MAJOR ARTICLE

Massive Increase, Spread, and Exchange of Extended Spectrum β-Lactamase–Encoding Genes Among Intestinal *Enterobacteriaceae* in Hospitalized Children With Severe Acute Malnutrition in Niger

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Background. From the time of CTX-M emergence, extended-spectrum β-lactamase–producing enterobacteria (ESBL-E) have spread worldwide in community settings as well as in hospitals, particularly in developing countries. Although their dissemination appears linked to *Escherichia coli* intestinal carriage, precise paths of this dynamic are largely unknown.

Methods. Children from a pediatric renutrition center were prospectively enrolled in a fecal carriage study. Antibiotic exposure was recorded. ESBL-E strains were isolated using selective media from fecal samples obtained at admission and, when negative, also at discharge. ESBL-encoding genes were identified, their environments and plasmids were characterized, and clonality was assessed with polymerase chain reaction–based methods and pulsed-field gel electrophoresis for *E. coli* and *Klebsiella pneumoniae*. *E. coli* strains were subjected to multilocus sequence typing.

Results. The ESBL-E carriage rate was 31% at admission in the 55 children enrolled. All children enrolled received antibiotics during hospitalization. Among the ESBL-E–negative children, 16 were resampled at discharge, and the acquisition rate was 94%. The $bla_{\text{CTX-M-15}}$ gene was found in >90% of the carriers. Genetic environments and plasmid characterization evidenced the roles of a worldwide, previously described, multidrug-resistant region and of IncF plasmids in CTX-M-15 *E. coli* dissemination. Diversity of CTX-M-15–carrying genetic structures and clonality of acquired ESBL *E. coli* suggested horizontal genetic transfer and underlined the potential of some ST types for nosocomial cross-transmission.

Conclusions. Cross-transmission and high selective pressure lead to very high acquisition of ESBL-E carriage, contributing to dissemination in the community. Strict hygiene measures as well as careful balancing of benefit–risk ratio of current antibiotic policies need to be reevaluated.

In developing countries, children who are hospitalized for malnutrition are at high risk of dying of severe bacterial infections [1–3]. Thus, the World Health Organization (WHO) recommends empiric systemic broad-spectrum antibiotics, whenever they are suspect of infection [4]. However, this can promote intestinal colonization by multiresistant bacteria, especially gram-negative bacilli [5, 6]. Recently, focus has been directed on intestinal carriage by extended -spectrum β-lactamase–producing enterobacteria (ESBL-E), because it is the primum movens for multidrug-resistant bacterial infections, which leave few therapeutic options open, negatively affect outcome, and promote

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resistance dissemination [7, 8]. Among ESBL, CTX-M are of major concern because they have spread worldwide, in community settings as well as in hospitals [9]. These plasmid-borne genes impair efficacy of all β-lactams except carbapenems and cephamycins and are most often associated with multiple-associated resistances. Intestinal colonization is the cornerstone of their dissemination [9]. This may be the consequence of their preferential association with *Escherichia coli*, which is not only the main enterobacteria of the human intestinal microbiota, but also a facultative pathogen [10]. However, although *E. coli* may play a reservoir role, plasmids carrying CTX-M enzymes can also disseminate in other commensal enterobacteria, such as *Klebsiella pneumoniae*, particularly in hospitals [11], or even to more pathogenic species, such as *Shigella* [12] or *Salmonella* [13]. Sparse data indicate that rates of ESBL–*E. coli* intestinal carriage are high in African communities even when antibiotic pressure is low [14]. This shows their diffusion capacities in the community, whereas ESBL–*E. coli* cross-transmission remains scarcely reported between hospitalized patients from developed countries [15]. However, the precise paths of this overall dynamic are largely unknown. In this study, we evaluated prevalence of ESBL-E at entry and the acquisition rate for ESBL-E in children with severe acute malnutrition hospitalized in an intensive care unit (ICU) of a renutrition center.

MATERIALS AND METHODS

Study Design

This work was a substudy of a general study on the prevalence of infections among children with severe acute malnutrition that was run by a large international nongovernmental organization (Médecins Sans Frontières) and the results of which will be published elsewhere. This general study took place between November 2007 and July 2008 in a 300-bed pediatric renutrition center located in Maradi (Niger). This center was composed of a 60-bed ICU with 6 full-time nurses, a transition phase with 1 nurse for 20–30 children, and a nutritional rehabilitation phase with 1 nurse for 40–50 children. Children were included in the general study if they were 0.5–5 years old (60–110 cm in height) with severe acute malnutrition (bilateral edema and/or mid-upper arm circumference $<$110 mm, weight-for-height less than 3 *z* score of the median) and a severe medical condition [16]. They were also not referred from another health institution and had not received antibiotics for at least 1 week according to their parents. Informed consent to participate was obtained from children's parents. Every fifth child included in the general study was included in the intestinal carriage substudy, which is the subject of this article.

Clinical data were recorded at admission. Records of vomiting and diarrhea were based on the mother's report. Fecal samples were obtained for detection of ESBL-E (see below) at admission and before discharge. Antibiotic policy in the ICU was based on WHO recommendations, which includes a short course of amoxicillin for all children with severe acute malnutrition [4]. This systematic antibiotic therapy was replaced by parenteral ceftriaxone (100 mg/kg/day) when there was suspicion of severe or complicated lower respiratory tract infection, meningitis, septic shock, hypothermia, or diarrhea. Oral amoxicillin (80 mg/kg/day for 5 days) was given to children with upper respiratory tract infection, and clavulanate was added in those with treatment failure. Oral ciprofloxacin (10–15 mg/kg/day) was available on medical request. Antibiotic exposure for each child was defined as the total number of antibiotic treatment days divided by the number of days of hospitalization. This study adhered to the principles that govern biomedical research involving human subjects [17]. Ethical approval was granted by the Ethical Committee of Niger and the Conseil de Protection des Personnes, Saint-Germain en Laye, France. Written informed consent was obtained from the legal representatives of included children after oral and written information delivered in French or in Haussa, the native language of the representatives.

Microbiology

The prevalence of ESBL-E carriage at admission was measured from fecal samples obtained within 24 hours of admission, and the acquisition rate was measured from samples obtained at discharge from the children who were free of ESBL-E at admission. Aliquots (~200 mg) of freshly passed stools were inoculated by central puncture in conservation agar tubes (Bio-Rad) and transported to France at room temperature. They were then plated on ChromID ESBL agar plates (BioMérieux) for detection of ESBL-E and on Drigalski agar for control of viable enterobacteria in the sample. All colonies with different morphotypes on ESBL agar were identified to the species level using standard techniques (API20E, Biomérieux). Antibiotic susceptibility was determined using the agar diffusion method, as recommended by the French Society for Microbiology (www.sfm-microbiologie.org). ESBL content was characterized as described elsewhere [18]. Resistance genes, including $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM}, were amplified with specific primers according to phenotypic results, as described elsewhere [18]. Finally, cephalosporinase bla_{CMY} genes were screened in *E. coli* or *K. pneumoniae* strains expressing a cephalosporinase phenotype, using ad hoc primers (AmpCU-F: 5′-GCARACSCTGTTYGAGMTDGG-3′; AmpC-R: 5′-CTCCCARCCYARYCCCTG-3′). Amplification products were sequenced and submitted to the National Center for Biotechnology Information library for identification (http://blast.ncbi.nlm.nih.gov). The transferability of $bla_{\text{CTX-M-15}}$ genes from *E. coli* and *K. pneumoniae* strains was assessed by mating with *E. coli* $J53^{\text{rif}}$, as described elsewhere [18]. When mating was negative, transformation into *E. coli* TOP10 (Invitrogen) was

attempted by electroporation of whole plasmid DNA extracted, as recommended by the manufacturer (Macherey Nagel). Transformants were selected on Drigalski agar with 2 mg/L cefotaxime.

CTX-M-15 *E. coli* ESBL strains were typed by repetitive extragenic palindromic polymerase chain reaction (rep-PCR), as described elsewhere, and *K. pneumoniae* ones by enterobacterial repetitive intergenic consensus (eric-PCR), also as described elsewhere [19, 20]. Amplification products were separated by electrophoresis at 70 V for 3 hours on 1% agarose gel and stained by SYBR Safe dye (Invitrogen). When identical *E. coli* patterns were observed, representative strains were selected for multilocus sequence typing (MLST), performed as recommended (http://mlst.ucc.ie/). To maximize discrimination among CTX-M-15 *E. coli* and *K. pneumoniae* strains, all strains with similar PCR patterns were also typed by pulsed-field gel electrophoresis (PFGE) as well as acquired strains with a single PCR pattern. Results were analyzed and interpreted, as described elsewhere [21]. FII, FIA, FIB, I1/Iγ, A/C, and L/M plasmid replicons from parental *E. coli* and *K. pneumoniae*, as well as from transconjugants or transformants, were PCR typed, as described elsewhere [22, 23]. The genetic environment of $bla_{\text{CTX-M-15}}$ genes from *E. coli* and *K. pneumoniae* was compared with that of pandemic plasmid pC15-1a, using *E. coli* strain TN03 as a positive control, as described elsewhere [23].

Data Analysis

Characteristics of children included in the study were compared with those of the other children admitted to the ICU, with use of R software (version 2.12.1; http://www.cran.r-project.org). Univariate comparison of discrete variables was performed using the 2-sided Pearson χ^2 test and Fisher exact test; Student's *t* test and the Wilcoxon test were used for continuous variables. Because of the large number of explanatory variables tested, results of univariate analysis were adjusted using the Holm adjustment for multiple testing [24, 25].

RESULTS

Patients and Samples

A total of 2567 children were admitted directly to the renutrition center during the study period. Three hundred eleven were included in the general study, which will be described elsewhere. Among these children, we included 55 (18%) in the intestinal carriage study. The geographic origins of the children were diverse; only 12.5% came from the city of Maradi, 64.0% from neighboring districts, and 23.5% from Nigeria. There were no significant differences in any characteristic, including antibiotic exposure and geographic origin, between the children included in the intestinal carriage study and the 256 other children included in the general study (Table 1). Among the included children, 92.7% had weight-for-height *z* scores < -3, confirming the high rate of severe malnutrition. Vomiting (41.8%), diarrhea (50.9%), and axillary temperature >38°C (47.3%) suggested the high prevalence of acute bacterial infections (Table 1). Antibiotic exposure was very high, with an exposure score of 0.96 treatment days/hospitalization days. All children included in the intestinal carriage study received antibiotic treatment during hospitalization, and 74.5% received more than one type of antibiotic.

The ESBL-E carriage rate at admission in the 55 children in the study was 30.9% (17/55). Five children were carrying 2 strains (Table 2), including 2 strains of *E. coli* in 1, *E. coli* plus *Enterobacter cloacae* in 2, *E. coli* plus *K. pneumoniae* in 1, and *K. pneumoniae* plus *E. cloacae* in 1. Thus, a total of 22 ESBL-E strains were isolated, often coresistant to other antibiotics (data not shown), including *E. coli* in 71% (12/17) of the children, *Enterobacter* sp. in 29% (4/17 for *E. cloacae* and 1/17 for *Enterobacter asburiae*), and *K. pneumoniae* in 24% (4/17). The $bla_{\text{CTX-M-15}}$ gene was present in 91% of the strains (20/22) when $bla_{\text{SHV-2a}}$ and $bla_{\text{SHV-12}}$ were found in 1 *K. pneumoniae* strain each. The $bla_{\text{CTX-M-15}}$ strains were diverse, except for 3 *E. coli* strains isolated from 3 children admitted on 3 April, 29 April, and 15 July, who shared the same PCR-based pattern (data not shown). They were each from a distinct geographic area (data not shown). Ten of the 13 CTX-M-15–carrying plasmids from *E. coli* (66%) were typable with 1 of 4 replicon combinations: FIA/FIB (5 cases), FII/I1/Iγ (3 cases), FII/FIA (1 case), and I1/Iγ (1 case). Multidrug resistance (MDR) regions analogous to that of the pandemic pC15-1a plasmid were found in 7 of 13 (54%) of the CTX-M-15 *E. coli* strains isolated at admission, with minor variation consisting of deletion of J5 junction (2 strains), J6 junction and bla_{TEM} (1 strain), or *tet(A)* and J5 junction (1 strain). In addition, FIA/FIB multireplicons were present in 5 of these 7 strains, suggesting that the MDR region was carried by related plasmids. In contrast, the 2 CTX-M-15 *K. pneumoniae* strains were MDR negative, and no marker of any plasmid incompatibility group was detected. MLST of the *E. coli* strains identified 10 sequence types (types 354, 5, 131, 10, 101, 68, 448, 196, 410, and 361). Moreover, although isolated from 3 unrelated children from different geographic origins and at different dates, all 3 ST361 strains carried CTX-M-15, contained the pC15-1a MDR, and had identical plasmid markers, suggesting circulation of the clone in the community.

Acquisition Rate and Characteristics of Strains at Discharge

The acquisition rate of ESBL-E strains was calculated in 16 children in whom findings were negative at admission and samples were obtained again at discharge (median, 8 days later; range, 3–13 days later). It was as high as 94% (15/16 children). The antibiotic exposure in this group was not significantly higher than in the other children included in the general study (1.00 treatment days/day of hospitalization [95% confidence interval

Table 1. Characteristics of ICU Children Included or Not Included in the Intestinal Carriage Study

Characteristics	Inclusion in carriage study		Bivariate odds ratio (95% CI)[a]	*P*	Adjusted *P*[b]
	No ($n = 256$)	Yes ($n = 55$)			
Sociodemographic characteristics					
Age, mean (range), months	17.04 (6–59)	16.25 (7–36)	…	.52	1.00
Female-male ratio	1.18	1.29	…	.88	1.00
Characteristics at admission					
Hospital stay, median (range), days	9 (1–49)	10 (2–40)	…	.27	1.00
Weight, mean (range), kg	6.0 (3.1–12.1)	6.2 (3.3–9.6)	…	.33	1.00
Height, mean (range), cm	70.54 (60.0–97.0)	70.65 (60.5–82.5)	…	.91	1.00
Weight-for-height *z* score					
≥−2	6 (2.4)	1 (1.8	1.0	.46	1.00
<−2	7 (2.7)	3 (5.5)	2.4 (0.1–156.9)		
<−3	242 (94.9)	51 (92.7)	1.3 (0.1–59.3)		
Edema	37 (14.5)	11 (20.0)	1.5 (0.6–3.2)	.31	1.00
Axillary temperature >38°C	116 (45.3)	26 (47.3)	1.1 (0.6–2.0)	.88	1.00
Dehydration					
None	202 (78.9)	49 (89.1)	1.0	.29	1.00
Moderate	44 (17.2)	5 (9.1)	0.5 (0.1–1.3)		
Severe	10 (3.9)	1 (1.8)	0.4 (0.0–3.0)		
Diarrhea	149 (58.2)	28 (50.9)	0.7 (0.4–1.4)	.37	1.00
Vomiting	113 (44.1)	23 (41.8)	0.9 (0.5–1.7)	.77	1.00
Outcome and antibiotic treatment					
Death	23 (9.0)	6 (10.9)	1.2 (0.4–3.4)	.61	1.00
Antibiotic exposure (cumulative treatment days/days in hospital) (range)	0.93 (0.1–3.2)	0.96 (0–2.5)		.66	1.00
No. of antibiotics received					
1	113 (44.3)	14 (25.5)	1.0	.05	.95
2	90 (35.3)	28 (50.9)	2.5 (1.2–5.5)		
3	38 (14.9)	9 (16.4)	1.9 (0.7–5.2)		
4	14 (5.5)	4 (7.3)	2.3 (0.5–8.8)		
Treatment duration, days					
Any antibiotic[c]					
0	1 (0.4)	0 (0.0)	…	.32	1.00
1–3	26 (10.2)	4 (7.3)	1.0		
4–8	125 (48.8)	21 (38.2)	1.1 (0.3–4.7)		
≥9	104 (40.6)	30 (54.5)	1.9 (0.6–7.9)		
Ceftriaxone					
0	63 (24.6)	12 (21.8)	1.0	.87	1.00
1–3	31 (12.1)	6 (10.9)	1.0 (0.3–3.3)		
4–8	130 (50.8)	28 (50.9)	1.1 (0.5–2.6)		
≥9	32 (12.5)	9 (16.4)	1.5 (0.5–4.3)		
Cloxacillin					
0	214 (83.6)	45 (81.8)	1.0	.92	1.00
1–3	11 (4.3)	2 (3.6)	0.9 (0.1–4.2)		
4–8	20 (7.8)	5 (9.1)	1.2 (0.3–3.5)		
≥9	11 (4.3)	3 (5.5)	1.3 (0.2–5.2)		
Amoxicillin					
0	165 (64.5)	25 (45.5)	1.0	.05	1.00
1–3	54 (21.1)	19 (34.5)	2.3 (1.1–4.8)		
4–8	35 (13.7)	11 (20.0)	2.1 (0.8–4.9)		
≥9	2 (0.8)	0 (0.0)	0.0 (0.0–36.1)		

Table 1 continued.

Characteristics	Inclusion in carriage study: No (n = 256)	Yes (n = 55)	Bivariate odds ratio (95% CI)[a]	P	Adjusted P[b]
Amoxicillin clavulanate					
0	185 (72.3)	43 (78.2)	1.0	.84	1.00
1–3	20 (7.8)	3 (5.5)	0.6 (0.1–2.3)		
4–8	46 (18.0)	9 (16.4)	0.8 (0.3–1.9)		
≥9	5 (2.0)	0 (0.0)	0.0 (0.0–4.9)		
Ciprofloxacin					
0	197 (77.0)	40 (72.7)	1.0	.40	1.00
1–3	9 (3.5)	3 (5.5)	1.6 (0.3–7.0)		
4–8	47 (18.4)	10 (18.2)	1.0 (0.4–2.3)		
≥9	3 (1.2)	2 (3.6)	3.3 (0.3–29.4)		
Gentamicin					
0	249 (97.3)	52 (94.5)	1.0	.39	1.00
1–3	7 (2.7)	3 (5.5)	2.0 (0.3–9.3)		

Where not otherwise specified, data represent no. (%) of patients.

[a] Bivariate analysis was performed using Pearson χ^2, Fisher exact, Student's t, and Wilcoxon tests ($\alpha = 0.05$). CI, confidence interval.

[b] P after adjustment by Holm's method [26, 27]

[c] One nonincluded child did not receive any antibiotic treatment.

(CI), 0.12–1.27] vs 0.86 [95% CI, 0.33–2.50]) (data not shown). Acquired strains consist of 39 ESBL-E strains (2.3/colonized child; range, 1–4), including 19 *E. coli*, 11 *K. pneumoniae*, 5 *E. cloacae,* and 4 *Salmonella Typhimurium* strains (Table 2). Acquisition rates were 94% (15/16), 69% (11/16), 31% (5/16) and 25% (4/16) for each species, respectively. Ninety percent of these strains (35/39) produced CTX-M-15 (17 *E. coli*, 10 *K. pneumoniae*, 4 *E. cloacae*, and 4 *Salmonella* strains), whereas CMY-2 (*E. coli*), SHV-2a (*E. cloacae*), SHV-44 (*E. coli*), a combination of CTX-M-15 and CMY-30 (*E. coli*), and a combination of CTX-M-15 and SHV-12 (*K. pneumoniae*) were found in 1 strain each.

Typable plasmids were detected in 53% (9/17) of the acquired CTX-M-15 *E. coli* strains. FII/FIA/FIB multireplicons were found in 3, and FII/I1/Iγ, FIA/FIB, and I1/Iγ multireplicons in 2 each. MDR region markers were present in only 29% (5/17) of the CTX-M-15 *E. coli*. No particular association between a bacterial host genotype and a particular plasmid incompatibility group was identified. Just as for those at admission, plasmids from *K. pneumoniae* could not be typed.

Among the 17 acquired *E. coli* strains that produced CTX-M-15 (isolated from 14 children), 13 clustered only in 4 PFGE patterns labeled B (5 strains), C (3 strains), D (3 strains), or F (2 strains). Singletons were labeled (a), (b), or (c). Finally, 1 *E. coli* with PFGE pattern H produced CTX-M-15 plus the plasmid-borne cephalosporinase CMY-30. Interestingly, 2 other *E. coli* strains with PFGE pattern H were producing CMY-2 and SHV-44, respectively. These PFGE patterns corresponded to ST354 for PFGE patterns B and F, and to ST410, 101, 131, 617, 1284, and 216, respectively, for patterns H, C, D and singleton strains (a), (b), and (c) (Table 2). As stated earlier, ST354, 410, 101, and 131 were also identified at entry, suggesting that these clones were circulating both in the community and in the hospital. In contrast, among the 11 CTX-M-15 *K. pneumoniae* strains, which were from 11 children, only 3 were grouped in 1 PFGE pattern, and the others were singletons.

DISCUSSION

We showed that ESBL-E acquisition rate was dramatic during hospitalization of children with severe acute malnutrition, although they were discharged after a median of only 10 days. Although the sample was small, which may limit the significance of the observation, the acquisition rate reached 94%, higher than the already high 48% reported in a pediatric hospital in Madagascar [26]. Several factors may have contributed to the high acquisition rate. First, the antibiotic exposure of the children hospitalized in the Maradi center was massive and possibly facilitated acquisition. Indeed, many reports have shown the link between β-lactam exposure and intestinal colonization by enterobacteria resistant to cephalosporins [27, 28]. This is worrisome, because far from being administered without guidelines, antibiotic regimens were chosen in accordance with WHO recommendations for malnutrition cases [4]. Second, cross-transmission probably played a role, as evidenced by the fact that children from the renutrition center shared identical strains. This was the case not only for *K. pneumoniae* strains, which are well known to be highly diffusible in an ICU setting [29, 30], but also for *E. coli,* which is much less common, at least in hospitals in developed countries [15, 31]. This cross-transmission was probably favored by suboptimal hygiene and the high density of patients in the center. We accumulated molecular evidence

Table 2. Microbiologic Characteristics of ESBL Strains

Time of isolation and child identification no.	Strain no.	Strain characteristics				Resistance characteristics		
		Species	PCR-based pattern	PFGE pattern[a]	*Escherichia coli* MLST	ESBL type	Incompatibility group[b]	pC15-1a type MDR[c]
Admission								
M145	1	*Escherichia coli*	IX	G	361	CTX-M-15	FIA/FIB	Positive
M177	1	*E. coli*	IX	G	361	CTX-M-15	FIA/FIB	Positive (ΔJ5)
M301	1	*E. coli*	IX	G	361	CTX-M-15	FIA/FIB	Positive
M001	1	*E. coli*	I	ND	354	CTX-M-15	FIA/FIB	Negative
M005	1	*E. coli*	II	ND	5	CTX-M-15	FII/I1/Iγ	Positive (ΔJ6Δbla_{TEM})
M009	1	*E. coli*	III	ND	131	CTX-M-15	FII/FIA	Positive (ΔJ5)
M057	1	*E. coli*	IV	ND	10	CTX-M-15	NT	Negative
M089	1	*E. coli*	V	ND	101	CTX-M-15	FII/I1/Iγ	Negative
M113	1	*E. coli*	VI	ND	68	CTX-M-15	FII/I1/Iγ	Negative
M113	2	*E. coli*	VII	ND	448	CTX-M-15	I1/Iγ	Negative
M125	1	*E. coli*	VIII	ND	196	CTX-M-15	NT	Positive
M201	1	*E. coli*	X	ND	617	CTX-M-15	FIA/FIB	Positive (Δ*tet(A)*ΔJ5)
M229	1	*E. coli*	XI	ND	410	CTX-M-15	NT	Negative
M025	1	*Klebsiella pneumoniae*	I	ND	NA	SHV-2a	ND	ND
M141	1	*K. pneumoniae*	II	ND	NA	SHV-12	ND	ND
M157	1	*K. pneumoniae*	III	ND	NA	CTX-M-15	NT	Negative
M301	2	*K. pneumoniae*	IV	ND	NA	CTX-M-15	NT	Negative
M009	2	*Enterobacter cloacae*	ND	ND	NA	CTX-M-15	ND	ND
M125	2	*E. cloacae*	ND	ND	NA	CTX-M-15	ND	ND
M141	2	*E. cloacae*	ND	ND	NA	CTX-M-15	ND	ND
M217	1	*E. cloacae*	ND	ND	NA	CTX-M-15	ND	ND
M213	1	*Enterobacter asburiae*	ND	ND	NA	CTX-M-15	ND	ND
Discharge								
M049	1	*E. coli*	XIII	F	354	CTX-M-15	FII/FIA/FIB	Negative
M105	1	*E. coli*	XIII	B	354	CTX-M-15	FII/FIA/FIB	Negative
M109	1	*E. coli*	XIII	B	354	CTX-M-15	I1/Iγ	Negative
M117	1	*E. coli*	XIII	B	354	CTX-M-15	FIA/FIB	Negative
M121	1	*E. coli*	XIII	F	354	CTX-M-15	NT	Negative
M129	1	*E. coli*	XIII	B	354	CTX-M-15	FII/I1/Iγ	Negative
M129	2	*E. coli*	XIII	B	354	CTX-M-15	FII/FIA/FIB	Negative
M137	1	*E. coli*	XV	C	101	CTX-M-15	FII/I1/Iγ	Negative
M161	1	*E. coli*	XV	C	101	CTX-M-15	I1/Iγ	Negative
M165	1	*E. coli*	XV	C	101	CTX-M-15	NT	Positive
M137	2	*E. coli*	XVI	D	131	CTX-M-15	NT	Positive
M149	1	*E. coli*	XVI	D	131	CTX-M-15	TF	Negative
M169	1	*E. coli*	XVI	D	131	CTX-M-15	FIA/FIB	Positive
M105	2	*E. coli*	XIV	H	410	CMY-2	ND	ND
M221	2	*E. coli*	XIV	H	410	SHV-44	ND	ND
M237	1	*E. coli*	XIV	H	410	CTX-M-15 + CMY-30	NT	Negative
M169	2	*E. coli*	XVI	S (a)	617	CTX-M-15	NT	Negative
M193	1	*E. coli*	XVII	S (b)	1284	CTX-M-15	NT	Positive
M233	1	*E. coli*	XVIII	S (c)	216	CTX-M-15	NT	Positive
M117	2	*K. pneumoniae*	V	A	NA	CTX-M-15	NT	Negative
M121	2	*K. pneumoniae*	V	A	NA	CTX-M-15	NT	Negative
M193	2	*K. pneumoniae*	V	A	NA	CTX-M-15 + SHV-12	NT	Negative

Table 2 continued.

Time of isolation and child identification no.	Strain no.	Species	Strain characteristics PCR-based pattern	PFGE pattern[a]	*Escherichia coli* MLST	Resistance characteristics ESBL type	Incompatibility group[b]	pC15-1a type MDR[c]
M109	2	*K. pneumoniae*	III	S	NA	CTX-M-15	TF	Negative
M137	3	*K. pneumoniae*	VI	S	NA	CTX-M-15	NT	Negative
M149	2	*K. pneumoniae*	VI	S	NA	CTX-M-15	NT	Negative
M161	2	*K. pneumoniae*	VII	S	NA	CTX-M-15	NT	Positive (Δ*tet(A)*)
M165	2	*K. pneumoniae*	VII	S	NA	CTX-M-15	NT	Positive (ΔJ2Δ*tet(A)*ΔJ5)
M169	3	*K. pneumoniae*	VI	S	NA	CTX-M-15	NT	Negative
M221	1	*K. pneumoniae*	V	S	NA	CTX-M-15	NT	Negative
M237	2	*K. pneumoniae*	V	S	NA	CTX-M-15	NT	Negative
M049	2	*E. cloacae*	ND	ND	NA	CTX-M-15	ND	ND
M117	3	*E. cloacae*	ND	ND	NA	SVH-2a	ND	ND
M149	3	*E. cloacae*	ND	ND	NA	CTX-M-15	ND	ND
M233	2	*E. cloacae*	ND	ND	NA	CTX-M-15	ND	ND
M237	3	*E. cloacae*	ND	ND	NA	CTX-M-15	ND	ND
M161	3	*Salmonella* Typhimurium	ND	ND	NA	CTX-M-15	ND	ND
M165	3	*S.* Typhimurium	ND	ND	NA	CTX-M-15	ND	ND
M233	3	*S.* Typhimurium	ND	ND	NA	CTX-M-15	ND	ND
M237	4	*S.* Typhimurium	ND	ND	NA	CTX-M-15	ND	ND

Abbreviations: ESBL, extended-spectrum β-lactamase–producing; NA, not applicable; ND, not done; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis.

[a] The *E. coli* and *K. pneumoniae* clones (≥2 strains) are named A, B, C, D, E, F, G, and H, according to PFGE patterns. S indicates singletons (strains with unique PFGE pattern); *E. coli* singletons were named (a), (b), and (c).

[b] NT indicates that the plasmid was not typable; TF, that the transfer in the *E. coli* recipient failed.

[c] Multidrug resistance (MDR) region analogous to that of the pC15-1a plasmid was considered present (positive) when ≥10/13 of the mapping PCR performed were positive and otherwise were considered absent (negative); deletions are in parentheses.

that transmission of a few clones of CTX-M-15 *E. coli* was responsible for acquisition in most cases. MLST showed that they were grouped in a small number of ST types, namely, ST354, ST101, and ST131, which were not only carried by children at admission but also acquired in the center. These 3 *E. coli* ST types have been found to predominate among *E. coli* CTX-M clinical isolates elsewhere and are considered highly virulent [32]. Here, we showed that they are also good gut colonizers with high potential for nosocomial dissemination.

We also observed that some children were colonized with CTX-M-15 *E. coli* belonging to the less studied ST617, 1284, 410, and 216 clones, suggesting that gene exchange was intense between strains. This was further confirmed by plasmid characterization. A minimum of 5 CTX-M-15–carrying plasmids, identified by replicon typing, were identified among the 7 acquired ST354 *E. coli* strains. In the same way, the 3 acquired ST410 *E. coli* strains were found in association with 3 different ESBL enzymes. We also confirmed the previously suggested role of IncF plasmids, known to be associated with *E. coli*, in the dissemination of $bla_{\text{CTX-M-15}}$, which has been described elsewhere [23]. Besides plasmids, gene exchange was also evidenced by our identification in *E. coli* strains, both at entry and at discharge, of MDR $bla_{\text{CTX-M-15}}$ genetic environments similar to that of plasmid pC15-1a, described in communities worldwide, particularly from several African countries [14, 33, 34]. It is suspected to have a major role in the worldwide dissemination of CTX-M-15. None of these features was observed in *K. pneumoniae* strains, suggesting that other means of dissemination of $bla_{\text{CTX-M-15}}$ exist for that species.

Cross-transmission of resistant ESBL-E in ICUs can be prevented only by implementing strict control measures. In developed countries, recommendations for the control of cross-contamination include increased ratios of paramedical workers to patients (at least triple the ratios of the renutrition center) [35] along with screening for rectal colonization. Widespread use of alcohol-based hand rub solution, recently promoted by WHO as the single most efficient measure for safety of care [36], is also recommended and can be applied in developing countries. Indeed, WHO provides recipes for preparing such solutions easily (WHO Guidelines on Hand Hygiene in Health Care, http://whqlibdoc.who.int/publications/2009/9789241597906_eng.pdf). Documenting whether colonization was followed by clinical infection was beyond the scope of the study, but it has been demonstrated repeatedly in the past [37–39]. It is also known that children are a key ESBL-E reservoir [40]. Thus, we believe that the dramatic colonization rate observed in the Maradi center, in addition to providing a source of infection for the hospitalized children, may also contribute to ESBL-E dissemination, not

only in the renutrition center but also in the community after discharge [41]. When the choice of antibiotics is narrowed to carbapenems, consequences of that dissemination may be of extreme concern, especially in developing countries, where these treatments are not usually available. Although our study was only descriptive, its results are a strong incentive to review the benefit–risk ratios of current antibiotic policies in children with severe acute malnutrition, given the concerns associated with the future of antibiotics and the current dissemination of carbapenemase-producing enterobacteria, which appear to follow the same paths as ESBL-E [42].

Notes

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