Clinical Microbiology and Infection 22 (2016) 891.e1–891.e4

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Research note

High rate of faecal carriage of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in healthy children in Bangui, Central African Republic

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ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form
30 June 2016
Accepted 2 July 2016
Available online 9 July 2016

Editor: C. Pulcini

Keywords:
Africa
Antimicrobial resistance
Central African Republic
CTX-M-15
Enterobacteriaceae
ESBL faecal carriage
IncFIb

ABSTRACT

The aim of this study was to estimate the prevalence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) in faeces of healthy children aged 0–59 months in Bangui (Central African Republic). Stool samples of 134 children, recruited for a matched case-control study, were cultured on a commercial ESBL-selective chromogenic medium (CHROMagar ESBL, France). The phenotypic resistance patterns of isolated strains were investigated, as well as the genetic basis for antibiotic resistance. The factors associated with increased risk for ESBL-E carriage were also studied. The prevalence of ESBL-E carriage was 59% (79/134), one of the highest reported worldwide. The only factor found to be associated with carriage was living in a highest-income family (p=0.03). In all, 83 ESBL-E were recovered as simultaneous carriage of two strains was detected in four children. $bla_{CTX\text{-}M\text{-}15}$ was found in all strains except two, frequently associated with *qnr* (54/81, 66%) and *aac*(6′)-*Ib-cr* (35/81, 43%) genes. *Escherichia coli*, the most commonly recovered species (51/83, 61%), was assigned mainly to the pandemic B2-O25b-ST131 group (39/51, 76%). Resistance transfer, which was studied in 20 randomly selected ESBL-E strains, was successful in 13 (13/20, 65%) isolates. In eight of these isolates (8/13, 62%), $bla_{CTX\text{-}M\text{-}15}$ genes were found in incompatibility group FIb conjugative plasmids. We found one of the highest prevalence rates of faecal carriage of ESBL-E reported worldwide, highlighting the need to improve control of the distribution of antibiotics in limited-resource countries. **A. Farra, CMI 2016;22:891.e1–891.e4**

Introduction

Enterobacteriaceae resistant to third-generation cephalosporins are a major threat, not only in hospitals but also in the community. Resistance is mediated mainly by acquired extended-spectrum β-lactamase (ESBL) enzymes, which can hydrolyse almost all β-lactams. In limited-resource countries, poor hygiene has been suspected to be one of the main reasons for the spread of multi-resistant bacteria as human excreta reach the environment directly. Indeed, the digestive tract is the main reservoir for enterobacteria [1]. Few data on asymptomatic faecal carriage of ESBL-producing *Enterobacteriaceae* (ESBL-E) in the community are available in Sub-Saharan Africa [1]. We studied their prevalence in faeces of children in the Central African Republic (CAR)'s capital city, Bangui, the phenotypic resistance patterns, and the genetic basis for antibiotic resistance. In addition, the factors associated with increased risk for ESBL carriage were investigated.

Methods

This study was conducted among controls recruited for a matched case–control study of severe diarrhoea in children aged

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http://dx.doi.org/10.1016/j.cmi.2016.07.001
1198-743X/

0–59 months to estimate the pathogen burden in Bangui (428 controls and 428 cases recruited between December 2011 and November 2013) [2]. Only the controls selected between March and November 2013 were included in this study (134 children). Controls selected in Bangui's urban districts were defined as healthy age- and sex-matched children without diarrhoea and antibiotic use during the previous 7 days, living in the same urban quarter as the case. The study protocol was approved by the national ethics committee of CAR. Written informed consent was obtained from the parents or guardians of all children.

Stool samples were cultured on a commercial ESBL-selective chromogenic medium (CHROMagar ESBL, France). All colonies that differed by colour and/or morphology were selected. *Enterobacteriaceae* species identification, antimicrobial susceptibility by the disc diffusion method, detection of the production of ESBL using the double-disc synergy test, genomic extraction, screening for $bla_{\text{CTX-M}}$ and bla_{SHV} β-lactamase genes, the *aac*(6′)-*Ib* gene and its variant, the quinolone resistance *qnr*A/B/S and *qepA* genes were performed as previously described [3]. Duplex PCR targeting the *pabB* and *trpA* genes was used to determine whether the *E. coli* isolate belonged to the B2-O25b-Sequence Type (ST) 131 group [4], followed by confirmation on 10 randomly selected strains using Multilocus Sequence Typing (http://mlst.ucc.ie/), phylogroup B2 [5] and O25b typing [6] using PCR. Conjugation and transformation experiments were performed as described previously [3]. Data were collected on nutritional and socioeconomic status. The chi-square test and Student *t* test were used to compare categorical and continuous variables in univariate analysis, respectively. Factors associated with increased risk for ESBL carriage in the univariate analysis with p values <0.25 were retained for the multivariate analysis. We considered that p values <0.05 indicated significance.

Results and Discussion

A total of 134 children (78 boys, 56 girls; mean age 14.7 months; median age 10.5 months; 25th percentile, 7 months; 75th percentile, 16 months) were included. The overall prevalence of faecal carriage ESBL-E in children was 59% (79/134). This rate is one of the highest reported worldwide (1–69%), the highest being reported in Thailand (69% in 2010) and the lowest in Europe (<12%) [1]. Very few data are available on the prevalence of faecal carriage of ESBL-E in Africa, although it appears to be high, with 22% in Madagascar, 31% in Niger, and 33% in Guinea-Bissau among children admitted to hospital, and 63% in Egypt and 10% in Cameroon among healthy individuals [1,7–9].

Poverty has been associated with a higher incidence of ESBL-E rectal carriage [1,9], whereas we found an association with children in the highest-income families ($p < 0.03$) (Table 1). Our study was conducted in a very low-income country engulfed in a civil war during the study period. It is likely that many children in low socioeconomic families did not receive antibiotics because their parents could not afford them. However, this explanation cannot be tested as we were unable to collect reliable data. The antibiotics

Table 1
Risk factors for extended-spectrum β-lactamase *Enterobacteriaceae* rectal carriage in 134 children

Characteristic[a]	ESBL-E carriage		Univariate analysis		Multivariate analysis	
	No (n=55)	Yes (n=79)	OR (95% CI)	p	Adjusted OR (95% CI)	p
Sex						
Male	31 (39.7)	47 (60.3)	Reference	0.718	–	
Female	24 (42.9)	32 (57.1)	0.88 (0.44–1.77)			
Age (months)						
0–11	30 (39.5)	46 (60.5)	0.61 (0.21–1.76)	0.214	0.64 (0.19–2.12)	0.083
12–23	19 (51.4)	18 (48.5)	0.38 (0.12–1.19)		0.28 (0.08–1.00)	
24–59	6 (28.6)	15 (71.4)	Reference		Reference	
Nutritional status						
SAM[b] or MAM[c]	9 (64.3)	5 (35.7)	Reference	0.064	Reference	0.067
No malnutrition	46 (38.3)	74 (61.7)	2.90 (0.91–90.18)		3.13 (0.92–10.66)	
Socioeconomic class[d]						
Lowest income	7 (87.5)	1 (12.5)	Reference	0.013	Reference	0.028
Middle income	44 (39.3)	68 (60.7)	10.81 (1.29–90.97)		14.09 (1.59–124.93)	
Highest income	4 (28.6)	10 (71.4)	17.5 (1.60–191.89)		31.06 (2.49–387.13)	
Residence						
Urban	45 (38.8)	71 (61.2)	Reference	0.182	Reference	0.207
Rural	10 (55.6)	8 (44.4)	0.51 (0.19–1.38)		0.51 (0.18–1.45)	
Latrines						
None or traditional	53 (41.1)	76 (58.9)	Reference	0.961	–	
Modern	2 (40.0)	3 (60.0)	1.05 (0.17–6.48)			
No. of household members						
≤ 5	20 (41.7)	28 (58.3)	Reference	0.994	–	
5–10	24 (40.7)	35 (59.3)	1.04 (0.48–2.26)			
> 10	11 (40.7)	16 (59.3)	1.04 (0.40–2.71)			
Meals						
With the hand only	13 (46.4)	15 (53.6)	Reference	0.595	–	
With spoon and fork only	19 (43.2)	25 (56.8)	1.14 (0.44–2.96)			
Access to improved water[e]	29 (40.3)	43 (59.7)	1.07 (0.54–2.14)	0.846	–	
Chlorinated drinking-water	21 (46.7)	24 (53.3)	0.71 (0.34–1.46)	0.348	–	
Use of pacifier	5 (35.7)	9 (64.3)	1.29 (0.41–4.07)	0.666	–	
Mother completed primary school	31 (41.0)	45 (59.2)	1.02 (0.51–2.05)	0.945	–	

[a] Of note, contact(s) with healthcare centre in the last 6 months and consumption of antibiotics in the last 3 months were not studied as we were unable to collect reliable data.
[b] Severe acute malnutrition (SAM): mid-upper arm circumference < 115 mm.
[c] Moderate acute malnutrition (MAM): mid-upper arm circumference < 125mm and ≥ 115 mm.
[d] For socioeconomic level, lowest income was defined as having no cell phone; middle income as having a cell phone but no car, working refrigerator, or modern sanitation (flushing toilet inside the house); and highest income as having a car, a working refrigerator, and modern sanitation.
[e] Improved water was defined as access to water from a spring or running water.

Table 2
Resistance genes transferred to transconjugants

Species[a]	PMQR determinant[b]	Transconjugant[a]		
		Incompatibility group	PMQR determinant[b]	Non-β-lactam drug resistance
Escherichia coli	QnrS, AAC(6′)-Ib-cr	IncFIB	QnrS, AAC(6′)-Ib-cr	GEN, TOB, NAL
E. coli	AAC(6′)-Ib-cr	TF	–	–
E. coli	–	TF	–	–
E. coli	QnrS, AAC(6′)-Ib-cr	IncFIB	QnrS, AAC(6′)-Ib-cr	GEN, TOB, NAL
E. coli	–	TF	–	–
E. coli	–	IncY	–	GEN, TOB, STR, NAL, TET, SUL, SXT
E. coli	QnrS, AAC(6′)-Ib-cr	IncFIB	QnrS, AAC(6′)-Ib-cr	GEN, TOB, TET
E. coli	qnrS	IncFIB	qnrS	GEN, TOB, NAL
E. coli	qnrS	IncFIB	qnrS	GEN, TOB, NAL
E. coli	QnrS, AAC(6′)-Ib-cr	IncFIB	QnrS, AAC(6′)-Ib-cr	KAN, STR, TET, SUL, SXT
E. coli	QnrB, AAC(6′)-Ib-cr	IncFIB	QnrB, AAC(6′)-Ib-cr	KAN, STR, TET, SUL, SXT
E. coli	AAC(6′)-Ib-cr	IncFIB	AAC(6′)-Ib-cr	GEN, TOB, NAL
E. coli	–	IncY	–	GEN, TOB, NAL
E. coli	–	TF	–	–
E. coli	AAC(6′)-Ib-cr	IncFIB	–	GEN, TOB, STR, NAL, TET, SUL, SXT
Klebsiella pneumoniae	QnrB, AAC(6′)-Ib-cr	TF	–	–
K. pneumoniae	QnrS, AAC(6′)-Ib-cr	TF	–	–
K. pneumoniae	QnrB, AAC(6′)-Ib-cr	IncFIB	–	KAN, STR, TET, SUL, SXT
K. pneumoniae	QnrB, QnrS, AAC(6′)-Ib-cr	NT	–	–
Enterobacter cloacae	QnrB, AAC(6′)-Ib-cr	TF	–	–

TF, transfer to the *E. coli* recipient failed; NT, the plasmid could not be typed; PMQR, plasmid-mediated quinolone resistance; KAN, kanamycin; GEN, gentamicin; TOB, tobramycin; NET, netilmicin; STR, streptomycin; NA, nalidixic acid; TET, tetracycline; SUL, sulfamide; SXT, co-trimoxazole.
[a] $bla_{CTX\text{-}M\text{-}15}$ was found in all the studied strains and transconjugants.
[b] QepA and QnrA were not found in any of the isolates.

received by children in the highest-income families would have been obtained primarily without prescription from street vendors. They are much less expensive than in pharmacies, but inappropriate antibiotic prescription and substandard quality of some drugs favour the spread of antimicrobial resistance [10].

In all, 83 ESBL-E were recovered as simultaneous carriage of two strains was detected in four children. *Escherichia coli* was the most frequent species (51/83, 61%), as described in most of the studies [1], the other species being *Klebsiella pneumoniae* (20/83, 24%), *Enterobacter cloacae* (5/83, 6%), *Klebsiella oxytoca* (4/83, 5%), and *Morganella morgani* (1/83, 1%). None was resistant to imipenem. Most of the *E. coli* isolates (39/51, 76%) corresponded to the pandemic hypervirulent *E. coli* B2-O25b-ST131 group, in keeping with previous descriptions of infectious strains in CAR [3] and elsewhere. This group has contributed extensively to dissemination of $bla_{CTX\text{-}M15}$ [4,11], the most widely distributed ESBL worldwide. Further studies based on high-resolution genotyping methods are needed to determine its evolutionary history.

In our study, $bla_{CTX\text{-}M\text{-}15}$ was found in all strains except two ($bla_{CTX\text{-}M\text{-}127}$, n=1; $bla_{CTX\text{-}M\text{-}27}$, n=1), frequently associated with *qnr* (54/81, 66%) and *aac*(6′)-*Ib-cr* (35/81, 43%) genes. *qepA* and *qnrA* were not found. Transfer of ESBL was successful for 13 (13/20, 65%) ESBL-E out of the 20 randomly selected, only by conjugation, highlighting their potential to spread (Table 2). ESBL were carried mainly on plasmids of IncFIb incompatibility groups (8/13, 62%) that can be considered pandemic [12]. The *aac*(6′)-*Ib-cr* and *qnr* genes were systematically co-transferred with $bla_{CTX\text{-}M\text{-}15}$ in *E. coli*, except for one strain, but not in *K. pneumoniae* strains. Transfer of additional resistance genes was also detected (Table 2). Their aggregation on the same plasmid and its spread is of great concern.

Our cohort was not a random sample, but a matched control group of children with diarrhoea, which might have introduced a bias. However, the data reported here are particularly important. Children in the lowest-income families may not be at higher risk for harbouring an ESBL-E, suggesting that previous antibiotic exposure is probably the most important factor. Therefore, control of multidrug resistant bacteria spread should focus also on better control of antibiotic distribution.

Transparency declaration

This study was supported by funds from the Fondation Total. The authors have no conflict of interest to declare.

Acknowledgements

We express our sincere thanks to the children and parents who participated in this study. We also extend our thanks to the staff at the Institut Pasteur of Bangui, particularly Ernest José Gbao and Natacha Zato, Antoine Talarmin (Institut Pasteur of Guadeloupe) for many helpful discussions, and Benoit Garin (Institut Pasteur of Madagascar) for technical help.

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