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Original Article

Molecular characterization of beta-lactamase genes produced by community-acquired uropathogenic *Escherichia coli* in Nouna

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Abstract

Introduction: Extended-Spectrum Beta-Lactamases (ESBL) are a common mechanism of bacterial resistance in *Enterobacteriaceae*. The purpose of this study is to characterize the ESBL genes produced by community-acquired uropathogenic *Escherichia coli* strains in the Nouna District, in the West-African country, Burkina Faso.

Methodology: Samples were collected from non-hospitalized patients who came for consultation at the CMA (Centre Médical avec Antenne chirurgicale) in Nouna and were sent to the laboratory for a urine culture test. The detection of ESBL production by the bacteria was carried out with the double-disc synergy test and the extraction of the ESBL genes with the heat shock method. Molecular characterization of ESBL genes was performed with three sequential multiplex polymerase chain reaction (PCR) assays.

Results: One hundred and eighty-two (182) bacteriological cultures were analyzed and 29 *E. coli* isolated, between 01/07/2017 and 01/07/2018. The ESBL phenotype was found in 13/29 (44.8%). Multiplex PCR yielded many beta-lactamase genes, predominantly bla$_{CTX\text{-}M\text{-}1,3,15}$ (12/13; 92.3%) followed by beta-lactamase genes *bla*$_{OXA\text{-}1,4,30}$ (8/13; 61.5%) and beta-lactamase genes *bla*$_{TEM\text{-}1,2}$ (7/13; 53.8%).

Conclusion: This study showed that the *bla*$_{CTX\text{-}M\text{-}1,3,15}$ genes produced by uropathogenic *E. coli* were predominant. Sequencing of these genes would be needed to better characterize the different types of ESBL circulating in Nouna.

Key words: *Enterobacteriaceae*; *Escherichia coli*; extended-spectrum beta-lactamase (ESBL).

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Introduction

Urinary tract infections (UTIs) are a very common reason for consultation and medical prescription in routine practice [1]. Intensive and often abusive use of antibiotics such as beta-lactams in the treatment of bacterial infections like UTIs, has been rapidly followed by the development of Extended-Spectrum Beta-Lactamase-producing strains (ESBLs) [2].

ESBL production is the most common resistance mechanism developed by *Enterobacteriaceae* worldwide, such as the frequent urinary pathogen *E. coli* [3]. ESBLs are a group of enzymes that cleave the beta-lactam ring of antibiotics belonging to the beta-lactam groups like: penicillins (e.g. amoxicillin); first, second and third generation broad-spectrum oxyimino-cephalosporins (e.g. ceftriaxone, ceftazidime, cefotaxime) and monobactams (aztreonam), rendering them inactive against bacteria with the capacity to produce ESBL [2,3]. They are further characterized by the fact that: (i) they can generally be inhibited by clavulanic-acid, tazobactam and sulbactam; (ii) they are unable to hydrolyze cephamycins (e.g. cefoxitin) and carbapenems (e.g. imipenem, meropenem); (iii) and they are encoded by genes that are highly exchangeable between bacteria [2,3]. The largest groups of ESBLs are the TEM and SVH type beta-lactamases, the CTX-M type beta-lactamases and OXA type beta-lactamases, which can be grouped according to the molecular homology (amino acid similarity) or according to the functional properties of the enzymes, the latter of clinical relevance as it takes into account the substrate-enzyme specificity [4].

Recently, the global epidemiology of beta-lactamases in *Enterobacteriaceae* has shifted from hospital-onset *Klebsiella spp*. infections, carrying TEM and SVH types beta-lactamases, towards a massive increase of extended-spectrum CTX-M type beta-lactamases in community-onset *E. coli* infections [3-7]. Indeed, there is a spread of CTX-M ESBL in the community, further away from the nosocomial setting, with *E. coli* as the most frequently isolated species, which hints at a huge public health problem [3,8].

Worldwide many CTX-M producing organisms have been isolated, with various CTX-M beta-lactamases distributed differently across the globe. Among the ESBL genes circulating in West-Africa, $bla_{CTX\text{-}M\text{-}15}$ is the most commonly found gene both in the hospital and in the community environments [9]. In Burkina Faso a very high intestinal carriage rate of ESBL-producing *Enterobacteriaceae* in hospitalized patients (42% of 113 stool samples) and in healthy volunteers from the community (22% of 101 stool samples) was found [10]. Urinary *E. coli* infections are a priority for surveillance and antibiotic resistance studies because of their high frequency and severity. They serve as an epidemiologic marker of colonization and potential transfer or spreading in the community and are an important cause of community-acquired bloodstream infections [4,11,12].

Frequently, serious infections caused by ESBL-producing organisms are refractory to the majority of empirically started antibiotics, not only cephalosporins but also other classes of antibiotics like fluoroquinolones (e.g. ciprofloxacin), cotrimoxazole, aminoglycosides (e.g. gentamicin) and tetracyclines [2,3]. This fact highlights the need for specific detection of ESBL producing organisms in clinical laboratories, both by phenotypic and genotypic methods that provide essential information for the treatment, prevention and control efforts of these infections and for tracking of these organisms in surveillance systems [3].

The objective of this work is to describe the local epidemiology and to molecularly characterize the ESBL genes of *E. coli* obtained from community urinary tract infections at the Nouna District Hospital in order to assess the magnitude of different genes of ESBL circulating at the community level.

Methodology

This was a cross-sectional descriptive study of *E. coli* isolated from community-acquired urinary tract infections. The study population was composed of patients from the rural and semi-rural area around the town of Nouna. Nouna is located in the north-west of Burkina Faso, in the province of Kossi, about 300km from the capital Ouagadougou.

The samples for this study consisted of all outpatient's urines sent for cultural test between 01/07/2017 and 01/07/2018 at the Nouna District Hospital, samples obtained from inpatients were excluded from the study. Bacteriological analyses and molecular characterization of ESBL genes were performed in the CRSN (Centre de Recherche en Santé de Nouna) biological laboratory.

For the bacterial isolation, all urine samples were inoculated on culture media (CLED) and incubated at 37ºC for 18 hours to 24 hours. On the samples with a bacterial grow $\geq 1\times10^4$ cfu/mL and leucocyte count $\geq 1\times10^3/\mu L$ a well isolated colony was selected and subjected to the API20E (BioMérieux, Marcy-l'Etoile, France) biochemical test for identification and subsequent confirmation of *E. coli*. The antibiograms were performed on fresh and pure *E. coli* colonies re-isolated on Mueller–Hinton following the Kirby Bauer technique as recommended by the Antibiogram Committee of the French Microbiology Society (CASFM, 2016). *Escherichia coli* ATCC 25922 was used as the reference strain for quality control. The antibiotic discs (Liofilchem srl, Liofilchem srl, Roseto degli Abruzzi, Italy) cefepime (30µg), amoxicillin+clavulanic acid (30µg), amoxicillin (30µg), ceftriaxone (30µg), cefotaxime (30µg), imipenem (10µg), ciprofloxacin (5µg), gentamicin (10µg), cotrimoxazole (25µg) and tobramycin (10µg) were used.

Strains exhibiting resistance to third-generation cephalosporins were tested for synergy with a centric amoxicillin+clavulanic acid disc and three cefepime discs in a 30 mm radius. Furthermore, to exclude the possibility of hyperproduction of cephalosporinase, for instance, mediated by AmpC enzymes, all phenotypically identified ESBL-*E. coli* were subjected to an antibiogram on a Mueller-Hinton agar supplemented with cloxacillin at 500 mg/L. The recovery of the sensitivity to the antibiotics would confirm the presence of hyperproduced cephalosporinases. We detected no cephaloporinase hyperproduction.

Extraction of DNA from strains with an ESBL phenotype was done with the heat shock method. An Eppendorf Mastercycler® gradient thermal cycler and a migration vessel were used for amplification of beta-lactamase genes on a 1.5% migration agarose gel. The reagents (Solis BioDyne, Tartu, Estonia) for the amplification consisted of 100bp Ladder-ready Ladder DNA, FIREPol Master Mix and PCR Grade water.

Three multiplex PCRs were performed according to the method of Dallenne *et al.*, 2010 [13] for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. The primers TEM-1 and TEM-2, SHV-1, OXA-1, OXA-4 and OXA-30 were included in the multiplex PCR I. Next, the primers CTX-M-1 group (CTX-M-1, CTX-M-3 and CTX-M-15), CTX-M-2 group, CTX-M-9 group (CTX-M-9 and CTX-M-14), CTX-M-8, CTX-M-25, CTX-M-26 and CTX-M-39 to CTX-M-41 were included in the multiplex PCR II. Finally, the primers ACC-1 and ACC-2, FOX-1 to FOX-5, MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11 and CMY-19, DHA-1 and DHA -2, LAT-1 to LAT-3, BIL-1, CMY-2 to CMY-7, CMY-12 to CMY-18 and CMY-21 to CMY-23, ACT-1 and MIR-1 were part of the multiplex PCR III.

For each primer a mix forward primer and a mix reverse primer solution with a 10mM concentration were prepared; 20 µl of each forward primer solution were taken, assembled and homogenized in an Eppendorf tube to obtain the Mix primer forward at 10mM; the same was done for the Mix primer reverse. For each multiplex PCR reaction, the corresponding forward and reverse primers were mixed with the FIREPol Master Mix, PCR Grade water and 2 µL of DNA lysate extracted from the ESBL-producing *E. coli* strains.

Multiplex PCR uses multiple pairs of primers to simultaneously amplify a large number of sequences. For this type of PCR, primers with similar hybridization temperatures were chosen. The lengths of the amplified products should also be similar given that large differences in the length of the target DNAs would favor amplification of the short target relative to the long target, resulting in differences in the yield of amplified products. In addition, buffers used for multiplex PCR contained Taq polymerase, which decreases competition between amplicons and discrimination of longer DNA fragments during multiplex PCR. During PCR, sense and antisense primers hybridize to a specific sequence at the level of the amplicons. The primer contained in the same reaction mixture hybridizes to a target sequence. Thus, the target sequence can be read and amplified by Taq polymerase.

The following PCR program was used: a) Initial denaturation at 94ºC for 10 minutes; b) 2nd denaturation cycle at 94ºC for 40 seconds; c) Annealing at 60ºC for 40 seconds; d) Extension at 72ºC for 60 seconds; e) Thirty times cycle repetition; f) Final extension at 72ºC for 7 minutes; g) Final Holding at 4ºC.

Descriptive statistics and graphics were performed using Microsoft Excel 2010.

Results

One hundred and eighty-two (182) patients were included in the study, with 108 (59.3%) men and a sex ratio of 1.4. The median age of the patients was 43.5 years with an interquartile range of 29-60 years.

After microbiological culture of all 182 samples, it was possible to isolate and identify a pathogen organism in 60 (33.0%) of them (*E. coli* n = 29, *Klebsiella pneumoniae* n = 4, *Staphylococcus spp.* n = 8, *Staphylococcus aureus* n = 7, *Serratia fonticola* n = 1, *Acinetobacter baumannii* n = 2, *Citrobacter koseri* n = 1, *Proteus mirabilis* n = 1, *Pseudomonas aeruginosa* n = 2 and *Candida albicans* n = 5). *E. coli* were isolated in 29 of the 182 samples (16%) and 13 (44.8%) out of them were ESBL producers. Among the 29 *E. coli* positive urine cultures, 18 (62.1%) belonged to women and 9/18 (50%) of them carried ESBL producing *E. coli*, while 4/11 (36.4%) were carried by men.

Regarding the age distribution of patients bearing *E. coli* infection, all patients were adults (age ≥ 18 years), the median age was 39 years with an interquartile range of 32-59 years. The patients with urinary infection caused by ESBL *E. coli* were mostly women and were overall older than the patients with non ESBL *E. coli* infection. The characteristics of the population from where samples were obtained are summarized in Table 1.

ESBLs associated resistance to gentamicin was identified in 46.1% of the isolates, and to tobramycin in 53.8%. As for ciprofloxacin and cotrimoxazole, 38.4% and 100% of the ESBL producing *E. coli* were resistant, respectively. All strains of ESBL *E. coli* were sensitive to imipenem. This reflects the absence of carbapenemase-producing bacteria among isolated ESBL strains. From the 13 ESBL *E. coli* 69.3% were multidrug-resistant (resistant to three or more

Table 1. Characteristics of the population from where samples were obtained.

Characteristic	Urine positive cultures (n = 60)	*E. coli* (n = 29)	ESBL *E. coli* (n = 13)	Non ESBL *E. coli* (n = 16)
Age, median (IQR) years	37.5 (31-57)	39 (32-59)	43 (36-65)	37.5 (30.5-44)
Male, n (%)	29 (48.3%)	11 (37.9%)	4 (30.8%)	7 (43.8%)
Female, n (%)	31 (51.7%)	18 (62.1%)	9 (69.2%)	9 (56.2%)

IQR: interquartile range.

antimicrobial classes) [14]. Figure 1 shows the susceptibility profile of ESBL strains to associated antibiotics.

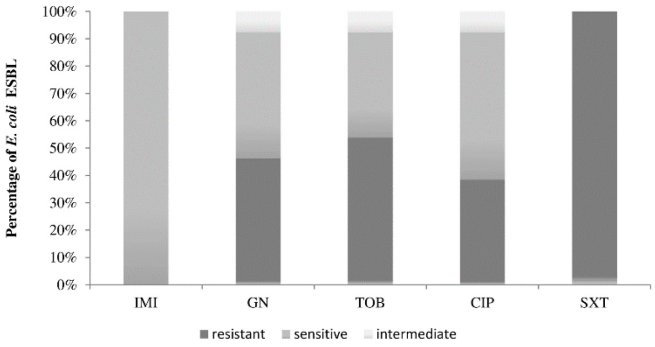
Molecular characterization by multiplex PCR confirmed the presence of extended-spectrum beta-lactamase genes $bla_{CTX\text{-}M\text{-}1}$ group in 12 out of 13 (92.3%) isolates. In addition to these genes, the beta-lactamase genes $bla_{TEM\text{-}1,2}$ were present in 7 isolates out of 13 (53.8%) and the beta-lactamase genes $bla_{OXA\text{-}1\text{-}like}$ in 8 out of 13 (61.5%). Co-expression of beta-lactamases was present in 11 out of 13 (84.6%) isolates. The combination of $bla_{CTX\text{-}M\text{-}1}$ group and $bla_{TEM\text{-}1,2}$ genes was detected in 4 isolates (30.8%); $bla_{CTX\text{-}M\text{-}1}$ group and $bla_{OXA\text{-}1\text{-}like}$ genes in 4 isolates (30.8%); $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{OXA\text{-}1\text{-}like}$ and $bla_{TEM\text{-}1,2}$ genes in 3 isolates (23.0%), and finally the presence of $bla_{OXA\text{-}1\text{-}like}$ and $bla_{CTX\text{-}M\text{-}1}$ group genes in only one isolate each (7.7%). On the other hand, we could not detect genes from the groups $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M8/25}$, nor were we able to detect amplified genes in multiplex PCR III. Table 2 shows the result of molecular characterization of ESBL genes by multiplex PCR and associated antimicrobial resistance.

As for the non-ESBL *E. coli* found in our study 15 out of 16 strains (93.8%) showed a decreased susceptibility to amoxicillin+clavulanic acid, 13 out of 16 isolates (81.3%) were resistant to ampicillin, 9 out of 16 (56.25%) to cotrimoxazole, and 1 (6.3%) to ciprofloxacin.

Discussion

In our study, the first of this type conducted at the Nouna district hospital, a high rate of 44.8% (13/29) of community-origin urinary ESBL *E. coli* was found.

Figure 1. Susceptibility profile of ESBL strains to associated antibiotics.



IMI: imipenem (10µg), GN: gentamicin (10µg), TOB: tobramycin (10µg), CIP: ciprofloxacin (5µg), STX: cotrimoxazole (25µg). Intermediate: 0.00% 7.60% 7.69% 0.00%; sensitive: 100.00% 46.15% 38.46% 53.85% 0.00%; resistant: 0.00% 46.15% 53.85% 38.46% 100.00%.

This is lower than a frequency of 58% previously reported by Ouedraogo *et al.*, 2016 [15] in a study carried at the three main hospitals in the capital city of Burkina Faso. This difference can result from many factors. First is the origin of the samples, because in the present study we only investigated urinary infections of community origin, whereas in the study of Ouedraogo *et al.* the samples were collected among inpatients and outpatients, and it is possible that a higher occurrence of urinary infections of hospital origin elevated the overall rate. Another factor is that our studied population comes from a rural setting, in contrast to a more urban setting investigated in 2016 [15], where it is more likely that the population will seek health care at a hospital and has an easier access to antibiotics [16]. Finally, and possibly most importantly, in the present

Table 2. Molecular Characterization of ESBL genes by multiplex PCR and associated antibiotic resistance.

E. coli ESBL isolates	Antimicrobial susceptibility testing (disk diffusion)										Beta-lactamase encoding genes		
	AML	AUG	CRO	CTX	FEP	IMI	GN	TOB	SXT	CIP	ESBL genes	Narrow-spectrum beta lactamase	
E. coli 1	R	R	R	R	R	S	R	R	R	R	CTX-M-1 group	TEM-1,2	-
E. coli 2	R	R	R	R	R	S	R	R	R	R	CTX-M-1 group	-	OXA-1-like
E. coli 3	R	R	R	R	R	S	R	R	R	R	CTX-M-1 group	-	OXA-1-like
E. coli 4	R	R	R	R	R	S	S	S	R	S	CTX-M-1 group	-	OXA-1-like
E. coli 5	R	R	R	R	R	S	S	S	R	R	-	-	OXA-1-like
E. coli 6	R	R	R	R	R	S	S	S	R	S	CTX-M-1 group	TEM-1,2	-
E. coli 7	R	R	R	R	R	S	R	R	R	S	CTX-M-1 group	TEM-1,2	OXA-1-like
E. coli 8	R	R	R	R	R	S	S	S	R	S	CTX-M-1 group	TEM-1,2	-
E. coli 9	R	R	R	R	R	S	S	I	R	S	CTX-M-1 group	TEM-1,2	-
E. coli 10	R	R	R	R	R	S	S	S	R	S	CTX-M-1 group	-	-
E. coli 11	R	R	R	R	R	S	S	R	R	R	CTX-M-1 group	-	OXA-1-like
E. coli 12	R	R	R	R	R	S	I	S	R	S	CTX-M-1 group	TEM-1,2	OXA-1-like
E. coli 13	R	R	R	R	R	S	R	R	R	S	CTX-M-1 group	TEM-1,2	OXA-1-like

AML: amoxicillin (30µg), AUG: amoxicillin + clavulanic acid (30µg), CRO: ceftriaxone (30µg), CTX: cefotaxime (30µg), FEP: cefepime (30µg), IMI: imipenem (10µg), GN: gentamicin (10µg), TOB: tobramycin (10µg), SXT: cotrimoxazole (25µg), CIP: ciprofloxacin (5µg), ESBL: Extended-spectrum beta-lactamase, R-resistant, I: intermediate, S: sensitive.

study *E. coli* were only retrieved from 16% of urine samples, which is a very low number, when compared with the usual prevalence of 65-75% of uropathogenic *E. coli* in UTIs [17]. This may be due to a recent intake of antibiotics by the patients, which could have reduced culture-based detection of bacterial infections, but unfortunately, we did not have information in this regard.

Nevertheless, our findings are in accordance with many recent studies carried across Africa, showing very high rates of ESBL-producing enterobacteria, ranging from 16.4–31.4% in Algeria, 11-42.9% in Egypt to 49.4% in Ghana in samples of both hospital and community origin [18,19]. The high frequency observed in samples of community origin is of special concern, as it denotes a high microbiological and ecological success of ESBL-producing strains outside the hospitals [3].

In Burkina Faso there are currently no guidelines for the treatment of complicated urinary infections. When available, treatment decisions are supported by the antibiograms, but generally third generation cephalosporins or quinolones are employed, which can explain the high development of resistance to these antibiotics. Additionally, an over the counter self-prescription of antibiotics is very common in Burkina Faso, further increasing the antibiotic selective pressure and development of resistance.

All these assumptions are backed by our results which show that, along with the resistance to third generation cephalosporins, associated resistances to gentamicin at 46.1%, tobramycin at 53.8%, ciprofloxacin at 38.4% and cotrimoxazole at 100% were present. In fact, 69.3% of ESBL *E. coli* were multidrug-resistant. Similar ESBL-associated resistances have been observed in many other studies [2-4,9,18].

This fact raises concerns for the treatment of these infections, as many of the commonly started empirical therapies are no longer effective and the failure and delay of adequate treatment often translates into bad outcomes for the patients and higher risk of transmission. If initial empiric therapy is inappropriate ESBL *E. coli* can pose a serious threat of urosepsis, in such cases it is crucial to rapidly isolate and perform AST on the microbial pathogen, in order to establish a definitive treatment, which significantly improves morbidity and mortality outcomes [3]. As such, it is extremely important that local epidemiology and institutional susceptibility data provide the empirical evidence for the selection of the most appropriate and cost-effective narrow-spectrum antibiotics for the treatment of serious infections when the patient's antibiogram is not available, followed by the development of prescribing guidelines. While fluroquinolones seem to still be a good alternative for the treatment of non-ESBL *E. coli* infection, many infections with strains harboring ESBL genes may be refractory to therapy with these agents, as it appears to exist a strong association between quinolone resistance and ESBL production [4]. Doctors must be aware of this relationship and take this into consideration for their prescription practice. Ciprofloxacin should not be used as empirical first-choice antibiotic for uncomplicated urinary tract infection, unless resistance to other antibiotics with a narrower spectrum is suspected and bacterial sensitivity is confirmed. Medical history, such as previous intake of antibiotics and AST results from past bacterial infection should be inquired. This information should be taken into account at the time of the prescription. The treatment regimens should be well documented as well as the results and outcomes for patients.

The present study is the first effort towards the implementation of a surveillance system of ESBL-producing organisms using molecular based techniques, such as PCR, at the Nouna hospital.

The migration of PCR products from our ESBL strains demonstrated the production of two types of narrow-spectrum beta-lactamases: the TEM (53.8%), and OXA (61.5%). This is lower than a previous report by Mousse *et al.*, 2018 [20] where 100% of TEM type beta-lactamase were found in a similar study of ESBL *E. coli* genes isolated from urine samples in Benin. Additionally, Kpoda *et al.*, 2018 [21] found an overall 24.6% prevalence of TEM encoding genes in *E. coli* isolates obtained from urine, pus, blood, stool, vaginal swab and pleural fluid samples of patients attending three major hospitals in the capital of Burkina Faso, which indicates a variability in the distribution of these genes among *E. coli* in Africa.

As for ESBL-encoding genes, they consisted of $bla_{CTX\text{-}M\text{-}1}$ group ESBL in 92.3% (12/13) of our ESBL-producing isolates. This frequency of CTX-M-1 type ESBL is similar to the one found previously in Burkina Faso (94%), by Ouedraogo *et al.*, 2016 [15] and it is congruent with the report of the same author [9] that CTX-M-15 is the most frequent ESBL found in West-Africa, as well as in many other parts of the world [3,4,20,21].

We could not detect other groups of CTX-M encoding genes such as $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}8/25}$ in our study. This is concordant with the results from D. Salah *et al.*, 2016 [22] in Togo, where groups

bla$_{CTX-M-2}$, and *bla*$_{CTX-M-9}$ were likewise not detected and Ouedraogo *et al.*, 2016 [15] that reported a low rate of 4% of *bla*$_{CTX-M-9}$ group. Finally, we were not able to detect ESBL genes in multiplex PCR III, conveying the absence of production of plasmid-encoded AmpC beta-lactamases [23].

Most of our strains co-harbored more than one type of beta-lactamase genes and the combination of *bla*$_{OXA-1-like}$ genes with *bla*$_{CTX-M-1}$ group genes predominated. The expression of class D OXA enzymes, besides the ubiquitous class A beta-lactamases is increasingly being reported worldwide and it is associated with an enhanced resistance to beta-lactam/beta-lactamase inhibitor combinations [23-26]. The *E. coli* strain 5 was positive for only *bla*$_{OXA-1-like}$ genes. Although these genes encode narrow spectrum beta-lactamases, this strain was resistant to the cephalosporins tested and was phenotypically identified as an ESBL strain with the double-disc synergy test. This could be due to a mutation and production of a new OXA-type beta-lactamase with increased enzymatic activity, such as OXA-31, that differs from the OXA-1 by only three amino acids [4,26-28].

Indeed, it is well known that mutations that affected a small number of pivotal amino acids from narrow-spectrum beta-lactamases, like TEM-1, TEM-2 and SHV-1, resulted in the emergence of extended-spectrum TEM and SHV beta-lactamases [4]. Sequencing of the beta-lactamase genes of *E. coli* strain 5 is needed for better characterization and assessment of this possibility. Fortunately, in the present study all strains showed good sensitivity to imipenem, indicating the absence of OXA-type carbapenemases.

The design, the small number of samples obtained and the lack of information about the patients does not allow any considerations regarding risk factors for acquiring an infection of community origin with ESBL-producing pathogens. However, investigating these and other aspects regarding the provenience of the strains and their resistance genes, along with the surveillance of asymptomatic carriers is crucial for the development of prevention and infection control efforts, for instance of the growing number of community-onset bloodstream infections caused by ESBL-producing bacteria, which has the potential to become similar to that of community-acquired methicillin-resistant *Staphylococcus aureus* [4,27,29].

Lastly, indiscriminate use of antibiotics in the treatment of uncomplicated infections must be controlled and antibiotic stewardship programs should be implemented. For the treatment of uncomplicated lower UTI in the community, use of beta-lactam/lactamase-inhibitor, such as amoxicillin + clavulanic acid, or other antimicrobial drugs like fosfomycin or nitrofurantoin are still considered useful, even in the presence of ESBL producing organisms (particularly if it possesses only a single beta-lactamase enzyme, and in the case of beta-lactam-/lactamase-inhibitor, most probably one other than OXA-1), due to positive pharmacokinetic effects of these drugs in treating localized infections and the high concentrations of drug achieved in the urinary tract [4,30]. Policies to regulate the access to antibiotics and promotion of health literacy and responsible use of antimicrobial drugs in the general population should also be undertaken.

Conclusions

The first results showed a high frequency of the blaCTX-M-1 group genes produced by uropathogenic *E. coli* isolated from community infections at the hospital laboratory. High levels of associated resistance to different antibiotic classes were also present. Sequencing of genes encoding ESBL is needed to better characterize the different types of ESBL circulating in Nouna, and to develop efforts toward infection control, optimal treatment and decrease the occurrence of resistance.

References

1. Magistro G, Marcon J, Schubert S, Gratzke C, Stief CG (2017) Pathogenesis of urinary tract infections. Der Urologe 56: 720-727. [Article in German]
2. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA (2015) Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci 22: 90-101.
3. Pitout JD, Laupland KB (2008) Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infect Dis 8: 159-166.
4. Rawat D, Nair D (2010) Extended-spectrum beta-lactamases in Gram-negative Bacteria. J Glob Infect Dis 2: 263-274.
5. Ruppé E (2010) Epidemiology of expanded-spectrum beta-lactamases: The rise of CTX-M 12: 3–16. [Article in French]
6. Ejaz H, Wang N, Wilksch JJ, Page AJ, Cao H, Gujaran S, Keane JA, Lithgow T, Ul-Haq I, Dougan G, Strugnell RA, Heinz E (2017) Phylogenetic Analysis of *Klebsiella pneumoniae* from Hospitalized Children, Pakistan. Emerg Infect Dis 23: 1872-1875.
7. Heinz E, Ejaz H, Bartholdson Scott J, Wang N, Gujaran S, Pickard D, Wilksch J, Cao H, Haq IU, Dougan G, Strugnell RA (2019) Resistance mechanisms and population structure of highly drug resistant Klebsiella in Pakistan during the introduction of the carbapenemase NDM-1. Sci Rep 9: 2392.
8. Zahar J-R, Bille E, Schnell D, Lanternier F, Mechai F, Masse V, Nassif X, Lortholary O (2009) Extension of β-lactamases producing bacteria is a worldwide concern. J Med Sci 25: 939-944. [Article in French]

9. Ouedraogo A-S, Jean Pierre H, Banuls AL, Ouedraogo R, Godreuil S (2017) Emergence and spread of antibiotic resistance in West Africa: contributing factors and threat assessment. Med Sante Trop 27: 147-154.
10. Ouedraogo, A-S (2016) Prevalence, circulation and characterization of multiresistant bacteria in Burkina Faso. Doctorate thesis in Biology-Health. Université Montpellier. 146 p. [Article in French]
11. El Bouamri MC, Arsalane L, Zerouali K, Katfy K, El kamouni Y, Zouhair S. (2015) Molecular characterization of extended spectrum β-lactamase-producing *Escherichia coli* in a university hospital in Morocco, North Africa. Afr J Urol 21: 161-166.
12. Ben-Ami R, Schwaber MJ, Navon-Venezia S, Schwartz D, Giladi M, Chmelnitsky I, Leavitt A, Carmeli Y (2006) Influx of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* into the hospital. Clin Infect Dis: 42: 925-934.
13. Dallenne C, Da Costa A, Decre D, Favier C, Arlet G (2010) Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother 65: 490-495.
14. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268-281.
15. Ouedraogo A-S, Sanou M, Kissou A, Sanou S, Solaré H, Kaboré F, Poda A, Aberkane S, Bouzinbi N, Sano I, Nacro B, Sangaré L, Carrière C, Decré D, Ouégraogo R, Jean-Pierre H, Godreuil S (2016) High prevalence of extended-spectrum ß-lactamase producing *Enterobacteriaceae* among clinical isolates in Burkina Faso. BMC Infect Dis 16: 326-326.
16. Okeke TA, Okeibunor JC (2010) Rural-urban differences in health-seeking for the treatment of childhood malaria in south-east Nigeria. Health Policy 95: 62-68.
17. Storberg V (2014) ESBL-producing *Enterobacteriaceae* in Africa - a non-systematic literature review of research published 2008-2012. Infect Ecol Epidemiol 13: 1-16.
18. Obeng-Nkrumah N, Twum-Danso K, Krogfelt KA, Newman MJ (2013) High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. Am J Trop Med Hyg 89: 960-964.
19. Paterson DL, Bonomo RA (2015) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18: 657-686.
20. Moussé W, Sina H, Wélé M, Nouvlessounon DD, Bade FT, Chabi F, Anagonou SY, Baba-Moussa L (2018) Molecular characterization and Antibiotic resistance profiles of *Escherichia coli* extended-spectrum β-lactamases producer strains isolated from urine samples in Benin. Eur Sci J 14: 1857-7881
21. Kpoda DS, Ajayi A, Somda M, Traore O, Guessennd N, Ouattara AS, Sangare L, Traore AS, Dosso M (2018) Distribution of resistance genes encoding ESBLs in *Enterobacteriaceae* isolated from biological samples in health centers in Ouagadougou, Burkina Faso. BMC Res Notes 11: 471.
22. Philippon A (2013) Extended-spectrum beta-lactamases. Immuno-Anal Biol Spe 28: 287-296. [Article in French]
23. Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, Baquero F, Canton R, Nordmann P (2008) Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerg Infect Dis 14: 195-200.
24. Salah F, Diagbouga S, Dabire A, Sadji A, Nadembega C, Moumouni A, Soubeiga RST, Ouattara A, Zohoncon TM, Kere A, Karou SD, Simpore J (2016) First Detection of Resistance Genes Encoding Extended Spectrum Beta-Lactamase Producing *Escherichia coli* at Lome, Togo. Arch. Clin. Microbiol 7: 6.
25. Pfeifer Y, Cullik A, Witte W (2010) Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. Int J Med Microbiol 300: 371-379.
26. Barguigua A, El Otmani F, Talmi M, Bourjilat F, Haouzane F, Zerouali K, Timinouni M (2011) Characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from the community in Morocco. J Med Microbiol 60: 1344-1352.
27. Livermore DM, Day M, Cleary P, Hopkins KL, Toleman MA, Wareham DW, Wiuff C, Doumith M, Woodford N (2019) OXA-1 beta-lactamase and non-susceptibility to penicillin/beta-lactamase inhibitor combinations among ESBL-producing *Escherichia coli*. J Antimicrob Chemother 74: 326-333.
28. Antunes NT, Fisher JF (2014) Acquired Class D β-Lactamases. Antibiotics (Basel) 3: 398-434.
29. Guzman M, Salazar E, Cordero V, Castro A, Villanueva A, Rodulfo H, De Donato M (2019) Multidrug resistance and risk factors associated with community-acquired urinary tract infections caused by *Escherichia coli* in Venezuela. Biomedica 39 Suppl 1: 96-106 .
30. Hawkey PM, Warren RE, Livermore DM, McNulty CAM, Enoch DA, Otter JA, Wilson APR (2018) Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party. J Antimicrob Chemother 73 Suppl 3: 2-78

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