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Original article

Neonatal intestinal colonization with extended-spectrum β-lactamase–producing *Enterobacteriaceae*—a 5-year follow-up study☆

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ABSTRACT

Objectives: To analyse *Klebsiella pneumoniae* (KP) isolates from an outbreak of extended-spectrum β-lactamase (ESBL)-producing KP and *Escherichia coli* (EC) among infants admitted to neonatal intensive care units and to determine the duration of the intestinal colonization.
Methods: We performed a prospective cohort study of intestinal ESBL-KP/ESBL-EC colonized neonates after a 5-month outbreak in two neonatal intensive care units. Whole genome sequencing, multilocus sequence typing, core genome multilocus sequence typing, pulsed-field electrophoresis and PCR for bla_{CTX-M} were performed on the first isolates. Stool cultures were performed every second month after discharge until 2 years after discharge and at 5 years of age. The last positive samples were analysed with pulsed-field gel electrophoresis and PCR for bla_{CTX-M}. The intestinal relative dominance of ESBL-producing *Enterobacteriaceae* was determined.
Results: Thirteen of 17 patients colonized with ESBL-KP/ESBL-EC survived. Isolates from 16 of 17 patients were available for analysis and featured the same strain type of ESBL-KP: sequence type 101. The strain had capsule type K29 and harboured $bla_{CTX-M-15}$. The virulence genes *irp1, irp2, iutA, kfu* and *mrk* were detected in all isolates. The median length of colonization was 12.5 months (range, 5–68 months). After 2 years, two of 13 patients were carriers of ESBL-KP and one of 13 of ESBL-EC. At 5 years of age, one neonate was colonized with ESBL-EC. No infant experienced an ESBL-KP/EC-infection during follow-up.
Conclusions: Two years after discharge, almost one fourth of the study participants were ESBL/KP-EC carriers. ESBL-KP sequence type 101 persisted in two of 13 children for 23 to 26 months. One patient was colonized with ESBL-EC at age 5 years. **V. Nordberg, Clin Microbiol Infect 2018;▪:1**

Introduction

Neonatal invasive infections caused by extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacilli are increasing and challenge treatment possibilities for neonatal Gram-negative bloodstream infections [1,2]. These infections are largely hospital acquired and are associated with increased mortality and higher healthcare costs [3,4]. ESBL-producing *Enterobacteriaceae* (EPE) colonization has in several studies been associated with subsequent invasive infection caused by the same strain of colonized neonates [5,6].

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A number of neonatal risk factors for intestinal EPE colonization have been proposed, such as low birth weight, low gestational age, long hospital stay, early-onset pneumonia, caesarean section, maternal-to-neonatal transmission and prior treatment with antibiotics [7,8]. There is sparse knowledge on whether acquired colonization with EPE during neonatal care is a transient or persisting condition. Premature neonates at neonatal intensive care units (NICUs) show a similar intestinal bacterial composition of Gram-negative bacteria compared to full-term breast-fed neonates that mainly harbours less harmful bifidobacteria. Theoretically, premature neonates may have a prolonged duration of ESBL-producing *Klebsiella pneumoniae* (KP) and ESBL-producing *Escherichia coli* (EC) colonization compared to full-term neonates [9]. However, this is not evaluated in our study. The duration of colonization is of importance to assess the risk of later EPE infection in the carrier and the risk of EPE dissemination both in the community and in the healthcare setting [10]. The characteristics of the bacterial strain play an important role in understanding the dynamics of the outbreak and the clinical aspects of infection [4]. The surveillance of the EPE outbreak in our NICU indicated dissemination of one *K. pneumoniae* clone.

The study did not aim to describe the nature of the outbreak in detail. Instead, our objectives were to characterize the ESBL-KP and to determine the duration of the intestinal colonization with ESBL-KP/EC in the surviving cohort after discharge from the NICU.

Methods

Study design and ethics

This was a prospective cohort study between November 2008 and August 2015. Written informed consent was obtained from all parents. The study was approved by the regional ethical review board in Stockholm, Sweden (2009/734-31/4 and 2014/491-31/3).

Setting and patients

The Karolinska University Hospital in Stockholm had two NICU sites (Solna and Huddinge) with 22 and 24 beds, respectively. At the time of the outbreak, they served approximately 1000 admissions with 10 500 inpatient-days per year. Before the outbreak, there was no routine for surveillance for ESBL colonization. After the first identified case, we implemented a surveillance protocol consisting of faecal sampling at admission and every other week during the hospital stay.

The patient group consisted of all neonates colonized with intestinal ESBL-KP at discharge from the NICU during an ESBL-KP/ESBL-EC outbreak lasting from November 2008 to March 2009. The intended follow-up period was every second month for 2 years after discharge; the children were cultured again three times during 6 successive weeks at age 5 years. We collected data on risk factors for colonization during the hospital stay. After discharge, the caregivers filled in a form about antibiotic therapy, international travel, hospital visits and the use of probiotics. The study participants were assigned numbers to maintain confidentiality.

Detection of EPE and epidemiologic typing

Faecal samples were inoculated on ChromID ESBL agar (bioMérieux, Marcy l'Etoile, France). Phenotypic confirmation of ESBL production was done with VITEK 2 (bioMérieux). Antibiotic susceptibility testing was done by disc diffusion (Oxoid, Basingstoke, UK). European Committee on Antimicrobial Susceptibility Testing clinical breakpoints were used [11]. Epidemiologic typing with pulsed-field gel electrophoresis (PFGE) was performed according to the PulseNet protocol for non-O157 *E. coli* using *Xba*I for restriction of DNA [12].

Determination of relative dominance of EPE

To determine the dominance of the EPE strain in the microbiota on each sampling occasion, a qualitative culture approach using MacConkey agar was adopted to investigate the two dominating colony morphologies. If one of the colonies was the EPE strain then we regarded the dominance as high, and if none of the colonies was the EPE strain then the dominance was considered low. This provided a crude estimate of the dominance of the resistant EPE strain. We considered three consecutive negative cultures as a confirmation of gut clearance of colonization.

Molecular and genetic characterization

The first positive ESBL-KP isolate from all 17 neonates was examined with molecular identification. Whole genome sequencing was performed on all isolates except one, which was not viable at the time for the analysis. The molecular identification of $bla_{\text{CTX-M}}$ group was done with a probe-based PCR assay [13]. We conducted *in silico* multilocus sequence typing (MLST) in the Bigsdb database, hosted by Institute Pasteur [14]. Core genome MLST (cgMLST) was performed by a previously published cgMLST protocol [15]. The bacteria DNA was extracted using MagNA Pure 96 (Roche Molecular Diagnostics, Pleasanton, CA, USA). Measurement of the DNA concentration was done with Qubit (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was carried out on the HiSeq platform (Illumina, San Diego, CA, USA) at SciLifeLab, Karolinska Institutet, Solna, Sweden. Raw reads were assembled into a

Table 1
Characteristics of patients with colonization with ESBL-producing *Enterobacteriaceae* at discharge from NICU

Characteristic	*n* (%)
History of PPROM	2 (14)
Antibiotics during pregnancy	3 (21)
Cesarean section	7 (50)
Gestational age	
>37 weeks	3 (21)
32–37 weeks	3 (21)
28–32 weeks	2 (14)
<28 weeks	6 (42)
Sex	
Female	6 (42)
Male	8 (57)
Birth weight	
>2500 g	4 (29)
1000–2499 g	7 (50)
<1000 g	3 (21)
Nasal CPAP treatment	11 (79)
Endotracheal tube	7 (50)
Ever central venous catheter	4 (29)
Ever central arterial catheter	7 (50)
Ever peripheral central venous catheter	7 (50)
Antibiotic treatment in NICU	11 (79)
Any parenteral nutrition	8 (57)
Enteral feeding, exclusive breast milk	9 (64)
Enteral feeding, formula feed only	0
Enteral feeding, breast milk and formula	5 (36)
Length of stay	
1–20 d	3 (21)
21–60 d	7 (50)
61–160 d	4 (29)

CPAP, continuous positive airway pressure; ESBL, extended-spectrum β-lactamase; NICU, neonatal intensive care unit; PPROM, prolonged premature rupture of membrane.

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