Carriage of extended spectrum beta-lactamase-producing Enterobacteriaceae by healthy school children from two remote villages in western Cameroun

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Research article

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Carriage of extended spectrum beta-lactamase-producing *Enterobacteriaceae* by healthy school children from two remote villages in western Cameroun

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Abstract

Background: Higher carriage rate of extended spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae have already been reported among healthy community children, thus can increases the risk of developing pathological infection. Since children are the most exposed population due to lack of hygiene knowledge, determining their carriage prevalence will limit the progression or development of those pathologies. The objective of this study was to determine the prevalence of ESBL-producing Enterobacteriaceae carriage among children in remote villages of western Cameroon where healthcare structures are absent and the use of antibiotic consumption rare.

Methods: A total of 110 fresh stool samples were collected from 110 healthy primary school children between ages 2 to 5 years old from two remote villages. Upon screening using selective agar media for ESBL, Enterobacteriaceae were identified using the Api 20E gallery. Antibiotic susceptibility was investigated using the disc diffusion technique and the ESBL production was determined using the double-disc synergy test. Chi-square test was used for comparison.

Results: Children had no history of hospitalization and had not been subjected to antibiotic treatment three months prior to this study. Data analysis indicated a 22% carriage rate for ESBL-producing Enterobacteriaceae among school children. Overall, 24 (67%) out of 36 isolates were ESBL producers and 15 (61%) out of 24 being Escherichia coli. Other ESBL-producing bacteria were Klebsiella pneumoniae (3%) and Kluyvera spp (3%). We also isolated small proportion of bacteria showing resistance to high level cepholosporinase, which overall represented 33% of the total bacteria isolate.

Conclusions: The higher carriage of ESBL-producing Enterobacteriaceae in children from some isolated villages devoid of health care structure highlights the risk for resistance transmission between pathogenic and non-pathogenic bacteria. This study also indicates that farming conditions can induces resistance. The current result may contribute to design a therapeutic policy to curtail the emergence of ESBL-producing Enterobacteriaceae in remote villages in western Cameroon.

Keywords: Carriage, Enterobacteriaceae, ESBL, Healthy children, Remote villages, Western Cameroon.
Introduction

The resistance of Gram-negative bacilli to antibiotics is considered as a global challenge for healthcare due to limited treatment options and is also associated to high mortality [1, 2, 3]. The production of extended-spectrum β-lactamases (ESBL) is the mechanism by which the Enterobacteriaceae species induce the antibiotic resistance [4]. Whereas the infection with multidrug-resistant organisms have been initially associated with the hospital environment, there is now increasing evidence of high rates carriage of ESBL-producing microorganisms identified in community settings [5, 6]. It is therefore evident that the communities are becoming important reservoirs for antibiotic-resistant bacteria. Recent investigations suggest that Escherichia coli strains producing ESBL are the Enterobacteriaceae responsible for community infections [7, 8, 9] and its prevalence is increasing in resource-limited countries where infectious diseases, poverty and malnutrition are endemic [10].

Whether the infection is acquired in hospital or community, the digestive tract is the main reservoir from which Enterobacteria originate [11, 12]. Moreover, the digestive tract is where exchange of resistance genes between bacteria happens and antibiotic treatment select for the overgrowth of resistant bacteria [5, 13]. Intestinal carriage of bacteria is common in resource-limited countries due to poverty and poor hygiene conditions [14]. Therefore, persons colonized are at risk of subsequent infection [5, 15, 16] and this impact on the prevalence of ESBL-producing Enterobacteriaceae among adult in rural Africa where hygiene is almost inexistent [17]. Moreover, high prevalence of fecal carriage ESBL-producing Enterobacteriaceae is also observed among children living in rural Africa where poverty is high and hygienic conditions absent [17, 18, 19] and are therefore one of the reason for higher mortality among children observed in rural Africa.

From the above informations’, we thought it wise to determine the prevalence of carriage of ESBL-producing Enterobacteriaceae among school children from two remote villages in western Cameroon. Indeed, in those villages the absence of basic healthcare exposures was queried and no antibiotic treatments during the last three (3) months were the main selection criteria. Recovered Enterobacteriaceae isolates were tested for susceptibility to relevant antibiotic classes. Data analysis indicated 22% carriage of ESBL-producing Enterobacteriaceae among investigated children. In addition, the data gathered in this study is of paramount importance since it may contribute to design strategies to curtail the emergence and spread of ESBL-producing Enterobacteriaceae among children in rural Africa and devise innovative therapeutic approaches against multidrug-resistant organisms.
Material and Methods

Ethical consideration

Permission to undertake at both remote villages was granted by the ministry of public health (Reference N°: 185/AR/MINSANTE/DRSPO/DS Bgité) and the ministry of basic education (Reference N°: LETTRE N°22/16/L/MINEDUB/DRO/NDE/IAEB-BGTE/BAG). In addition, ethic approval for the current study was given by the “Université des Montagnes” Ethical Committee (Autorization N°2017/087/UdM/PR/CAB/CIE). Written informed consent was obtained from the parents or guardian on behalf of all the children enrolled in the study.

Study setting and population

This prospective study was performed between October 2017 and July 2018 in two primary schools (Moineaux de Bafou Ballefer and oiselets de Nzi) of the Bafou village near Dschang, the largest city of the Menoua subdivision and in one primary school (École publique projet route du Noun 2) of a remote village (route du Noun) near Bangangté, the largest city of the Ndé subdivision. Healthy children between 2 to 5 years old were included (n = 110). A standardized questionnaire was performed for collection of demographic information on children (age, gender, antibiotic treatment during the last 3 months and never been hospitalized).

Sample collection and bacterial isolation

A freshly emitted stool specimens from each child and contained in the coproculture pots was stored in icebox and send to the laboratory microbiology at the “Clinique Universitaire des Montagnes (CUMs)” for analysis. Fecal specimen from each child was collected and cultured on MacConkey agar within six hours as follow. A total of 0.5 g of fecal sample was suspended in 5 mL of sterile 0.9 % saline. Each suspension was seeded on McConkey agars supplemented with céfotaxime a 1mg/L in order to select the Enterobacteriaceae resistant to third-generation cephalosporins (3GC). After seeding, the plates were incubated for 48h at 37°C. One colony representing each distinct colonial morphotype was isolated from supplemented MacConkey agar and further analyzed by gram coloration and oxidase test. Bacilli gram-negative and negative in oxidase test were seeded on a nutrient agar and incubated for 24 hours at 37°C. After 24 hours we collected the colonies and prepared a suspension having a turbidity equivalent to that of the 0.5 standard of the McFarland range for carrying out the biochemical identification and performing the antibiotic susceptibility tests.

Biochemical identification of Enterobacteriaceae
The Biochemical identification was carried out according to the recommendations of the manufacturer on gallery Api 20E (Biomérieux, Marcy l'Etoile, France), which constitutes a standardized system of identification of Enterobacteriaceae.

**Antimicrobial Susceptibility testing**

Susceptibility tests were carried out by the Kirby-Bauer disk diffusion susceptibility test using 15 conventional antibacterial agents that are commonly used in Cameroon. In short, this was conducted on 24h bacterial pure culture obtained by streaking bacterial isolates on fresh nutrient agar and allowing for an overnight aerobic incubation at 37°C. From the resulting bacterial population, a suspension to the density of a McFarland 0.5 turbidity standard prepared in 0.9% saline was adjusted to the final opacity recommended for susceptibility tests by agar diffusion technique on Mueller Hinton agar. Test procedures and interpretations were done according to the standard guidelines recommended by the "Comité de l’Antibiogramme de la Société Française de Microbiologie [20]". We used 30 μg of each antibiotic disc that included amoxicillin, cefoxitin, cefotaxime, ceftazidime, nalidixic acid. In addition, discs of 10 μg were used for cefepime and ertapenem while discs of 5 g were used for gentamicin, kanamycine, amikacin, ciprofloxacine and ofloxacin. A fosfomycin disc was used at 50 μg. The combination of trimethoprim-sulfamethoxazole and amoxicillin/clavulanic acid were used at 23.75/1.25 g and 20/10 g, respectively. Escherichia coli (25922) from American Type Culture Collection (Manassas, Virginia, USA) was used as reference for quality control.

**Phenotypic screening for ESBL-producing Enterobacteriaceae**

The detection of ESBL(s) production in Enterobacteriaceae was performed using a double-disc synergy testing as described [21]. Briefly, Amoxicillin-Clavulanic acid (20/10 μg) antibiotic disc was placed at the center of an agar Mueller-Hinton plate. Around Amoxicillin-Clavulanic acid disc was placed the cefotaxime, ceftazidime and cefepime discs at a distance of 3.0 centimeter (cm) to the center. Development of the zone of inhibition (in a form of "champagne stopper") towards the Clavulanic acid disc following incubation at 37°C for 24 hours was indicative of a potential ESBL positive Enterobacteriaceae.

**Statistical analysis**

Statistical analysis was performed using EPI Info version 7.1.3.3 software (USD, Inc., Stone Mountain, GA, USA). Chi-square test was used to compare proportions. Uni-variate and multivariate analysis were performed using logistic regression. Multivariate analysis of characteristic features for carriage of ESBL-producing Enterobacteriaceae included the
following nine variables: sex, age, parent level of education, underweight, stunting, wasting, use of antibiotics. A P-value <0.05 was considered statistically significant.
Results

Demographic Characteristics of school children

Out of 110 school children enrolled, 36.36% (40 children) were from “école publique projet route du Noun 2” in the Nde subdivision and 63.63% (70 children) were from “Moineaux de Bafou Ballefer and Oiselets de Nzi” schools in the Menoua subdivision. The children age varied from 2 to 5 years old and 41% (45 children) were male and 59% (65 children) were female. In addition, all parents reported that their children had never been hospitalized and had not taken antibiotics during the last 3 months prior to the study.

Bacteria isolation and identification

Screening of the fecal flora of 110 school children resulted in 31 positive culture indicating the presence of at least one bacteria strain. Among these cultures, 17 (15%) of the fecal sample were collected in the Menoua subdivision (Moineaux de Bafou Ballefer and oiselets de Nzi) and 14 (13%) were from samples collected in the Nde subdivision (école publique projet route du Noun 2). In total, bacteria susceptibility to cefotaxime allowed the isolation of 36 strains among which 64% were *E. coli*. Moreover, the other bacteria strain identified were *K. Pneumonia* (9%), *E. sakazaki* (6%), *S. liquefaciens* (6%), *Kluyvera spp* (6%), *E. agglomerans* (6%) and the least represented *E. intermedium* (3%) (Table 1). Further analysis showed that some patients were colonized by more than one micro-organism.

Table 1: Distribution of *Enterobacteriaceae* strains isolated from school children’s stools.

<table>
<thead>
<tr>
<th>Bacteria strains</th>
<th>Size (n = 36)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>23</td>
<td>64</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td><em>Enterobacter sakazaki</em></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td><em>Serratia liquefaciens</em></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td><em>Kluyvera spp</em></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td><em>Enterobacter Agglomerans</em></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td><em>Enterobacter intermedium</em></td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
Antimicrobial susceptibility and carriage of extended spectrum beta lactamase-(ESBL)-producing *Enterobacteriaceae*

The disc diffusion test is a method that confirms the presence of the ESBL-producing *Enterobacteriaceae* [22] and in the current investigation, 15 different antibiotic discs were used and the results of these tests are presented in table 2. Data analysis showed that all bacteria isolated from feces were susceptible to ertapenem and resistant to amoxicillin. In addition, these bacteria showed a high level of resistance to ciprofloxacin (90%) and ofloxacin (81%). Moreover, resistance to kanamycin (43%), amikacin (42%) and gentamicin (45%) was also observed (Table 2).

Table 2: Antibiotic susceptibility rates of *Enterobacteriaceae* isolated from healthy school children feces.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Phantotypes (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>0</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>67</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>100</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>44</td>
</tr>
<tr>
<td>Kanamycine</td>
<td>24</td>
</tr>
<tr>
<td>Amikacin</td>
<td>24</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10</td>
</tr>
<tr>
<td>Triméthoprine/sulfamethoxazole</td>
<td>23</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>13</td>
</tr>
<tr>
<td>Nalidixicacid</td>
<td>31</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>60</td>
</tr>
</tbody>
</table>

After analysis of the antimicrobial susceptibility tests, 24 of 36 (67%) of the isolated *Enterobacteriaceae* were ESBL-producing. Detailed analysis showed that 17 of the 24 (47%) were from the samples collected in the Ménoua subdivision and 7 of the 24 (20%) were from
sample collected in the NDE subdivision. Further analysis indicated that *Escherichia coli* was the most abundant *Enterobacteriaceae* isolated with ESBL phenotype (61%). Other ESBL-producing bacteria species were *Klebsiella pneumoniae* (3%) and *Kluyvera spp* (Table 3). Finally, additional analysis of the data collected from participant indicated that 22% of the community children analyzed were carrier of ESBL-producing *Enterobacteriaceae*.

**Table 3:** Distribution of resistance phenotypes by *Enterobacterial* isolates resistant to third-generation cephalosporin.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Phenotype (%)</th>
<th>HLC(^a)</th>
<th>ESBL(^b)</th>
<th>Carbapenemase</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
<td>9</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td>3</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterobacter sakazaki</em></td>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Serratia liquefaciens</em></td>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Kluyveraspp</em></td>
<td></td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterobacter intermedium</em></td>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td><strong>33</strong></td>
<td><strong>67</strong></td>
<td><strong>0</strong></td>
</tr>
</tbody>
</table>

\(^a\)High-level cephalosporinase; \(^b\)Extended Spectrum Beta-Lactamas

**Phenotypic characterizations of the isolated *Enterobacteriaceae***

Further analysis of data obtained upon antibiotic susceptibility test showed that some of the isolated *Enterobacteriaceae* were weakly resistant to the high level cephalosporinase (HLC) (33%). In contrast, all of the isolated *Enterobacteriaceae* showed no resistance to carbapenemase (0%). Moreover, the majority of the isolated *E. coli* (61%) was resistant to ESBL while only 3% was resistant to the HLC. The other microorganism which showed a resistance to both HLC and EBSL was *Kluyvera spp*. Distribution of resistance phenotypes displayed by *Enterobacterial* isolates are shown in Table 3.
Discussion

The current study was undertaken to determine the prevalence of community-based extended spectrum beta lactamase-(ESBL)-producing *Enterobacteriaceae* in healthy school children of 02-05 years old in two remote villages of west Cameroon. The main features of the investigated children that they have never been hospitalized and had not been treated with antibiotic 3 months prior to the study. The main finding is a high carriage rate of ESBL-producing *Enterobacteriaceae* by children in a region where no healthcare structure is present and the use of antibiotic is not common. In addition, weak resistance to high level cephalosporinase was observed and no resistance to carbapenemase was observed. This is the first study in Cameroonian rural community reporting on ESBL carriage among healthy children. Faecal carriage of ESBL-producing *Enterobacteriaceae* has been documented in both children and in adults [23, 24, 25, 26, 27, 28, 29]. A global prevalence of 14% ESBL carriage among healthy individuals has been reported [28] and in sub Sahara Africa, few studies have investigated the prevalence of ESBL-carriage among children [30, 17, 26].

This work reveals the presence of a significant reservoir of ESBL-*Enterobacteriaceae* in the community. The results obtained show an overall prevalence of ESBL-*Enterobacteriaceae* colonization of 67% among healthy children in the studied community. This prevalence is very high compared to 16% reported by Lonchel et al. [31] in community setting in Ngaoundere, Cameroon and 4.6% community carriage among healthy French children [32]. The lower prevalence obtained among healthy French children may be explained by the difference in hygienic conditions between France and Cameroon. In contrast, the prevalence obtained in this study is lower than the one obtained in Chad (38%) in community children [33]. The current data indicating higher prevalence of ESBL-*Enterobacteriaceae* (22%) in the community children is surprising and was not expected given the fact that the area investigated has no hospital and inhabitant are not use to antibiotic consumption. One of the possible explanations of the appearance of ESBL phenotype in such region is that the phenotype may arise from pesticides which are highly used by farmers and contaminate foods available to the inhabitants. Indeed, published reports have already indicated that pesticides or herbicide can induces change in antibiotic susceptibility of microorganisms including *E. coli* and *Salmonella* [34, 35].

Further data analysis found carriage of more than one bacteria species more common among children in the community investigated, increasing the risk of transfer of genetic material responsible for the resistance to other bacteria. This idea is supported by finding suggesting that carriage of more than one bacteria species might increase the risk of transfer
of genetic elements responsible the resistance [36]. In this study we did not carry out extensive molecular characterization in order to determine the genotype of each isolate. However, it is demonstrated that the CTX-M-15 like genotype is the dominant CTX-Ms enzyme among carriers worldwide [28]. Therefore the CTX-M-15-like genotype might be the one present in ESBL-positive isolates recorded in our community. In addition, SHV-type ESBL might also be considered as one of the possible genotype of ESBL-positive isolates.

The main ESBL-producing Enterobacteriaceae strains isolated in this study was E. coli which is the frequently reported Enterobacteriaceae in hospital-based [37, 38, 39] and community-based [40, 41] studies in other African countries. Although the current study found a significantly higher prevalence of ESBL-producing Enterobacteriaceae among healthy community children in remote region of western Cameroon, the clinical impact of multidrug-resistant bacteremias has yet to be investigated. Our study seems to be the only one conducted in Cameroon which has targeted children in remote villages where the use of antibiotics is rare. In addition, the clinical consequences of ESBL Bacteremia in such remote region have to be evaluated and the significant impact of these multi-resistant infections on mortality of children in that region has to be shown. These results should encourage health authorities to investigate whether multidrug-resistant bacteremia are among the causes of death in children aged 0-5 years recorded in remote villages or rural Cameroon.

**Conclusion**

Our study shows a presence of Enterobacteriaceae having a high level of antibiotic resistance among subject who had in all probability never taken antibiotics. This result can be explained by the fact that environmental conditions have a high roll in the transmission of Enterobacteriaceae resistant. Therefore, we believe on one hand that the farmers must avoid anarchic use of pesticides and herbicides for plants treatment, and on the other hand that all peoples in such area must apply strict rule of hygiene to avoid infection.
List of abbreviations: ESBL, extended spectrum beta-lactamases; HLC, High level cephalosporinase; *E. coli*, *Escherichia coli*; CUMs, Clinique Universitaire des Montagnes; CA-SFM, Comité de l’Antibiogramme de la Société Française de Microbiologie; CTX-M-15, cefotaximase Munich 15.

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Availability of data and materials
The datasets generated and analyzed during the current study are available from the corresponding author. In addition, all data generated and analyzed in this study are included in this manuscript.

Authors’ contributions
The conception and design of this study was made by SNF and CID. Patient recruitment and data collection were done by LWNN and ITL. The study was coordinated by SNF. Data analysis and interpretation was performed by SNF. SNF and LWNN wrote the manuscript. All authors read and approved the final manuscript.

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

Consent for publication
Not applicable.

Ethics approval and consent to participate
Permission to undertake at both remote villages was granted by the ministry of public health (Reference N°: 185/AR/MINSANTE/DRSPO/DS Bgté) and the ministry of basic education (Reference N°: LETTRE N°22/16/L/MINEDUB/DRO/NDE/IAEB-BGTE/BAG). In addition,
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