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BRIEF REPORT

Duration of Colonization by Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* in Healthy Newborns and Associated Risk Factors: A Prospective Cohort Study

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Duration of colonization by extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) and factors associated with it were studied in 20 newborns in Seville, Spain. Median duration of colonization was 7.5 months; factors associated with prolonged colonization were delivery by caesarean section, colonization of the mother, and phylogroup B2 *Eschericha coli* isolate.

Keywords. colonization; *Escherichia coli*; extended-spectrum β-lactamases; newborns; risk factors.

Extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) have rapidly spread worldwide. Extended-spectrum β-lactamase-producing *Enterobacteriaceae* are usually resistant to penicillins and cephalosporins, and because ESBL production is frequently associated with resistance to other non-β-lactam drugs, they are also multidrug resistant. Intestinal colonization with ESBL-E is important because colonized persons are key reservoirs of these organisms, and colonization generally precedes infection [1, 2].

The natural history of intestinal colonization by ESBL-E has mostly been studied in adults. In our area, the prevalence of colonization with ESBL-E in pregnant women at delivery is 6.7% (95% confidence interval [CI], 5.2–8.7) [3].

A systematic review showed that colonization was maintained for at least 6 months in 19% (95% CI, 9%–34%) of adults in the community, mostly travelers returning from endemic countries colonized with *Escherichia coli* [4]. However, the data on duration of colonization in neonates are limited, to the best of our knowledge, to 2 studies including only patients discharged after hospital outbreaks [5, 6]. After a literature search in PubMed, no data were found on healthy newborns, and there is very limited information about the human and microbiological factors associated with prolonged colonization [5]. The objective of our study was to investigate the duration of colonization with ESBL-E in healthy newborns during the first year of life and the risk factors for prolonged colonization.

METHODS

A prospective cohort study of children colonized by ESBL-E during the first year of life was undertaken. This study is part of a project investigating different aspects of the epidemiology of ESBL-E during pregnancy and in children. In summary, rectal colonization with ESBL-E was studied in a convenient sample of newborns and their mothers (see below) attended at Virgen Macarena University Hospital (Seville, Spain), a 900-bed tertiary hospital serving a population of 550 000, with 3200 births per year. All pregnant women who gave birth at the hospital (and their children) were eligible if attended for delivery on predefined random days and offered to participate. No exclusion criteria applied. The recruitment was completed from August 1, 2013 to June 30, 2014.

Rectal swabs were taken from the children and their mothers in the first 48 hours of life (or the first 48 hours after delivery in the mothers). Subsequent routine visits were made at 3, 6, 9, and 12 months of life, during which time the data were collected, the babies were explored, and a rectal sample was taken from the children and their mothers. The data were collected using a predesign questionnaire in a secured electronic database. Children detected as colonized with ESBL-E in any rectal sample were included in this analysis. The study was approved by the local ethics committee. All of the mothers provided written informed consent.

The main outcome variable was clearance of colonization (CoC) of ESBL-E in colonized children, which was defined as 2 negative rectal swabs after any positive one. The CoC date was arbitrarily considered as the intermediate date between the last positive sample and the first negative one (because samples were taken every 3 months, this was typically 6 weeks after the last positive sample). Negative samples with the same clone between 2 positives were considered false negatives. Exposure

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to children, mother-related variables, and microbiologic features of the isolates were collected.

Rectal swab specimens were directly inoculated onto MacConkey agar with 4 mg/L cefotaxime and after 18 hours enrichment in peptone-enriched broth. All colonies morphotypes isolated compatible with *Enterobacteriaceae* (with or without enrichment) were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Extended-spectrum β-lactamase screening was performed by the combination disc test [7]. Characterization of ESBL genes was determined by in-house polymerase chain reaction (PCR) with group-specific primers for the CTX-M-1, CTX-M-9, TEM, and SHV groups [8] and Sanger sequencing in all cases. XbaI pulsed-field gel electrophoresis was used to study genetic relationships among the isolates [9]. Fingerprinting 2.0 was used to generate dendograms, using the Dice index under 1% tolerance. Isolates with >2 band differences were considered clonally unrelated [10]. *Escherichia coli* phylogroups were assigned by quadruplex PCR for *chuA*, *yjaA*, *TspE4.C2*, and *arpA* genes [11]. B2 isolates were screened for belonging to the O25b:H4/ST131 clonal group, using PCR for O25b rfb and allele 3 of the *pabB* gene and multiplex PCR for phylogroup B23 typing [12].

Due to the difficulties for screen, recruit, and follow healthy newborns, we planned to include at least 20 colonized by ESBL-E. To do so, we screened for ESBL-E colonization the newborns of 50 ESBL-E colonized mothers and the newborns of 50 randomly selected noncolonized mothers which had been studied for another project investigating ESBL-E colonization in pregnancy [3]. Time until CoC was studied by Kaplan-Meier curves. The association between different exposures (considered until CoC or censoring) and time to CoC was investigated by Cox regression. Different multivariate models were constructed using a forward stepwise process. Variables included initially were those with a crude $P \leq .01$. Those causing significant changes in the hazard ratio were kept, and those with an adjusted P value ≥0.1 were excluded at each step. Interactions were also investigated. SPSS 17.0 was used for the analyses.

RESULTS

Twenty children were detected as colonized with ESBL-E and were included (Supplementary Figure S1): 11 (55%) were colonized at birth, and 9 (45%) acquired the colonization during the follow-up; acquisition of ESBL-E after birth was detected after a median of 6 months (range, 4.5–9 months). Among the 20 colonized newborns, 13 were detected among the 46 screened children with a colonized mother (4 with a colonized mothers were excluded because of missing data), and 7 among the 50 screened with a noncolonized mother (randomly selected among the 756 noncolonized mothers studied).

Thirty species/clones of ESBL-E were isolated: 2 were *Klebsiella pneumoniae* and the rest were *E coli*; 5 children were colonized with 2 different ESBL-E *E coli* clones, 1 child had 3 clones and 1 had 4 different clones. Overall, 13 isolates obtained from the children were clonally related to ESBL-E isolated from their mothers (43.3% of isolates). The ESBL types were CTX-M-14 in 14 isolates (46.6%), SHV-12 in 8 isolates (26.6%), and CTX-M-1 in 7 isolates (23.3%). One isolate produced both CTX-M-1 and SHV-12. Among the *E coli* tested, 12 isolates (42.8%) belonged to phylogroup A, 7 (25%) belonged to D, 6 (21.4%) belonged to B2 (1 of them belonged to ST131), and 3 (10.8%) belonged to B1.

Duration of colonization is shown in Figure 1. Overall, 17 children cleared colonization during their follow up: 6 at 1.5 months, 2 at 4.5 months, 6 at 7.5 months, and 3 at 10.5 months; 3 remained colonized at the end of follow up. Overall, the mean and median times to clearance were 5.5 and 7.5 months, respectively. Table 1 shows the univariate analysis of child, mother, and microbiologic variables associated with CoC. Multivariate analysis showed that delivery by caesarean section, a colonized mother, and being colonized with phylogroup B2 ESBL-producing *E coli* were associated with prolonged colonization, whereas the opposite was found for colonization with CTX-M-producing ESBL-E (Table 1). No infections due to ESBL-E were detected.

DISCUSSION

Mean duration of rectal colonization with ESBL-E during the first year of life in unselected newborns was found to be approximately 6 months, and some children were persistently colonized. Delivery by caesarean section and colonized mothers were factors associated with prolonged colonization. It is interesting to note that ESBL-producing *E coli* belonging to phylogroup B2 was associated with longer colonization, and the opposite was found in isolates producing CTX-M enzymes.

In our area, the prevalence of colonization with ESBL-E in pregnant women at delivery is 6.7% (95% CI, 5.2–8.7) [3]. Previous studies conducted with newborns were performed in completely different epidemiological situations and included patients discharged from neonatal intensive care units during ESBL-E outbreaks in the unit [5, 6]. Löhr et al [5] found that median duration of carriage was 12.5 months in 51 infants (mostly preterm) colonized with CTX-M-15-producing *K pneumoniae*; risk factors for prolonged carriage were delivery by caesarean section (as in our study) and use of antibiotics during hospitalization. They also found household transmission of ESBL-E in 32% of households. Nordberg et al [6] found that median duration of colonization was 12.5 months in 14 children (13 colonized with ESBL-producing *K pneumoniae*). Tandé et al [13] found that median duration of colonization with ESBL-E in 22 adopted children from Mali living in France was 9 months. To the best of our knowledge, this is the first study to evaluate both the duration and the risk factors for clearance of ESBL-E colonization (mostly *E coli*) in unselected newborns in a non-outbreak situation.

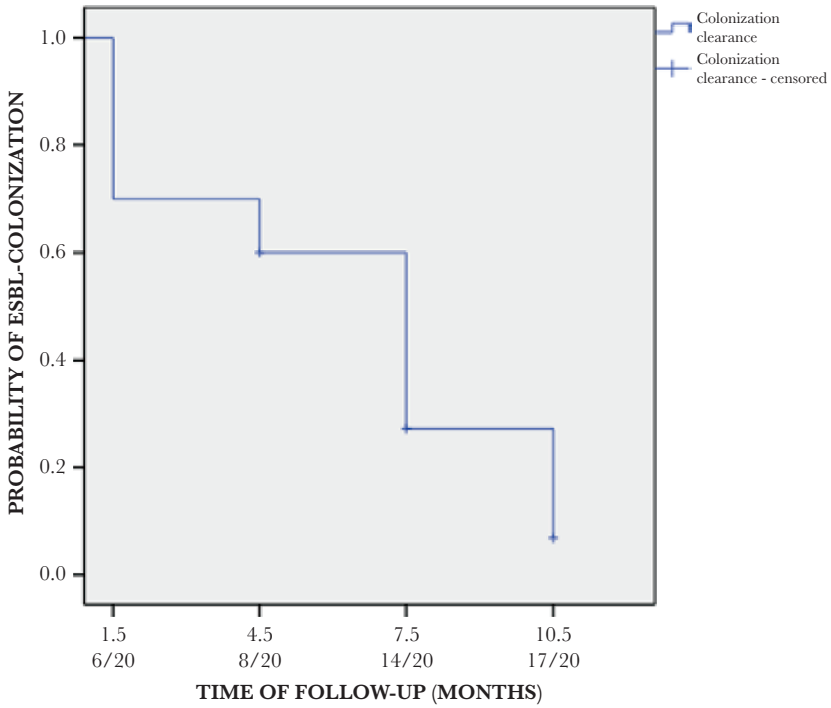

Figure 1. Probability of clearance of colonization in children colonized with extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*.

The association with delivery by caesarean section and prolonged carriage could be explained by the potential protective influence of the composition of gut microbiota acquired during vaginal delivery. Babies born vaginally are colonized by the mother's vaginal and fecal flora during delivery, whereas those born by caesarean section would have more influence from environment to form their microbiota; the latter also have lower counts of *Bacteroides* spp and bifidobacteria. Such impaired composition of protective gut microbiota might contribute to longer fecal colonization by ESBL-E if colonized early in life [5, 14]. Colonization of the mother was also associated, possibly reflecting person-to-person transmission between mother and newborn or common acquisition from an external source. It is interesting to note that phylogroup B2 ESBL-producing *E coli* was also associated with longer carriage. Titelman et al [15] also found that isolates from this phylogroup were more frequent among those with carriage at 12 months in a recent unadjusted prospective study of 61 colonized hospitalized adults, many of whom had a previous infection; 14 of the 19 B2 isolates in that study belonged to ST131, which may have been part of an outbreak, whereas this was the case in only 1 of 6 in our study. We did not study the virulence factors of the isolates, although Titelman et al [15] found no association with typical uropathogenic virulence factors. Therefore, B2 isolates may have some features unrelated to *E coli* uropathogenesis that favor prolonged intestinal colonization in adults and newborns. Finally, we found that isolates producing CTX-M enzymes were associated with shorter duration of colonization in neonates. We do not have an explanation for this; in fact, Titelman et al [15] found that CTX-M-9-producing isolates were associated with prolonged carriage, although it is possible that most of the B2 isolates were CTX-M-9 producers. If so, the phylogroup-associated feature would be more important than the ESBL in this regard. Therefore, the association between type of enzyme and carriage duration requires further studies.

CONCLUSIONS

The most important limitations of this study include the small number of patients, the fact that the date for CoC was arbitrarily chosen as the intermediate between the last positive and first negative samples, and the possible limited sensitivity for detection of colonization. In addition, our sample may have overestimated the estimation for mother colonization. Although these data improve our understanding of the epidemiology of ESBL-E in healthy newborns, more studies in this population are needed.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Table 1. Univariate and Multivariate Analysis of Variables Associated With Clearance of ESBL-E Colonization in Children[a]

Variable	Category	Colonization Clearance/Exposed (Percentage)	Crude HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Child Variables						
Gender	Male Female	9/10 (90) 8/10 (80)	2.01 (0.75–5.36)	.1		
Delivery by caesarean section	Yes No	2/4 (50) 15/16 (93.8)	0.43 (0.09–1.88)	.2	0.18 (0.03–0.91)	.03
Travel outside Spain	Yes No	2/2 (100) 15/18 (83.3)	0.69 (0.15–3.06)	.6		
New household member	Yes No	1/1 (100) 16/19 (84.2)	0.53 (0.06–4.17)	.5		
Pets	Yes No	6/7 (85.7) 11/13 (84.6)	0.89 (0.32–2.44)	.8		
Breastfeeding	Yes No	11/11 (100) 6/9 (66.7)	1.17 (0.43–3.22)	.7		
Sterilization of feeding bottles	Yes No	11/13 (84.6) 6/7 (85.7)	0.50 (0.17–1.48)	.2		
Fed with homemade puree	Yes No	14/17 (82.4) 3/3 (100)	0.73 (0.20–2.62)	.6		
Nursery attendee	Yes No	2/4 (50) 15/16 (87.5)	0.67 (0.15–2.99)	.6		
Any chronic disease	Yes No	1/2 (50) 16/18 (88.9)	0.67 (0.08–5.17)	.7		
Any pediatric visit for acute illness[b]	Yes No	5/7 (71.4) 12/13 (92.3)	0.64 (0.22–1.84)	.4		
Hospitalization[b]	Yes No	1/2 (50) 16/18 (88.9)	0.32 (0.04–2.44)	.2		
Antibiotic use[b]	Yes No	3/5 (60) 14/15 (93.3)	0.39 (0.11–1.38)	.1		
Colonization with ESBL-E at birth	Yes No	11/11 (100) 6/9 (66.7)	1.15 (0.42–3.17)	.7		
Time of acquisition of ESBL-E	≤3 months >3 months	12/13 (92.3) 5/7 (71.4)	0.46 (0.14–1.50)	.2		
Mother colonized with the same ESBL-E clone	Yes No	9/10 (90) 8/10 (80)	1.4 (0.53–3.69)	.5		
Mother Variables						
Caregiver of a disabled person	Yes No	2/3 (66.7) 15/17 (88.2)	0.72 (0.16–3.22)	.6		
Any chronic disease	Yes No	2/3 (66.7) 15/17 (88.2)	0.47 (0.10–2.08)	.3		
Any doctor's visit for acute illness	Yes No	1/1 (100) 16/18 (88.9)	0.21 (0.02–1.69)	.1		
Hospitalization	Yes No	0/0 (0) 17/20 (85)	–	–		
Antibiotic use	Yes No	1/2 (50) 16/18 (88.9)	0.21 (0.02–1.69)	.1		
Mother colonized	Yes No	11/13 (84.6) 6/7 (85.7)	0.62 (0.22–1.71)	.3	0.31 (0.10–1.01)	.05
Microbiologic Variables						
CTX-M producer	Yes No	12/15 (80) 5/5 (100)	1.23 (0.42–3.55)	.6	5.15 (1.29–20.55)	.02
SHV-12 producer	Yes No	7/8 (87.5) 10/12 (83.3)	0.70 (0.25–1.91)	.4		
Phylogroup A *Eschericha coli*	Yes No	10/11 (90.9) 7/9 (77.8)	1.06 (0.40–2.79)	.9		
Phylogroup B1 *E coli*	Yes No	2/3 (66.7) 15/17 (88.2)	1.18 (0.26–5.31)	.8		
Phylogroup B2 *E coli*	Yes No	4/6 (66.7) 13/14 (92.9)	0.51 (0.16–1.58)	.2	0.20 (0.05–0.81)	.02
Phylogroup D *E coli*	Yes No	6/7 (85.7) 11/13 (84.6)	1.01 (0.37–2.77)	.9		

Abbreviations: CI, confidence interval; ESBL-E, extended-spectrum β-lactamase-producing *Enterobacteriaceae*; HR, hazard ratio.

[a]Exposures are considered for the whole period from detection of colonization to clearance, or censoring, except where specified.

[b]None of these were related to infection due to ESBL producers.

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