Intestinal carriage of Extended Spectrum Beta-Lactamase producing *E. coli* in women with urinary tract infections, Cameroon

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Abstract

Introduction: During the last decade, the prevalence of the intestinal carriage of extended spectrum beta-lactamases – producing *Escherichia coli* (ESBL-*E. coli*) has continued to increase worldwide in the community, especially in developing countries. Hence, we undertook a study to determine the ESBL-*E. coli* fecal carriage rate and the associated risk factors in Cameroonian women.

Methodology: A total of 86 women suspected of community-acquired urinary tract infections (UTI) were included in 10 health structures from May 2011 to April 2012. After filling a questionnaire, they provided a stool sample that was plated on selective media for ESBL-producing bacteria. The identification of strains was obtained with mass spectrometry and the antibiotic susceptibility by disk diffusion in agar media. The ESBL type was determined by PCR. The relative abundance of ESBL-*E. coli* was measured for positive samples. Eventually, the presence of antibiotics in stool was assessed.

Results: The carriage rate of ESBL-*E. coli* was 57/86 (66.3%). Phenotypic and molecular characterization showed that all ESBL-*E. coli* strains contained group 1 CTX-M enzymes. Multivariate analysis showed that ESBL-*E. coli* fecal carriage was associated with the presence of antibiotics in stools (p < 0.05). Although not significant, mean ESBL relative abundance tended to be higher in patients with antibiotic exposure.

Conclusions: Our results show that the carriage of ESBL-*E. coli* fecal carriage in women with UTI suspicion from the Cameroonian community is extremely high and associated with recent antibiotic intake.

Key words: ESBL; carriage; UTI; Cameroon; community.


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Introduction

The global dissemination of extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (ESBL-*E. coli*) is a major public health concern. The ESBL diffusion is mainly due to the rapid and global spread of plasmid-borne *blaCTX-M* genes in *E. coli* which is the most common and abundant *Enterobacteriaceae* in the human intestinal gut [1]. Nowadays, the prevalence of ESBL-*E. coli* carriage in the community setting is estimated to range between 10% and 50% according to the geographical areas. This prevalence has been especially observed to be the highest in developing countries, as a possible result of poorly controlled antibiotic consumption and suboptimal hygiene conditions [2]. Although limited to a few reports, data originating from Africa’s WHO region show that the carriage of ESBL-*E. coli* ranged from 5 to 30% according to the country and the studied populations [2]. A recent study in Cameroon reported that the intestinal carriage of ESBL-producing *Enterobacteriaceae* was as high as 23.1% [3]. In this context, we designed the present study in order to assess ESBL-*E. coli* fecal carriage rate in female patients with
suspicions of urinary-tract infection (UTI) in Yaoundé, Cameroon.

Methodology

Population

A cross-sectional descriptive study for which ethical clearance was obtained from the Cameroonian National Ethics Committee (approval No. 207/CNE/SE/2011) was carried out between May 2011 and April 2012 in 10 health structures in Yaoundé (Cameroon), including 3 public hospitals (General Hospital, Central Hospital and Yaoundé Gynecology, Obstetrics and Pediatric Hospital) and 7 private clinics. Outpatient women consulting for suspicion of UTI were approached by one of the investigators who provided information about the study. When the patient agreed to participate and gave her consent, she provided a freshly passed (less than 6hrs) fecal sample in the provided sterile container and answered a standardized anonymous questionnaire on clinical (including history of UTIs, antibiotic use and hospitalization during the last three months, diabetes and pregnancy) and demographic data (age, address, occupation, marital status, number of children). Stool samples were kept at 4°C and transported to the CREMER laboratory of the Ministry of Scientific Research Cameroon within the same day, where they were 1/10 diluted in brain heart infusion broth supplemented with 10 % glycerol and frozen at -80°C. The diluted samples were later air-transported in dry ice to the bacteriology laboratory of Bichat-Claude Bernard Hospital in Paris (France) for further analysis.

Microbiological analysis

Samples were thawed in batches, plated on Drigalski agar (Oxoid, Dardilly, France) supplemented or not with cefotaxime 1 mg/L (Mylan Pharmaceuticals, Saint-Priest, France) and incubated for 48 hours at 37°C. All distinct colonies (with respect to the colour and aspect of the colonies) that could grow on Drigalski agar with cefotaxime were subcultured on trypticase soy agar (Bio-Rad, Marne-la-Coquette, France) and identified by mass spectrometry (Bruker Daltonics, Bremen, Germany). E. coli isolates were tested for susceptibility to amoxicillin, tetracycline, amoxicillin + clavulanic acid, cefotaxime, cefoxitin, ceftazidime, cefepime, ertapenem, nalidixic acid, ciprofloxacin, gentamicin, amikacin, fosfomycin and co-trimoxazole (Bio-Rad, Marne-la-Coquette, France) using the disc diffusion method and presence of ESBL was determined using the double disc-diffusion phenotypic method, as recommended by the AntiBiogram Committee of the French Society for Microbiology [4]. To confirm the ESBL identification with molecular methods, total DNA was extracted from E. coli strains, as described [5] and blaCTX-M (group 1), blaTEM and blasIV genes were amplified by PCR using specific primers, as described [6].

Exposition to antibiotics at the time of fecal sampling was defined by the detection of fecal antimicrobial activity, as described [7]. Briefly, 10 μL of each defrosted stool sample was placed on antibiotic free sterile 6 mm diameter paper discs (Dutschner, Brumath, France). The discs were then placed on Mueller-Hinton agar (Oxoid, Dardilly, France) containing a 10^5 CFU / mL suspension of a fully susceptible E. coli strain (strain 25922, derived from the American Type Culture Collection, Manassas, VA, USA). Stool samples for which a zone of inhibition was observed around the disc following overnight incubation at 37°C were considered positive and the corresponding patient as exposed to antibiotics at the time of sampling.

Densities of total Enterobacteriaceae and of ESBL-E. coli were determined by plating serial dilutions of the broth on Drigalski agar, with or without 1 mg/L cefotaxime. CFUs were counted in decimal logarithms at the dilution in which 1–100 CFUs grew. ESBL-relative abundance (ESBL-RA) was calculated as the ratio of the ESBL-E. coli counts divided by the total number of Enterobacteriaceae [8]. For women who carried more than one ESBL E. coli, only the ESBL-RA of the dominant clone was considered.

Statistical analysis

We compared demographic and clinical characteristics in patients according to fecal ESBL-E. coli carriage. Characteristics tested included: season during which the suspicion of UTI occurred (dry/rainy), city of origin (Yaoundé/other), patient’s age, history of diabetes or pregnancy, use of antibiotics in the 3 months preceding the inclusion, hospitalization in the 3 months preceding the inclusion, retained diagnosis of UTI and antimicrobial activity detected in the stool. Comparisons between groups were performed using nonparametric tests (Wilcoxon or Fisher exact tests).

We searched for risk factors associated with fecal ESBL-E. coli carriage. Variables achieving P < 0.20 in nonparametric tests were considered for a multivariate logistic regression analysis to identify risk factors of fecal ESBL-E. coli carriage. Using a forward selection method, we obtained a final model in which all risk factors had P < 0.05. First-order interaction was tested for significant variables. The model discrimination was
assessed by the c-statistic and its 95% confidence interval (95% CI), and the model calibration was assessed by the Hosmer-Lemeshow goodness-of-fit test. Analyses were performed with SAS v9.3 (SAS Institute Inc., Cary, NC). All tests were 2-sided with a type-I error fixed to 0.05.

**Results**

A total of 86 patients provided a stool sample and were included. An *E. coli* isolate was identified from their stool on cefotaxime containing plates in 57 (66.3%) of them, including 5 carrying two *E. coli* strains with different morphologies and antibiotic susceptibility patterns. This ended in a total of 62 *E. coli* strains. All the 62 *E. coli* exhibited an ESBL phenotype and were tested positive for the CTX-M group 1 by PCR. ESBL-*E. coli* rates of resistance to non-beta-lactam antibiotics tested were as follows: ciprofloxacin (n = 56, 90%), gentamicin (n= 25, 41%), amikacin (n = 8, 13%) and co-trimoxazole (n = 62, 100%). Of note, all were susceptible to fosfomycin.

Among the 86 included patients, 43 (50%) were exposed to antibiotics at the time of sampling, as demonstrated by the detection of an antimicrobial activity in the fecal sample.

As shown in Table 1, fecal ESBL-*E. coli* carriage was not different according to age, pregnancy, antibiotics use during the last 3 months and hospitalization the last 3 months. Univariate analysis (Table 1) showed that detection of an antimicrobial activity in the stool sample was significantly associated with ESBL-*E. coli* carriage versus non carriage (*P*=0.001). Multivariate analysis showed that the detection of antimicrobial activity in the stool sample was the only independent risk factor associated with ESBL-*E. coli* carriage (OR=5.4; 95%CI [2.0-14.7]).

The mean ESBL-RA of 49 out of 57 patients was found to be higher in patients with antibiotic exposure than in patients without (-1.1 log [7.9%] vs. -1.5 log [3.3%]) but the difference was not significant (*P* = 0.3) (Figure 1). Of note, ESBL-RA in patients without antibiotic exposure was similar to that observed in European women [1.1%] [8]. ESBL-RA was not found to be associated with age, pregnancy, antibiotics use

| Table 1. Risk factor analysis for fecal ESBL-producing *E. coli* carriage. |
|---------------------------------|-------------------|---------|-------------|---------|
|                                 | ESBL - *E. coli* carriers | ESBL - *E. coli* non carriers | *P* | Multivariate OR | *P* |
| **TOTAL**                       | 57                | 29      |             |         |
| Season of the year              |                   |         |             |         |
| Dry                             | 39 (8.4)          | 23 (79.3) | 0.3       |         |
| Rainy                           | 18 (31.6)         | 6 (20.7)  |           |         |
| Patient's home origin           |                   |         |             |         |
| Yaoundé                         | 44 (77.2)         | 20 (69)  |           |         |
| Other regions                   | 13 (22.8)         | 9 (31)   | 0.4       |         |
| Age (median)                    | 32 [1-95]         | 36 [10-89] | 0.7     |         |
| Pregnancy                       | 15 (26.3)         | 11 (37.9) | 0.3       |         |
| Diabetes                        | 9 (15.8)          | 5 (17.2)  | >0.99     |         |
| Hospitalization in the past three months | 4 (7)            | 6 (20.7)  | 0.08      | -       |
| Use of antibiotics in the past three months | 15 (30.6)       | 8 (29.6)   | >0.99     |         |
| Presence of antimicrobial activity in the stool sample | 36 (63.2) | 7 (24.1) | 0.001 | 5.4 (2.0-14.7) | < 0.001 |

**Figure 1.** Extended-spectrum beta-lactamase (ESBL) relative abundance with regards to the antibiotic exposure.
during the last 3 months, hospitalization the last 3 months, diabetes or season.

Discussion

The rapid spread of CTX–M-type ESBL- \textit{E. coli} in the community is not fully explained yet [9]. Such strains have emerged worldwide and have been reported in many countries. However, in African countries, data on ESBL- \textit{E. coli} carriage in community patients are scarce. In our study, the prevalence of ESBL- \textit{E. coli} carriage in stool was as high as 66.3%. This is much higher than in a 2012 study in northern Cameroon which reported a prevalence of 23.1% among community outpatients [3] or in other African studies which reported rates of 30.9 % in Niger [10], 10% in Senegal [11] and 7.3 % in Tunisia [12]. This is even higher to what has been reported in China where a rate of 50.5% of CTX-M- \textit{E. coli} [13] was described. In industrialized countries, carriage rates are much lower, as for example in France (6 %) [14] or UK (11.3 %) [15]. The reasons why rates of carriage are generally higher in developing countries than in industrialized countries are still a matter of debate [2]. Many factors may have contributed to the high rates of resistance found in our patients. Mainly, our subjects were women with suspected UTI who were very often treated with antibiotics at the time of sampling, as demonstrated by the association between ESBL– \textit{E. coli} carriage and the fecal antibiotic activity. That antibiotic treatment sharply increases ESBL fecal carriage rates has already been observed [16]. In the above-mentioned study from another part of Cameroon [3] where the rates of carriage were lower, subjects were not suspected of UTI and thus putatively less exposed to antibiotics at the time of sampling. This may explain why the rates, although already very high when compared to that in industrialized counties, were much lower than in our patients.

All of the ESBL strains isolated in this work were positive for CTX-M group 1, which is in accordance with the local epidemiology characterized by the predominance of the CTX-M-15 allele (belonging to group 1 CTX-M) [2]. Strains were also very often co-resistant to other families of antibiotics such as aminoglycosides, fluoroquinolones and co-trimoxazole, in consistency with what was found elsewhere in Cameroon [3]. This situation may be the consequence of the high exposure of women with UTI to antibiotics and may favour dissemination and infections due to ESBL-producing \textit{Enterobacteriaceae}.

One limitation of this study is that complementary data from the culture of the urine samples might have helped to assess the consequences of the ESBL carriage in our population. This issue, which has already been studied in other settings [6], was however not explored in this work.

Conclusions

In conclusion, we observed that the prevalence of carriage of ESBL- \textit{E. coli} in women suspect of UTI is dramatically high in Yaoundé, Cameroon, and that this high prevalence was associated to recent antibiotic intake. All isolates produced a CTX-M-type ESBL. While all strains were susceptible to fosfomycin (a first-line antibiotic in UTI), most were resistant to the non-beta-lactam antibiotics that are used to treat UTI infections, which may have very significant clinical consequences in case of pyelonephritis.

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