16	14.42
[65-80[	11	9.91
>80	4	3.60
Total	**111**	**100**
Marital status		
Married	65	58.6
Divorced	10	9
Single	36	32.4
total	**111**	**100**
Professions		
Civile servants	24	17.3
Student	29	26.7
Informal sector	58	56
total	**100**	**100**
Regions		
Center	15	13.4
Littoral	53	47.8
West	43	38.8
total	**111**	**100**
Self medication		
Yes	11	9.91
No	100	90.09
total	**111**	**100**

Table 2: Frequency of isolated germs

Isolated germs	n	%
E. coli	1	70
Klebsiella pneumoniae	1	20
Enterobacter cloacae	4	5
Proteus mirabilis	14	5
Total	**20**	**100**

Table 3: Profile of resistance of *Escherichia coli* to β lactamins

Profile / Antibiotics	S n(%)	R n(%)	I n(%)
Augmentin	0	14 (100)	0
Cefipime	4 (28.57)	10 (71.43)	0
Ticarcilin	3 (21.43)	11 (78.57)	0
Cefotaxime	0	14 (100)	0
Cefoxtine	0	14 (100)	0
Amoxicicllin	1 (7.14)	13 (92.86)	0
Ceftazidim	6 (35.71)	9 (64.29)	0
Ertapenem	14 (100)	0	0

Table 4: Resistance profile of *Escherichia coli* to other families of antibiotics

Profile / Antibiotics	S n(%)	R n(%)	I n(%)
Fosfomycin	2 (14.23)	10 (71.43)	2 (14.23)
Gentamycin	1 (7.14)	13 (92.85)	0
Cotrimoxazol	3 (21.43)	11 (78.57)	0
Amykacin	0	14 (100)	0
Acide nalidixique	2 (14.29)	8 (57.14)	4 (28.57)
Ofloxacin	4 (28.57)	7 (50)	3 (21.43)
Ciprofloxacin	4 (28.57)	7 (50)	3 (21.43)
Aztreonam	11 (78.57)	3(21.43)	0

n=effective; %=frequence

Table 5: Multiple regression logistic analysis for risk factors associated with infection CTX-M type ESBLs *E. coli* in women

Variables	Size/Percentage N= 111 (100%)	CT-M type ESBLs *E. coli* in women		P-value
		OR	95% CI	
Profession				
No	32 (28.83)	1		
Yes	79 (77.17)	9.82	[1.25 ; 76.81]	**0,0295***
Self-medication				
No	100 (90)	1		
Yes	11 (10)	19.56	[4.55 ; 84.01]	**<0,0001***
Marital status				
No	36 (32.43)	1		
Yes	75 (67.57)	1.55	[0.52 ; 4.66]	0,4353

OR: Odd ratio; 95 % IC : 95% confidence interval