

Extended-spectrum β -lactamase-producing *Enterobacteriaceae* in Cameroonian hospitals

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Abstract Extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* have been described worldwide, but there are few reports on the carriage of these bacteria in Cameroon. In order to investigate the types of ESBLs and to analyse some risk factors associated with ESBL carriage, faecal samples were collected between 3 January and 3 April 2009 from hospitalised patients at Yaounde Central Hospital and at two hospitals in Ngaoundere, Cameroon. Enterobacterial isolates resistant to third-generation cephalosporins were screened for ESBL production using the double-disk synergy test. Polymerase chain reaction (PCR) and DNA sequencing were performed in order to find out the different types of ESBL genes in presumptive ESBL-positive isolates. During the study period, a total of 121 different patients were screened for ESBL carriage. The

prevalence among these patients whose faecal samples were found to contain ESBL-producers was 55.3 % (67/121). According to a univariate analysis, hospitalisation during the previous year was found to be associated with ESBL carriage. Of the 71 bacteria isolated, *Escherichia coli* was predominant and represented 48 % of all isolates. ESBL characterisation revealed two types of ESBLs, CTX-M-15 (96 %) and SHV-12 (4 %). The present study emphasises the importance of screening for ESBLs in laboratories in African countries. The monitoring and detection of ESBL-producing bacteria are important in the setting up of appropriate treatment of patients and to ensure effective infection control efforts.

Introduction

Extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* are mostly encoded by plasmids, although some of them are from chromosomal origin [1–3]. ESBLs are able to hydrolyse a wide range of β -lactam antibiotics, including second- and third-generation cephalosporins [4]. The first ESBLs described were derived from the two “classical” β -lactamase families—TEM and SHV—from which mutations have since occurred. In the last few years, however, new families of ESBL enzymes have been discovered. CTX-M-type ESBLs, a group of enzymes with an increased activity against cefotaxime, have become widespread in Europe and other continents [3, 5]. Since their first description in 1983 in Germany [6], ESBLs have been described worldwide, but in African countries, data are still very scarce. In Cameroon, although rare, studies have reported the presence of ESBL types SHV and CTX-M among inpatients in the city of Yaounde [7–9]. Moreover, although in Yaounde some data on ESBLs are available, in other cities in Cameroon, there is no information on antibacterial resistance. So, to increase the database on the subject, we thought it would be interesting to

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study and determine the prevalence of ESBL-producing *Enterobacteriaceae* isolated from the faecal flora of inpatients more than ten years after the first study [9] at the same hospital (Yaounde Central Hospital) and in two other Cameroonian hospitals located in the Adamawa province (city of Ngaoundere, located 858 km from Yaounde).

Materials and methods

Study setting

During a period of three months (from 3 January 2009 to 3 April 2009), faecal samples were collected from hospitalised patients for more or less 48 h in three different hospitals in Cameroon. Written informed consent was obtained from each patient who volunteered to participate in the study. All participants were asked to fill out a standardised questionnaire, including general demographics data, diagnosis at admission, hospital stays in the previous year and antibiotic use in the past three months. The three participating hospitals were defined as follows:

1. Yaounde Central Hospital is the largest medical institution located in the city of Yaounde (political capital of Cameroon) in the central province, covering a population of about 3,525,664 inhabitants. This hospital has 650 beds, annually admits more than 20,000 inpatients and is comprised of 20 wards divided into five unit specialisations (medicine and specialties; surgery; intensive care unit; emergency; gynaecology; obstetrics and maintenance unit). The recruitment of patients was done in the medical unit and specialties (in cardiology/diabetes and gastroenteritis wards), emergency and intensive care units.
2. Ngaoundere Protestant Hospital and (3) Ngaoundere Regional Hospital are the two largest hospitals located in the city of Ngaoundere (in the Adamawa province, covering a population of about 1,015,622 inhabitants). These hospitals are intermediate-level hospitals in the health system in Cameroon, with about 150 beds each. The annual number of hospitalisations ranges from 5,000 to 10,000 patients across many wards (medicine, surgery, medical imaging, maternity, paediatrics, emergency, intensive care unit, operating room and maintenance unit). In these two hospitals, patients were recruited in the medicine, surgery, intensive care unit and emergency wards.

Detection of ESBL-producing isolates in faecal samples

A total of 0.5 g of each faecal sample was suspended in 5 mL of sterile saline, and aliquots of 50 μ l were streaked onto two selective media, Drigalski and MacConkey agars,

supplemented respectively with cefotaxime (1.5 mg/L) and ceftazidime (2 mg/L), enabling the detection of Gram-negative bacteria resistant to these antibiotics. Plates were incubated for 24 h at 35 ± 2 °C. One colony representing each distinct colonial morphotype was subcultured on MacConkey agar plates and was analysed further. All isolates that showed growth were screened for ESBL production by using both the resistance phenotype and the combined double-disk synergy test with 30 μ g amoxicillin/clavulanate (20 μ g of amoxicillin; 10 μ g of clavulanic acid), 30 μ g cefotaxime, 30 μ g ceftazidime and 30 μ g cefepime disks (BBL, USA), which were applied at a 30 mm distance from amoxicillin/clavulanate [10].

Identification and antimicrobial susceptibility testing

Only the isolates with a positive synergy test—the “presumptive ESBL-producers”—were identified by mass spectrometry using the BioTyper MALDI-TOF mass spectrometer (Bruker). For such ESBL-positive isolates, the minimal inhibitory concentration (MIC) to the following drugs was determined with the Vitek 2 system (bioMérieux, France): temocillin, meropenem, amikacin, gentamicin, ciprofloxacin, nitrofurantoin and trimethoprim/sulphamethoxazole. The results enabled us to define the isolates as being susceptible or resistant according to the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) [11]. All the presumptive ESBL-producers were further analysed by polymerase chain reaction (PCR) aimed at detecting ESBL genes (one isolate of each morphotype from each patient).

Automated DNA isolation

Bacteria were grown overnight on MacConkey agar plates at 35 ± 2 °C. Subsequently, colonies were picked up using a sterile 1- μ l plastic loop and transferred into 400 μ l sterile water. Total DNA was extracted with the Maxwell automat (Promega, USA) according to the manufacturer’s protocol. DNA was eluted in a final volume of 200 μ l. The extracted DNA was stored at -70 °C for further analysis.

PCR detection and sequencing of ESBLs

All isolates were initially screened for the presence of *bla*_{TEM}, *bla*_{SHV} and *bla*_{OXA}-like genes using a multiplex PCR protocol previously described by Dallenne et al. [12]. PCR amplification of *bla*_{CTX-M-1} group alleles was carried out with primers CTX-M3G-F and CTX-M3G-R, as described previously using a simplex PCR according to a previously published method [13]. The primers used for PCR amplification are presented in Table 1. Specific PCR amplification and DNA sequencing of the PCR products were used to determine whether the *bla*_{TEM}, *bla*_{SHV},

Table 1 Primers used for polymerase chain reaction (PCR) amplification

PCR name	β -lactamase(s) targeted	Primer name	Sequence (5'-3')	Amplicon size (bp)	Primer concentration (pmol/ μ l)	Reference
Multiplex <i>TEM</i> , <i>SHV</i>	TEM variants including TEM-1 and TEM-2 SHV variants, including SHV-1	MultiTSO-T_for	CATTTCCGTGTGCGCCCTTATTC	800	0.4	[12]
		MultiTSO-T_rev	CGTTCATCCATAGTTGCCTGAC			
and <i>OXA-1-like</i>	OXA-1, OXA-4 and OXA-30	MultiTSO-S_for	AGCCGCTTGAGCAAATTAAC	713	0.4	
		MultiTSO-S_rev	ATCCCGCAGATAAATCACCCAC			
CTX-M group 1	Variants of CTX-M group 1, including CTX-M-1, CTX-M-3 and CTX-M-15	MultiTSO-O_for	GGCACCAGATTCAACTTTCAAG	564	0.4	
		MultiTSO-O_rev	GACCCCAAGTTTCTCTGTAAGTG			
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	[13]
		CTX-M3G_rev	CCGTTTCCGCTATTACAAAC			

*bla*_{OXA} and *bla*_{CTX-M-1} group alleles were present and to characterise the type of β -lactamase belonging to each family. PCR products (multiplex and simplex PCR) were visualised after electrophoresis at 100 V for 1 h on a 2 % agarose gel containing ethidium bromide. A 100-bp DNA ladder (Promega, USA) was used as a marker size. PCR products were purified using the Wizard[®] kit (Promega, USA) and sequenced using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequence alignment and analyses were performed online using the BLAST program available on the National Center for Biotechnology Information web site (<http://blast.ncbi.nlm.nih.gov/>).

Statistical analysis

Statistical analysis was performed with Epi Info version 3.5.3 [Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA]. Pearson's chi-square analysis or Fisher's exact test, when appropriate, was used for the univariate comparison of all variables. Associations between ESBL carriage and the variables studied were determined by calculation of the odds ratios (ORs) along with 95 % confidence intervals (CIs). A value of $p < 0.05$ was considered to be statistically significant.

Results

Patient characteristics

During the study period, a total of 121 patients were screened for ESBL production: 56 patients were from Yaounde Central Hospital (cardiology/diabetes, 28 patients; gastroenteritis, 26; emergency, 1; and intensive care unit, 1) and 65 patients were from the two Ngaoundere hospitals (medicine, 41 patients; surgery, 20; emergency, 2; and intensive care unit, 2). Overall, for these patients, the mean age was 46.81 ± 19.86 years and males accounted for 57 % of the study participants. The

diagnosis at admission is presented in Table 2. Of all the diseases recorded, diarrhoea and diabetes mellitus were the most prevalent, representing 27.2 and 22.3 %, respectively. Before inclusion in the study, 26 patients had been admitted to hospital during the previous year and 49 patients had received antibiotics during the last 3 months. The names of the types of antibiotics used are also reported in Table 2.

Prevalence of ESBL-producers

Of the 121 cultures tested, 67 grew on both of the selective media and were designated presumptive ESBL-producers. All presumptive ESBL-producing *Enterobacteriaceae* were confirmed as ESBL-producers by PCR.

Overall, the prevalence of ESBL-producers among inpatients was 55.3 % (67/121). Among those patients found to be carrying ESBL-producers, ten were carriers within 48 h of admission to hospital and were, therefore, probably already colonised before admission. The other 57 carriers had already been hospitalised for more than 48 h when screening was done. Of the 67 positive cultures tested, 71 bacteria were isolated (we found one patient colonised with three isolates and two patients colonised with two isolates at Yaounde Central Hospital). The prevalence of ESBL-producers at Yaounde Central Hospital was significantly higher than the prevalence of ESBL-producers in the two Ngaoundere hospitals [73.2 % (41/56) and 40 % (26/65), respectively, $p < 0.000$] (see Table 2). According to the wards where patients were hospitalised, the prevalence of ESBL-producers at the Yaounde Central Hospital was 67.8 % (19/28) in cardiology/diabetes, 76.9 % (20/26) in gastroenteritis and 100 % (1/1) in the remaining services (emergency and intensive care unit). Similarly, in the two Ngaoundere hospitals, the prevalence of ESBL-producers was 39 % (16/41) in medicine, 40 % (8/20) in surgery and 50 % (1/2) in the remaining services (emergency and intensive care unit).

Table 2 Univariate analysis of clinical characteristics for extended-spectrum β -lactamase (ESBL) carriage among the 121 hospitalised patients

Characteristics of patients	ESBL-producers (n=67) No. (%)	Non-ESBL-producers (n=54) No. (%)	Odds ratio	95 % CI	p-value
Male gender	40 (59.7)	29 (53.7)	1.007	0.989–1.025	0.432
Age, years (mean, SD)	47.9, 19.7	45, 20	1.189	0.580–2.436	0.636
YCH	41	15			
NH	26	39	4.10	1.77–9.59	0.000
Diagnosis at admission					
Accidents	5 (7.4)	6 (11.1)	0.65	0.16–2.58	0.351
Diabetes mellitus	19 (28.3)	8 (14.8)	2.28	0.84–6.34	0.075
Diarrhoea	18 (26.8)	15 (27.7)	0.96	0.40–2.30	0.910
Infections	8 (11.9)	17 (31.4)	0.30	0.10–0.82	0.008
Other*	13 (19.4)	6 (11.1)	1.93	0.62–6.22	0.212
Unknown	4 (5.9)	2 (3.7)	1.65	0.25–13.58	0.568
Hospitalisation (previous year)	19 (28.3)	7 (12.9)	4.13	1.37–12.78	0.004
Use of antibiotics (<3 months)	29 (43.2)	20 (37)	1.59	0.51–5.06	0.372
Class of antibiotic used					
Penicillins	8 (27.5)	3 (15)	2.16	0.42–12.30	0.248
Tetracycline	1 (3.4)	–	–	–	–
Penicillins+aminoglycosides+ third-generation cephalosporin	14 (48.2)	14 (70)	0.40	0.10–1.54	0.130
Fluoroquinolones+aminoglycosides+ third-generation cephalosporin	3 (10.3)	2 (10)	1.04	0.12–10.07	0.675
Sulphamides	4 (13.7)	1 (5)	3.04	0.28–77.60	0.317

YCH=Yaounde Central Hospital; NH=Ngaoundere hospitals

*Malignancy, neurological, respiratory

Penicillins (ampicillin, amoxicillin, cloxacillin); tetracycline (doxycycline); aminoglycosides (gentamicin); third-generation cephalosporin (ceftriazone); fluoroquinolones (ciprofloxacin); sulphamides (trimethoprim/sulphamethoxazole)

Potential associated factors for ESBL carriage

As shown in Table 2, when comparing ESBL-producers and non-ESBL-producers among the 121 hospitalised patients, hospitalisation during the previous year appeared to be associated with ESBL carriage ($p<0.000$). However, no significant association was found between ESBL carriage and wards where the patient was hospitalised.

Bacterial identification and susceptibility

Of the 71 bacteria isolated, 34 (47.8 %) were *Escherichia coli*, 28 (39.4 %) *Klebsiella* spp. (27 *K. pneumoniae* and 1 *K. oxytoca*) and 9 (12.6 %) *Enterobacter cloacae*. ESBL-producing *E. coli* was the species frequently isolated in the two Ngaoundere hospitals (61.5 %), whereas at Yaounde Central Hospital, *Klebsiella* spp. were predominant (51.1 %).

The susceptibility profile of strains from the different hospitals to the antibiotics tested was relatively similar. All isolates were susceptible to temocillin, meropenem and amikacin.

Regardless of the hospital, *E. cloacae* was resistant to gentamicin (100 %), ciprofloxacin (90 %), trimethoprim/sulphamethoxazole (100 %) and nitrofurantoin (90 %).

Klebsiella spp. showed resistance to gentamicin (74.5 %), trimethoprim/sulphamethoxazole (100 %) and nitrofurantoin (67.8 %) but a good susceptibility to ciprofloxacin (60.5 %).

E. coli showed resistance to gentamicin (85 %), ciprofloxacin (93.7 %) and trimethoprim/sulphamethoxazole (100 %). However, the susceptibility of strains to nitrofurantoin was different between the hospital sites. Strains from Yaounde Central Hospital showed a good susceptibility (77.8 %) compared to strains from the two Ngaoundere hospitals (43.8 %).

β -lactamase characterisation

Patients shared the same ESBL types regardless of their hospital, and ESBL characterisation revealed two types of ESBLs: CTX-M-15 (96 %) and SHV-12 (4 %). The CTX-M-15 gene was always associated with other β -lactamase genes: OXA-1 and TEM-1 (34 %); TEM-1 alone (22 %); OXA-1 alone (19 %); TEM-1 and SHV-1 (13 %); TEM-1, OXA-1 and SHV-1 (10 %); OXA-1 and SHV-1 (2 %) (Table 3).

Table 3 Bacterial strains, ESBL types and antimicrobial susceptibility

Bacterial strains (number)		Origin (number)	ESBL types (number)	Other β -lactamases (number)	MIC ₉₀ : minimal inhibitory concentration (μ g/ml) of								
					Temocillin	Meropenem	Amikacin	Gentamicin	Ciprofloxacin	Trimethoprim/ sulphamethoxazole	Nitrofurantoin		
<i>Enterobacter cloacae</i> (9)	NPH (3)	CTX-M-15 (2)	TEM-1, OXA-1 (2)		16	0.125	8	32	8	>256		128	
		CTX-M-15 (1)	TEM-1 (1)										
	NRH (2)	CTX-M-15 (2)	TEM-1, OXA-1 (2)		2	0.125	4	32	8	>256		128	
		YCH (4)	CTX-M-15 (2)	TEM-1, OXA-1 (2)		16	0.125	4	32	8	>256		64
	<i>Escherichia coli</i> (34)	NPH (13)	CTX-M-15 (1)	TEM-1 (1)									
			SHV-12 (1)	TEM-1 (1)									
		NRH (3)	CTX-M-15 (7)	TEM-1, OXA-1 (7)		16	0.125	4	32	8	>256		128
			CTX-M-15 (4)	TEM-1 (4)									
		YCH (18)	CTX-M-15 (2)	TEM-1, OXA-1 (2)		16	0.125	4	32	2	>256		64
			CTX-M-15 (1)	OXA-1 (1)									
<i>Klebsiella</i> spp. (28)		NPH (4)	CTX-M-15 (10)	TEM-1, OXA-1 (10)		16	0.125	8	32	8	>256		128
			CTX-M-15 (5)	TEM-1 (5)									
	YCH (23)	CTX-M-15 (3)	OXA-1 (3)										
		SHV-12 (1)	None										

Discussion

The present study was conducted to evaluate the prevalence of ESBL-producing *Enterobacteriaceae* among inpatients in three Cameroonian hospitals. Our results showed high levels of gut colonisation by ESBL-producing *Enterobacteriaceae* among inpatients (55.3 %), much higher than that observed in hospitals in other countries: Philippines (29.9 %) [14], Croatia (9.9 %) [15], Iran (16.8 %) [16], Portugal (39 %) [17], Indonesia (21 %) [18] and in some hospitals in low-income countries: Benin (22 %) [19], Tanzania (28.2 %) [20] and Ethiopia (15 %) [21].

The high prevalence of ESBL-producing *Enterobacteriaceae* described here was also due to the number of asymptomatic carriers entering hospitals. Indeed, in this study, 10 (14.9 %) patients were carriers of ESBL-producing *Enterobacteriaceae* within 48 h of admission to hospital and were, therefore, probably colonised before their admission, while for the other 57 carriers, the source of ESBL infection was unknown. Some studies have demonstrated the role of community transmission of ESBL-producing *Enterobacteriaceae*. Mirelis et al. observed a significant increase in the frequency of ESBL carriers in faecal samples from outpatients attending hospital. These patients came to hospital from the community, carrying strains that expressed ESBL [22]. Moreover, the accumulation of carriers in a catchment population links all of the institutions that serve it; carriers may shed antibiotic-resistant bacteria (ARB) for years but remain undetected, transmitting ARB to others as they move between hospitals, long-term care facilities and the community [23]. Recently, a publication by Lonchel et al. found a prevalence of ESBL-producers of approximately 23.1 % among outpatients (who visited the bacteriology laboratory of Ngaoundere Protestant Hospital with a stool sample requested by their general practitioner) and a prevalence of 6.7 % in faecal samples from student volunteers (the student volunteers at the University of Ngaoundere were attending the clinic for their annual medical examination) [24].

In the present study, the prevalence of ESBL-producers at Yaounde Central Hospital was significantly higher than the prevalence obtained at the two Ngaoundere hospitals ($p < 0.000$). Moreover, an earlier study conducted between 1995 and 1998 at Yaounde Central Hospital found a prevalence of about 12 % of ESBL-producers [9]. In 2009, in the same hospital, we found a dramatic increase in the prevalence of ESBL-producing *Enterobacteriaceae* to around 72.3 % (personal communication) from two wards of this hospital (cardiology/diabetes and gastroenteritis). Our results are likely to reflect the nosocomial spread and dissemination of ESBL-producing *Enterobacteriaceae* within the hospital. In the absence of infection control measures, ESBL-producing *Enterobacteriaceae* could readily pass horizontally from patient to patient. Due to a number of limitations,

we were not able to determine the relatedness between isolates recovered from different patients.

In the two Ngaoundere hospitals, the prevalence of ESBL-producing *Enterobacteriaceae* found was also high: 44 % in Ngaoundere Protestant Hospital and 30 % in Ngaoundere Regional Hospital. To our knowledge, the present study is the first survey in the area and underlines the great need to apply proper empiric therapy through the implementation of methods of screening for ESBL-producing bacteria, which are currently lacking in laboratories in Cameroon.

We also investigated the potential associated factors for ESBL carriage and found no association between ESBL carriage and wards where the patient was hospitalised. However, hospitalisation during the previous year was found to be associated with ESBL carriage. This factor has been widely reported as a major cause of the development of infection by ESBL-producing *Enterobacteriaceae* in many studies [25–28].

In our study, we should sound the alarm regarding antibacterial resistance. Indeed, most of the patients were admitted with a history of antibiotic consumption. Patients had used an antibiotic or a combination of several antibiotics for empiric treatment. Some antibiotics were prescribed by medical professionals, while others were not. In general, there are no restrictions to the use of antibiotics in Cameroon or in many African countries, leading to a greater consumption of broad-spectrum antibiotics by the population. Prior antibiotic use has previously been described in many studies as a risk factor associated with ESBL carriage [25, 28–30].

Among the collected isolates, *E. coli* and *K. pneumoniae* were the main species identified. ESBL-producing *E. coli* and *K. pneumoniae* remain the predominant organisms harbouring ESBL worldwide [5]. The majority of our ESBL-producing *Enterobacteriaceae* was multiresistant; specifically, most were resistant to third-generation cephalosporins, ciprofloxacin, gentamicin and trimethoprim/sulphamethoxazole. These results are in line with the findings of previous studies in other African countries [16, 19, 31]. However, we found that drugs such as amoxicillin, gentamicin, trimethoprim/sulphamethoxazole and ceftriaxone were often used by patients in our study. These antibiotics are widely prescribed in Cameroon because of their widespread availability and low cost on the market. The previous use of fluoroquinolone (ciprofloxacin) appeared to have been an associated factor for ESBL carriage ($p < 0.005$) [24]. The reduction of efficacy of these drugs in the treatment of infections caused by ESBL-producing *Enterobacteriaceae* has been shown in several studies [16, 19, 21, 26, 32, 33] and their use should, therefore, be limited.

The results of PCR and DNA sequencing of ESBL genes found that all of the cefotaxime- and ceftazidime-resistant

isolates had the CTX-M-15 enzyme. CTX-M enzymes (especially CTX-M-15) have emerged worldwide and have been identified in European, American and Asian countries [17, 34–37]. In African countries, the CTX-M-15 gene has been found in Tanzania [20, 38], South Africa [34, 39], Algeria [40–42], Nigeria [43], Egypt [31], the Central African Republic [44] and Tunisia [45]. The gene has also been found in Hungary [46] and Colombia [47]. In our study, the CTX-M-15 gene was found to be always associated with other β -lactamase genes (TEM-1, OXA-1 and SHV-1). The CTX-M-15 gene was found in 96 % of the isolates. Our results are similar to those regarding the distribution of the CTX-M-15 ESBL in *Enterobacteriaceae* in studies performed in other countries: Indonesia, 94.5 % of isolates [18]; Tunisia, 91 % [45]; Egypt, 89 % [31]; Mali, 83 % [48] and Algeria, 81 % [49]. It is clear that CTX-M-15 ESBLs are becoming widely distributed throughout Africa.

The other reported ESBL gene in the present study was SHV-12. This gene was found in *K. pneumoniae* and *E. cloacae*. The CTX-M-15 ESBL and SHV-12 ESBL were previously described in Cameroon [9, 24, 50] and, recently, types SHV-5 and -27 were also found [7]. As far as other African countries are concerned, type CTX-M-12 has been found in Kenya [51], CTX-M-14, -15 and -27 in Egypt [52] and CTX-M-3 and -15 in the Central African Republic [44].

Our results highlight a high prevalence of the faecal carriage of ESBL-producing *Enterobacteriaceae* in Cameroonian hospitals and denote the role of the community as a reservoir for the dissemination of ESBLs within hospitals. The present study emphasises the importance of screening for ESBLs in the laboratory. The monitoring and detection of ESBL-producing bacteria are important to ensure the appropriate treatment of patients and effective infection control efforts.

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Conflict of interest The authors declare that they have no conflict of interest.

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References

- Song W, Kim J, Bae IK, Jeong SH, Seo YH, Shin JH, Jang SJ, Uh Y, Shin JH, Lee MK, Lee K (2011) Chromosome-encoded AmpC and CTX-M extended-spectrum beta-lactamases in clinical isolates of *Proteus mirabilis* from Korea. *Antimicrob Agents Chemother* 55(4):1414–1419
- Fabre L, Delaune A, Espie E, Nygard K, Pardos M, Polomack L, Guesnier F, Galimand M, Lassen J, Weill FX (2009) Chromosomal integration of the extended-spectrum beta-lactamase gene *bla*CTX-M-15 in *Salmonella enterica* serotype Concord isolates from internationally adopted children. *Antimicrob Agents Chemother* 53(5):1808–1816
- Cantón R, González-Alba JM, Galán JC (2012) CTX-M enzymes: origin and diffusion. *Front Microbiol* 3:110
- Rahal JJ (2000) Extended-spectrum beta-lactamases: how big is the problem? *Clin Microbiol Infect* 6(Suppl 2):2–6
- Colodner R, Raz R (2005) Extended-spectrum beta-lactamases: the end of cephalosporins? *Isr Med Assoc J* 7(5):336–338
- Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S (1983) Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection* 11(6):315–317
- Breurec S, Guesseend N, Timinouni M, Le TA, Cao V, Ngandjio A, Randiriririna F, Thiberge JM, Kinana A, Dufougeray A, Perrier-Gros-Claude JD, Boisier P, Garin B, Brisse S (2012) *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. *Clin Microbiol Infect*. [Epub ahead of print]
- Gangoué-Piéboji J (2007) Caractérisation des beta-lactamases et leur inhibition par les extraits de plantes médicinales. BICTEL/e—ULG Serveur institutionnel des thèses de doctorat
- Gangoué-Piéboji J, Bedenic B, Koulla-Shiro S, Randegger C, Adiogo D, Ngassam P, Ndumbe P, Hächler H (2005) Extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* in Yaounde, Cameroon. *J Clin Microbiol* 43(7):3273–3277
- Jarlier V, Nicolas MH, Fournier G, Philippon A (1988) Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 10(4):867–878
- Clinical and Laboratory Standards Institute (CLSI) (2010) Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20. CLSI, Wayne, PA
- Dallenne C, Da Costa A, Decré 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(3):490–495
- Pagani L, Dell'Amico E, Migliavacca R, D'Andrea MM, Giacobone E, Amicosante G, Romero E, Rossolini GM (2003) Multiple CTX-M-type extended-spectrum beta-lactamases in nosocomial isolates of *Enterobacteriaceae* from a hospital in northern Italy. *J Clin Microbiol* 41(9):4264–4269
- Bomasang ES, Mendoza MT (2003) Prevalence and risk factors associated with extended spectrum beta lactamase (ESBL) production among selected *Enterobacteriaceae* isolates at the Philippine General Hospital. *Phil J Microbiol Infect Dis* 32:151–158
- Tonkic M, Goic-Barisic I, Punda-Polic V (2005) Prevalence and antimicrobial resistance of extended-spectrum beta-lactamases-producing *Escherichia coli* and *Klebsiella pneumoniae* strains isolated in a university hospital in Split, Croatia. *Int Microbiol* 8(2):119–124
- Ramazanzadeh R, Mansouri M (2009) Spread of extended-spectrum beta-lactamase producing *Escherichia coli* clinical isolates in Sanandaj hospitals. *J Biol Sci* 9:362–366
- Machado E, Coque TM, Cantón R, Novais A, Sousa JC, Baquero F, Peixe L; Portuguese Resistance Study Group (2007) High diversity of extended-spectrum beta-lactamases among clinical isolates of *Enterobacteriaceae* from Portugal. *J Antimicrob Chemother* 60(6):1370–1374
- Severin JA, Mertaniasih NM, Kuntaman K, Lestari ES, Purwanta M, Lemmens-Den Toom N, Duerink DO, Hadi U, van Belkum A,

- Verbrugh HA, Goessens WH; Study Group 'Antimicrobial Resistance in Indonesia: Prevalence and Prevention' (AMRIN) (2010) Molecular characterization of extended-spectrum beta-lactamases in clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates from Surabaya, Indonesia. *J Antimicrob Chemother* 65(3):465–469
19. Ahoyo AT, Baba-Moussa L, Anago AE, Avogbe P, Missihoun TD, Loko F, Prévost G, Sanni A, Dramane K (2007) Incidence of infections due to *Escherichia coli* strains producing extended spectrum beta-lactamase, in the Zou/Collines Hospital Centre (CHDZ/C) in Benin. *Med Mal Infect* 37(11):746–752
 20. Blomberg B, Jureen R, Manji KP, Tamim BS, Mwakagile DS, Urassa WK, Fataki M, Msangi V, Tellevik MG, Maselle SY, Langeland N (2005) High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. *J Clin Microbiol* 43(2):745–749
 21. Seid J, Asrat D (2005) Occurrence of extended spectrum beta-lactamase enzymes in clinical isolates of *Klebsiella* species from Harar region, eastern Ethiopia. *Acta Trop* 95(2):143–148
 22. Mirelis B, Navarro F, Miró E, Mesa RJ, Coll P, Prats G (2003) Community transmission of extended-spectrum beta-lactamase. *Emerg Infect Dis* 9(8):1024–1025
 23. Smith DL, Dushoff J, Perencevich EN, Harris AD, Levin SA (2004) Persistent colonization and the spread of antibiotic resistance in nosocomial pathogens: resistance is a regional problem. *Proc Natl Acad Sci USA* 101(10):3709–3714
 24. Lonchel CM, Meex C, Gangoué-Piéboji J, Boreux R, Okomo Assoumou MC, Melin P, De Mol P (2012) Proportion of extended-spectrum β -lactamase-producing *Enterobacteriaceae* in community setting in Ngaoundere, Cameroon. *BMC Infect Dis* 12(1):53
 25. Colodner R, Rock W, Chazan B, Keller N, Guy N, Sakran W, Raz R (2004) Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. *Eur J Clin Microbiol Infect Dis* 23:163–167
 26. Andriatahina T, Randrianirina F, Hariniana ER, Talarmin A, Raobijaona H, Buisson Y, Richard V (2010) High prevalence of fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a pediatric unit in Madagascar. *BMC Infect Dis* 10:204
 27. Calbo E, Romani V, Xercavins M, Gómez L, Vidal CG, Quintana S, Vila J, Garau J (2006) Risk factors for community-onset urinary tract infections due to *Escherichia coli* harbouring extended-spectrum beta-lactamases. *J Antimicrob Chemother* 57(4):780–783
 28. Rodríguez-Baño J, Navarro MD, Romero L, Martínez-Martínez L, Muniain MA, Perea EJ, Pérez-Cano R, Pascual A (2004) Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. *J Clin Microbiol* 42:1089–1094
 29. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO (2001) Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis* 32(8):1162–1171
 30. Kuster SP, Hasse B, Huebner V, Bansal V, Zbinden R, Ruef C, Ledergerber B, Weber R (2010) Risks factors for infections with extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care university hospital in Switzerland. *Infection* 38(1):33–40
 31. Fam N, Leflon-Guibout V, Fouad S, Aboul-Fadl L, Marcon E, Desouky D, El-Defrawy I, Abou-Aitta A, Klena J, Nicolas-Chanoine MH (2011) CTX-M-15-producing *Escherichia coli* clinical isolates in Cairo (Egypt), including isolates of clonal complex ST10 and clones ST131, ST73, and ST405 in both community and hospital settings. *Microb Drug Resist* 17(1):67–73
 32. 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(7):925–934
 33. Gangoué-Piéboji J, Koulla-Shiro S, Ngassam P, Adio D, Ndumbe P (2006) Antimicrobial activity against gram negative bacilli from Yaounde Central Hospital, Cameroon. *Afr Health Sci* 6(4):232–235
 34. Peirano G, van Greune CH, Pitout JD (2011) Characteristics of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* from community hospitals in South Africa. *Diagn Microbiol Infect Dis* 69(4):449–453
 35. Drieux L, Brossier F, Duquesnoy O, Aubry A, Robert J, Sougakoff W, Lecso-Bornet M, Jarlier V (2009) Increase in hospital-acquired bloodstream infections caused by extended spectrum beta-lactamase-producing *Escherichia coli* in a large French teaching hospital. *Eur J Clin Microbiol Infect Dis* 28(5):491–498
 36. Silva J, Aguilar C, Becerra Z, López-Antuñano F, García R (1999) Extended-spectrum beta-lactamases in clinical isolates of enterobacteria in Mexico. *Microb Drug Resist* 5(3):189–193
 37. Ho PL, Poon WW, Loke SL, Leung MS, Chow KH, Wong RC, Yip KS, Lai EL, Tsang KW; COMBAT study group (2007) Community emergence of CTX-M type extended-spectrum beta-lactamases among urinary *Escherichia coli* from women. *J Antimicrob Chemother* 60(1):140–144
 38. Ndogulile F, Jureen R, Harthug S, Urassa W, Langeland N (2005) Extended spectrum beta-lactamases among Gram-negative bacteria of nosocomial origin from an intensive care unit of a tertiary health facility in Tanzania. *BMC Infect Dis* 5:86
 39. Ehlers MM, Veldsman C, Makgatho EP, Dove MG, Hoosen AA, Kock MM (2009) Detection of *bla*SHV, *bla*TEM and *bla*CTX-M antibiotic resistance genes in randomly selected bacterial pathogens from the Steve Biko Academic Hospital. *FEMS Immunol Med Microbiol* 56(3):191–196
 40. Touati A, Brasme L, Benallaoua S, Madoux J, Gharout A, de Champs C (2008) *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates producing CTX-M-15 recovered from hospital environmental surfaces from Algeria. *J Hosp Infect* 68(2):183–185
 41. Touati A, Benallaoua S, Forte D, Madoux J, Brasme L, de Champs C (2006) First report of CTX-M-15 and CTX-M-3 beta-lactamases among clinical isolates of *Enterobacteriaceae* in Béjaia, Algeria. *Int J Antimicrob Agents* 27(5):397–402
 42. Touati A, Benallaoua S, Djoudi F, Madoux J, Brasme L, De Champs C (2007) Characterization of CTX-M-15-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from hospital environments in Algeria. *Microb Drug Resist* 13(2):85–89
 43. Olowe OA, Grobbel M, Büchter B, Lübke-Becker A, Fruth A, Wieler LH (2010) Detection of *bla*(CTX-M-15) extended-spectrum beta-lactamase genes in *Escherichia coli* from hospital patients in Nigeria. *Int J Antimicrob Agents* 35(2):206–207
 44. Frank T, Arlet G, Gautier V, Talarmin A, Bercion R (2006) Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*, Central African Republic. *Emerg Infect Dis* 12(5):863–865
 45. Dahmen S, Bettaieb D, Mansour W, Boujaafar N, Bouallège O, Arlet G (2010) Characterization and molecular epidemiology of extended-spectrum beta-lactamases in clinical isolates of *Enterobacteriaceae* in a Tunisian University Hospital. *Microb Drug Resist* 16(2):163–170
 46. Damjanova I, Tóth A, Pászti J, Bauernfeind A, Füzi M (2006) Nationwide spread of clonally related CTX-M-15-producing multidrug-resistant *Klebsiella pneumoniae* strains in Hungary. *Eur J Clin Microbiol Infect Dis* 25(4):275–278
 47. Valenzuela de Silva EM, Mantilla Anaya JR, Reguero Reza MT, González Mejía EB, Pulido Manrique IY, Darío Llerena I, Velandia D (2006) Detection of CTX-M-1, CTX-M-15, and CTX-M-2 in clinical isolates of *Enterobacteriaceae* in Bogota, Colombia. *J Clin Microbiol* 44(5):1919–1920
 48. Duval V, Maiga I, Maiga A, Guillard T, Brasme L, Forte D, Madoux J, Vernet-Garnier V, De Champs C (2009) High prevalence of CTX-M-type beta-lactamases among clinical isolates of

- Enterobacteriaceae* in Bamako, Mali. Antimicrob Agents Chemother 53(11):4957–4958
49. Ramdani-Bouguessa N, Mendonça N, Leitão J, Ferreira E, Tazir M, Caniça M (2006) CTX-M-3 and CTX-M-15 extended-spectrum beta-lactamases in isolates of *Escherichia coli* from a hospital in Algiers, Algeria. J Clin Microbiol 44(12):4584–4586
 50. Gangoué-Piéboji J, Miriagou V, Vourli S, Tzelepi E, Ngassam P, Tzouvelekis LS (2005) Emergence of CTX-M-15-producing enterobacteria in Cameroon and characterization of a *bla*CTX-M-15-carrying element. Antimicrob Agents Chemother 49(1):441–443
 51. Kariuki S, Corkill JE, Revathi G, Musoke R, Hart CA (2001) Molecular characterization of a novel plasmid-encoded cefotaximase (CTX-M-12) found in clinical *Klebsiella pneumoniae* isolates from Kenya. Antimicrob Agents Chemother 45(7):2141–2143
 52. Mohamed Al-Agamy MH, El-Din Ashour MS, Wiegand I (2006) First description of CTX-M beta-lactamase-producing clinical *Escherichia coli* isolates from Egypt. Int J Antimicrob Agents 27(6):545–548