Prevalence and spread of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in Ngaoundere, Cameroon

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Abstract

During April 2010 and June 2010, 334 *Enterobacteriaceae* isolates from 590 participants (outpatients, inpatients, inpatient carers, hospital workers and members of their households) were collected from faecal samples. Based on β-lactamase pattern, origin of strains and the relationship between participants, 44 isolates of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* were selected from 44 participants (in Ngaoundere Protestant Hospital and Ngaoundere Regional Hospital, Cameroon). To determine the relatedness of bacterial strains, these isolates were fingerprinted using the automated, repetitive-sequenced-based PCR-based DiversiLab system. Subsequently, *E. coli* isolates that had undergone DiversiLab analysis were examined with respect to their phylogenetic group and detection of the ST131 clone to shed light on the epidemiology of these isolates in the Ngaoundere hospitals. The prevalence of faecal carriage of ESBL-producing *Enterobacteriaceae* among the study participants was 54.06%. According to participant groups, the prevalence of faecal carriage was also high (outpatients 45%; inpatients 67%; inpatient carers 57%; hospital workers 44%; and members of their households 46%). Analysis of the molecular epidemiology of ESBL-producing *E. coli* and *K. pneumoniae* showed a close relationship of the isolates between related and non-related individuals. In addition, DiversiLab results of *E. coli* identified four related isolates (4/22) from cluster III belonging to the epidemiologically important clone ST131. Our results highlight the importance of outpatients, inpatients, their carers, hospital workers and their families as reservoirs of ESBL-producing *Enterobacteriaceae*

Keywords: Cameroon, CTX-M-15, *Enterobacteriaceae*, epidemiology, extended-spectrum β-lactamases

Studies have shown the presence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* (E-ESBLs) in Cameroon, not only in hospitals but also in the community [1–4]. However, although E-ESBLs are present and increasingly prevalent in the country, little is known about their dissemination. To find out the prevalence of faecal carriage of E-ESBLs and how they are spreading, an epidemiological study of E-ESBLs involving outpatients, inpatients, inpatient carers, hospital workers and members of their households was performed in hospitals in Ngaoundere, Cameroon. Written informed consent was obtained from all participants. Participants' characteristics are shown in Table 1.

A total of 334 *Enterobacteriaceae* were isolated on two selective media, Drigalski and MacConkey agars, supplemented, respectively with cefotaxime and ceftazidime. Detection of ESBL producers was carried out by the double-disc synergy test [5]. Susceptibility of the isolates to antibiotics was determined using the Vitek system (bioMérieux, Marcy l'Etoile, France). All the presumptive ESBL producers were further analysed by PCR aimed at detecting ESBL genes (one isolate of each morphotype from each patient). PCR amplification of *bla* genes (bla_{TEM}, bla_{SHV}, bla_{OXA} and $bla_{CTX\text{-}M}$) was performed using primers and methods described previously [6,7] (Table 2). All PCR products were sequenced using a 3100 ABI Prism Genetic Analyser (Applied Biosystems, Foster City, CA, USA). Sequence alignment and analyses were performed online using the BLAST program available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

To determine the relatedness of bacterial strains, 44 isolates (22 *Escherichia coli* and 22 *Klebsiella pneumoniae*) were recovered from 44 participants and fingerprinted using the automated repetitive-sequence-based PCR DiversiLab system (bioMérieux). The strains were selected based on their β-lactamase pattern, their origin and the relationship between participants. The relationships between repetitive-sequence-based PCR profiles were designated as recommended by the manufacturer: different, 3+ band differences (similarity <95%);

TABLE 1. Characteristics of all participants included in the study

Characteristic	Outpatients (n = 232)		Inpatients (n = 208)		Inpatient carers (n = 63)		Hospital workers (n = 48)		Household members (n = 39)	
	E-ESBL$^+$ n = 104 (45%)	E-ESBL$^-$ n = 128 (55%)	E-ESBL$^+$ n = 140 (67%)	E-ESBL$^-$ n = 68 (33%)	E-ESBL$^+$ n = 36 (57%)	E-ESBL$^-$ n = 27 (43%)	E-ESBL$^+$ n = 21 (44%)	E-ESBL$^-$ n = 27 (56%)	E-ESBL$^+$ n = 18 (46%)	E-ESBL$^-$ n = 21 (54%)
Median age, years (±SD)	35 ± 15	36 ± 15.5	37 ± 17.4	41 ± 18	42 ± 14.8	39 ± 13.5	35 ± 6.72	37 ± 10.52	17 ± 13	14 ± 9.24
Male gender (n)	40	60	71	39	6	8	9	16	8	14
Previous hospital admission										
Yes	13	23	46	14	5	3	3	3	1	4
No	78	83	88	53	31	24	17	19	14	13
Unknown	13	22	6	1	0	0	1	5	3	4
Previous antimicrobial treatment										
Yes	48	56	73	28	7	6	11	6	5	3
No	44	54	52	32	27	19	8	16	11	14
Unknown	12	18	15	8	2	2	2	5	2	4

E-ESBL, extended-spectrum β-lactamase-producing *Enterobacteriaceae*; E-ESBL$^-$, E-ESBL-negative; E-ESBL$^+$, E-ESBL-positive; SD, standard deviation.

TABLE 2. Primers used for PCR amplification

PCR name	β-Lactamase(s) targeted	Primer name	Sequence (5′-3′)	Amplicon size (bp)	Primer concentration (pmol/μL)	Reference
Multiplex TEM,	TEM variants including, TEM-1 and TEM-2	MultiTSO-T_for	CATTTCCGTGTCGCCCTTATTC	800	0.4	[6]
		MultiTSO-T_rev	CGTTCATCCATAGTTGCCTGAC		0.4	
SHV	SHV variants, including SHV-1	MultiTSO-S_for	AGCCGCTTGAGCAAATTAAAC	713	0.4	
		MultiTSO-S_rev	ATCCCGCAGATAAATCACCAC		0.4	
and OXA-1- like	OXA-1, OXA-4 and OXA-30	MultiTSO-O_for	GGCACCAGATTCAACTTTCAAG	564	0.4	
		MultiTSO-O_rev	GACCCCAAGTTTCCTGTAAGTG		0.4	
CTX-M group 1	Variants of CTX-M group 1, including CTX-M-1, CTX-M-3 and CTX-M-15	CTX-M3G_for	GTTACAATGTGTGAGAAGCAG	1050	0.5	[7]
		CTX-M3G_rev	CCGTTTCCGCTATTACAAAC		0.5	

similar, 1–2 band differences (similarity 95–97%); and indistinguishable, no band differences (similarity >97%).

Subsequently, the 22 *E. coli* isolates that had undergone DiversiLab analysis were investigated by a series of PCRs [8–10] to determine the phylogenetic groups (A, B1, B2 and D), the presence of IS*Ecp1* elements and the *aac(6′)-Ib-cr* variant (the gene that can induce resistance to aminoglycoside and fluoroquinolone simultaneously).

All 22 *E. coli* ESBL-producing isolates were screened for sequence type 131 (ST131) using a PCR for the *pabB* allele, described by Clermont *et al.* [11]. Multilocus sequence typing was performed on positive *pab*B isolates using the Achtman typing scheme (http://www.mlst.ucc.ie/mlst/dbs/Ecoli).

Statistical analysis was performed with Epi Info version 3.5.3. Fisher's exact test, when appropriate, was used for the univariate comparison of all variables. A p value of $p < 0.05$ was considered to be statistically significant.

The overall prevalence of faecal carriage of E-ESBLs in this study was 54.06%. Regarding participant groups and their relatives, the prevalence was also high (see Table 2). However, the prevalence of faecal carriage among inpatients was not significantly different from that of their carers ($p > 0.05$). Similarly, the prevalence of faecal carriage among hospital workers was not significantly different from that of their household members ($p > 0.05$).

Of the 334 bacteria isolated, 216 (64.67%) were *E. coli,* 74 (22.15%) were *Klebsiella* spp., 23 (6.88%) were *Enterobacter* spp. and 21 (6.28%) were *Citrobacter freundii*. Regardless of group, participants presented similar ESBLs and CTX-M-15 was the most widespread ESBL found in all strains (Table 3).

As demonstrated by the DiversiLab results (see Supplementary material, Fig. S1 and Fig. S2), multiple clones were seen among ESBL-producing *E. coli* and using a cut-off of 95% similarity (vertical dashed red line, Fig. S1) it was possible to establish six distinct clusters (types I, III, V, VI, VII and X), indicating the overall close relationship of the isolates, and five singleton patterns (types II, IV, VIII, IX and XI) (Table 4). These six outbreaks occurred between related or non-related individuals. This finding confirms that in some cases the acquisition of E-ESBLs has occurred via a common source or a common environmental reservoir (hands of hospital workers, through contaminated surfaces or via food) as described previously [12–17].

Molecular typing of *K. pneumoniae* isolates showed them to be more genotypically diverse than the *E. coli* isolates. The repetitive-sequence-based PCR analysis allowed the identifica-

TABLE 3. **Overview of extended-spectrum β-lactamase (ESBL) types produced by *Enterobacteriaceae* isolated in all groups of participants**

	ESBL types (number of isolates)				
Strain (*n* = 334)	**Outpatients**	**Inpatients**	**Inpatient carers**	**Hospital workers**	**Household members**
Citrobacter freundii (*n* = 21)	CTX-M-15, OXA-1 (8) CTX-M-15, TEM-1 (2) SHV-12, TEM-1 (2)	CTX-M-15 (2) CTX-M-15, OXA-1, TEM-1 (2) CTX-M-15, TEM-1 (1) SHV-12, TEM-1 (1) SHV-12 (1)			CTX-M-15, TEM-1 (1) CTX-M-15, OXA-1, TEM-1 (1)
Enterobacter spp. (*n* = 23)	CTX-M-15, OXA-1, TEM-1 (14) SHV-12, TEM-1, OXA-1 (1)	CTX-M-15, OXA-1, TEM-1 (5) SHV-12, TEM-1 (1)			CTX-M-15, OXA-1, TEM-1 (2)
Escherichia coli (*n* = 216)	CTX-M-15, OXA-1 (26) CTX-M-15, TEM-1 (15) CTX-M-15, OXA-1, TEM-1 (14) CTX-M-15 (3)	CTX-M-15, OXA-1, TEM-1(42) CTX-M-15, OXA-1 (30) CTX-M-15, TEM-1 (24) CTX-M-15 (3) SHV-12, TEM-1 (1) SHV-12 (1)	CTX-M-15, OXA-1, TEM-1 (11) CTX-M-15, OXA-1 (9) CTX-M-15, TEM-1 (8)	CTX-M-15, OXA-1 (8) CTX-M-15, OXA-1, TEM-1 (4) CTX-M-15, TEM-1 (2) CTX-M-15, SHV-12, OXA-1, TEM-1(1) CTX-M-15 (1)	CTX-M-15, TEM-1 (8) CTX-M-15, OXA-1, TEM-1 (5)
Klebsiella spp. (*n* = 74)	CTX-M-15, SHV-1, TEM-1 (8) CTX-M-15, TEM-1 (8) CTX-M-15, OXA-1, TEM-1 (5) CTX-M-15, OXA-1 (2) CTX-M-15, SHV-1, OXA-1, TEM-1 (1) SHV-12 (1)	CTX-M-15, SHV-1, TEM-1 (13) CTX-M-15, TEM-1 (6) CTX-M-15, SHV-1, OXA-1, TEM-1(5) CTX-M-15, OXA-1, TEM-1 (4) CTX-M-15, SHV-12, TEM-1 (1) CTX-M-15 (1)	CTX-M-15, SHV-1, TEM-1 (7) CTX-M-15, SHV-1, OXA-1, TEM-1(1) CTX-M-15, TEM-1 (1) CTX-M-15, OXA-1 (1) SHV-12 (1)	CTX-M-15, SHV-1, TEM-1 (2) CTX-M-15, OXA-1, TEM-1 (2) CTX-M-15, OXA-1 (1) CTX-M-15, TEM-1 (1)	CTX-M-15, SHV-1, TEM-1 (1) CTX-M-15 (1)

TABLE 4. **Epidemiological data of the 22 selected strains of extended-spectrum β-lactamase (ESBL) -producing *Escherichia coli* in Ngaoundere Protestant Hospital**

Alternative identification	Type of participants (department in which the participant was at the time of sampling)	Type of relationship/ gender (age)	Phylogenetic group	Cluster type	ST131 PCR	PMQR	IS*Ecp1* element	ESBL types	Resistance to antibiotics (other than β-lactam)
f32	Household member of p3	Daughter (3)	D	I	–	–	–	CTX-M-15, TEM-1	GEN, SXT
20	Maintenance staff *(medicine)*	M (42)	D	I	–	*aac(6')Ib-cr*	–	CTX-M-15, OXA-1, TEM-1	GEN, SXT
hn50	Inpatient *(medicine)*	F (29)	D	II	–	*aac(6')Ib-cr*	+	CTX-M-15, OXA-1, TEM-1	CIP, GEN, SXT
hn89	Inpatient *(medicine)*	M (31)	B2	III	+	*aac(6')Ib-cr*	+	CTX-M-15, OXA-1	CIP, SXT
p13	Nurse *(medicine)*	F (28)	B2	IIIa	+	*aac(6')Ib-cr*	+	CTX-M-15, OXA-1	CIP, SXT
p3	Nurse *(surgery)*	F (41)	B2	IIIb	+	–	+	CTX-M-15, TEM-1	CIP, SXT
p25	Nurse *(intensive care unit)*	F (35)	B2	III	+	–	–	CTX-M-15, TEM-1	CIP, SXT
hn69	Inpatient *(surgery)*	M (40)	B2	IV	–	*aac(6')Ib-cr*	+	CTX-M-15, OXA-1, TEM-1	CIP, GEN, SXT
p5	Nurse *(medicine)*	F (30)	A	V	–	*aac(6')Ib-cr*	–	CTX-M-15, OXA-1	CIP, GEN, NIT, SXT
16	Maintenance staff *(medicine)*	M (38)	A	V	–	*aac(6')Ib-cr*	–	CTX-M-15, OXA-1	CIP, GEN, NIT, SXT
hn82	Inpatient *(medicine)*	M (39)	A	Va	–	*aac(6')Ib-cr*	–	CTX-M-15, OXA-1	CIP, GEN, NIT, SXT
12	Maintenance staff *(operating room)*	F (25)	A	Vb	–	*aac(6')Ib-cr*	–	CTX-M-15, OXA-1	CIP, GEN, NIT, SXT
p4	Nurse *(medicine)*	M (38)	A	Vc	–	*aac(6')Ib-cr*	–	CTX-M-15, OXA-1	CIP, NIT, SXT
p17	Nurse *(surgery)*	F (27)	A	VI	–	*aac(6')Ib-cr*	–	CTX-M-15, OXA-1	CIP, SXT
3	Maintenance staff *(operating room)*	M (41)	A	VIa	–	*aac(6')Ib-cr*	–	CTX-M-15, OXA-1	CIP, SXT
f2	Household member of 11	Sister (18)	A	VII	–	*aac(6')Ib-cr*	+	CTX-M-15, OXA-1, TEM-1	GEN, SXT
11	Maintenance staff *(maternity)*	F (35)	A	VII	–	*aac(6')Ib-cr*	+	CTX-M-15, OXA-1, TEM-1	GEN, SXT
ng33	Carer of hn57	Husband (53)	A	VIII	–	–	–	CTX-M-15, OXA-1, TEM-1	CIP, SXT
ng41	Carer of hn69	Wife (35)	B1	IX	–	–	–	CTX-M-15, OXA-1, TEM-1	CIP, SXT
ng32	Carer of hn57	Sister (55)	B1	X	–	*aac(6')Ib-cr*	+	CTX-M-15, OXA-1, TEM-1	CIP, GEN, SXT
p16	Nurse *(medicine)*	F (33)	B1	X	–	*aac(6')Ib-cr*	+	CTX-M-15, OXA-1, TEM-1	GEN, SXT
hn57	Inpatient *(medicine)*	F (43)	D	XI	–	–	–	CTX-M-15, OXA-1, TEM-1	CIP, GEN, SXT

M, male; F, female; PMQR, plasmid-mediated quinolone-resistance determinants; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; Nit, nitrofurantoin.

tion of two clusters and 17 unique profiles (see Supplementary material, Fig. S3 and Fig. S4). Our results emphasize the high endemicity of CTX-M-15 producers in the study setting (demonstrated by the very high prevalence in all participant categories). In addition, these findings could also explain the dramatic increase in the prevalence of faecal carriage of E-ESBLs previously observed in the same area during two non-outbreak periods separated by 1 year (in 2009 and 2010) [3,4] (and data of this study). Moreover, examination of the different clusters (III, V and VI) showed that different strains of *E. coli* produced the same type of β-lactamase, indicating that the spread does not occur by strain but by another mode of dissemination (e.g. dissemination by plasmids: further studies are ongoing to clarify this).

The PCR for the *pabB* allele of ST131 status identified cluster III with four related isolates as belonging to ST131. The ST131 status was confirmed by multilocus sequence typing. In addition, isolates from this cluster could be assigned to phylogenetic group B2. The remaining ESBL-producing *E. coli* isolates (negative by PCR for *pabB*) belonged to phylogenetic groups A, B2, D and B1 (ten, one, four and three isolates, respectively). Interestingly, strains that belonged to the same phylogenetic group and clustered together showed a similar resistance profile to the antibiotics tested (Table 4). Many of the *E. coli* carrying $bla_{\text{CTX-M-15}}$ from different countries in Europe and North America are homogeneously grouped as *E. coli* O25:H4-ST131 [11,18–20]. In addition, in all $bla_{\text{CTX-M-15}}$ tested, the *aac(6')-Ib-cr* gene was carried by 16 strains (16/22) and the IS*Ecp1* element was found in nine of the strains analysed (9/22). One study has reported that CTX-M ESBL genes are often associated with an IS*Ecp*1 element, which may provide a higher level of expression of the plasmid-located $bla_{\text{CTX-M}}$ genes and facilitate the spread of resistance [21]. Our study provides the first evidence for the presence of an *E. coli* ST131 clone in Cameroon. Strict hygiene measures, staff training to improve hand-washing procedures and changes to the antibiotic policy are essential to limit the spread of these E-ESBLs in hospitals and the community.

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Transparency Declaration

All the authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figures S1 and S2. DNA fingerprinting of the 22 investigated extended-spectrum β-lactamase (ESBL) -producing *Escherichia coli* isolates in Ngaoundere Protestant Hospital (NH). Dendrogram, virtual gel image and similarity matrix demonstrate strain clustering. The horizontal bar on the bottom left indicates the percentage similarity within the strains. A cut-off of 95% similarity (vertical dashed red line) was chosen for determination of clonal relatedness. ICU, Intensive care unit.

Figures S3 and S4. DNA fingerprinting of the 22 investigated extended-spectrum β-lactamase (ESBL) -producing *Klebsiella pneumoniae* isolates in Ngaoundere Protestant Hospital (NH) and Ngaoundere Regional Hospital (RH). *hn59: inpatient identified with a strain of *Escherichia coli*, but the two carers (ng36 and ng37) of this patient carried *K. pneumoniae* isolates. The horizontal bar on the bottom left indicates the percentage similarity within the strains. A cut-off of 95% similarity (vertical dashed red line) was chosen for determination of clonal relatedness.

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