Long-term faecal carriage in infants and intra-household transmission of CTX-M-15-producing Klebsiella pneumoniae following a nosocomial outbreak

Iren Høyland Løhr1,2*, Siren Rettedal3, Olav B. Natås1,4, Umaer Naseer5,6, Knut Øymar2,3 and Arnfinn Sundsfjord5,6

1Department of Medical Microbiology, Stavanger University Hospital, Stavanger, Norway; 2Department of Clinical Medicine, University of Bergen, Bergen, Norway; 3Department of Paediatrics, Stavanger University Hospital, Stavanger, Norway; 4Department of Infection Control, Stavanger University Hospital, Stavanger, Norway; 5Department of Medical Biology, University of Tromsø, Tromsø, Norway; 6Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway

*Corresponding author. Department of Medical Microbiology, Stavanger University Hospital, PO Box 8100, 4068 Stavanger, Norway. Tel: +47-51518829; Fax: +47-51519939; E-mail: iren.hoyland.lohr@sus.no

Received 1 October 2012; returned 12 November 2012; revised 26 November 2012; accepted 30 November 2012

Objectives: To investigate the duration of faecal carriage of CTX-M-15-producing Klebsiella pneumoniae in infants colonized during a nosocomial neonatal intensive care unit (NICU) outbreak after discharge from hospital, possible risk factors for long-term colonization and transmission to household contacts (HCs).

Methods: Fifty-one infants colonized with two unrelated clones of CTX-M-15 K. pneumoniae[sequence type (ST) 17 and ST485] during an NICU outbreak and 60 HCs provided faecal and rectal samples, respectively, every 1–3 months after hospital discharge. Extended-spectrum β-lactamase (ESBL)-producing strains of K. pneumoniae were identified on Chrom ID ESBL agar and examined by antimicrobial susceptibility testing. blaCTX-M-15 was detected by PCR and DNA sequencing. Clonal relationship was examined by PFGE.

Results: The median carriage time in infants after discharge was 12.5 months (IQR 9.5–17.5). Stable antimicrobial susceptibility patterns in PFGE-related strains confirmed the intestinal persistence of both outbreak strains. Risk factors for prolonged faecal carriage in infants were delivery by caesarean section [hazard ratio (HR) 2.4, 95% CI 1.1–5.5, P=0.029] and treatment with antibiotics during hospitalization (HR 4.5, 95% CI 1.6–12.6, P=0.004). Transmission of CTX-M-15 K. pneumoniae was observed in 9/28 (32%) households. Median carriage length in parents was 2.5 months (IQR 1.0–5.0) (P<0.001 compared with infants).

Conclusions: Infants may be long-term faecal carriers of ESBL-producing K. pneumoniae after colonization during hospitalization in the neonatal period. Delivery by caesarean section and antibiotic treatment during hospitalization are possible risk factors for prolonged carriage. Faecal ESBL carriage in infants represents a reservoir for intra-household spread of ESBL-producing K. pneumoniae.

Keywords: extended-spectrum β-lactamases, ESBLs, Enterobacteriaceae, children, risk factors

Introduction

The prevalence of extended-spectrum β-lactamase (ESBL)-expressing clinical isolates of Enterobacteriaceae is increasing worldwide, causing limited treatment options in commonly occurring infections.1–3 The successful dissemination of CTX-M-type ESBLs in multiresistant genetic lineages of Escherichia coli and Klebsiella pneumoniae has caused the most serious concern.4–6 CTX-M-type enzymes are also by far the most prevalent ESBLs in Scandinavian countries.7–11 K. pneumoniae is a common cause of nosocomial infections and outbreaks in neonatal intensive care units (NICUs), and NICU-associated outbreaks due to multiresistant ESBL-producing K. pneumoniae strains are increasingly reported.12–14 We have recently described an outbreak caused by multiresistant CTX-M-15-producing K. pneumoniae (CTX-M-15 K. pneumoniae) in the NICU at Stavanger University Hospital in Norway between November 2008 and April 2009.15 Two distinct pulsotypes of CTX-M-15 K. pneumoniae[sequence type (ST) 17 and ST485] were identified. Fifty-eight infants were colonized during the outbreak and were putative faecal carriers of CTX-M-15 K. pneumoniae when discharged from the hospital. Limited knowledge exists concerning the duration of faecal carriage of ESBL-producing Enterobacteriaceae in infants and their potential
spread to household contacts (HCs). Long-term faecal carriage and intra-household spread of ESBL-producing Enterobacteriaceae have been examined previously. However, in these studies both the ESBL carriers and the HCs were adults or older children.

The main objectives of this study were to investigate the duration of faecal ESBL carriage in infants colonized with CTX-M-15 K. pneumoniae during a nosocomial NICU outbreak after discharge from hospital, possible risk factors for long-term faecal colonization in infants and the transmission rate of CTX-M-15 K. pneumoniae to HCs.

**Methods**

**Study population**

One of the 58 infants colonized by CTX-M-15 K. pneumoniae during the outbreak died soon after discharge for reasons not related to the outbreak. Thus, we invited 57 infants from 53 families (twins in four families) and their HCs to participate in a prospective cohort study. The intended follow-up period was up to 36 months after discharge. Infants with fewer than three follow-up samples were excluded from the study. HCs that did not provide follow-up samples until elimination of CTX-M-15 K. pneumoniae from their respective colonized infant were also excluded. The study was approved by the Norwegian Data Inspectorate and the Regional Committee on Medical Research Ethics (096.09). Signed statements of informed consent were obtained from all parents.

**Definitions**

Index cases were infants colonized with CTX-M-15 K. pneumoniae during the NICU outbreak. HCs were family members (parents and siblings) living in the same household as index cases. Three or more consecutive negative faecal samples were defined as CTX-M-15 K. pneumoniae elimination in colonized infants. Identification of one or more CTX-M-15 K. pneumoniae-positive rectal samples from HCs was defined as intra-household transmission.

**Detection of ESBL-producing Enterobacteriaceae**

The intended sampling schedule included a monthly sample from the infants and their HCs during the first 12 months and thereafter a sample every third month for another 2 years. Both faecal samples from the infants and rectal samples from HCs were collected with cotton swabs. Sampling was performed by the parents and the swabs were transported in Stuart’s medium. Samples were inoculated on modified MacConkey agar with a 30 μg cefotaxime tablet (Rosco Diagnostics AS, Taastrup, Denmark) and on Chrom ID ESBL agar (bioMérieux, Marcy l’Étoile, France). Swabs were also cultured in brain heart infusion (BHI) broth (Becton Dickinson, Sparks, MD, USA) supplemented with cefotaxime (1 mg/L) directly after plating. Agar plates and broth samples were incubated for 24–48 h at 35°C under aerobic conditions. Any putative ESBL-producing strain (one colony of each phenotype) growing on the Chrom ID ESBL agar was subcultured on modified MacConkey agar for further identification. Colonies growing within the cefotaxime inhibition zone on the primary modified MacConkey agar were subcultured if no growth was detected on the Chrom ID ESBL agar after 48 h of incubation. Bacterial growth outside the cefotaxime inhibition zone was used as a control for a valid sample. If no growth of ESBL-producing bacteria was suspected on either of the two plates after 48 h of incubation, the corresponding BHI broth sample was re-inoculated on a Chrom ID ESBL plate. Antimicrobial susceptibility testing was performed by agar disc diffusion (Becton Dickinson) on all putative ESBL-producing isolates. An ESBL phenotype was confirmed by the double-disc approximation test using cefotaxime, cefazidine and clavulanic acid. Interpretations were according to clinical breakpoints recommended by the Norwegian Working Group on Antibiotics (NWGA) 2009–11, in line with the clinical breakpoints of EUCAST. The first ESBL-producing isolate from each infant and HC was identified to the species level using Vitek 2 ID-GN (bioMérieux). K. pneumoniae ATCC 700603 (ESBL-positive) and E. coli ATCC 25922 (ESBL-negative) were used as control strains. All ESBL-producing K. pneumoniae isolates were stored at −70°C for molecular analysis.

**Molecular characterization**

Molecular characteristics of the first ESBL-producing K. pneumoniae isolate from each of the colonized infants have been described recently. Briefly, 56 infants were colonized by a major pulsotype I (ST17), whereas two infants were colonized by a distinct pulsotype II (ST485). ST17 and ST485 are unrelated clones, but they were both CTX-M-15 producers. In the present study, similar molecular analysis was performed on the last faecal isolate of ESBL-producing K. pneumoniae recovered from all colonized infants and on the first and last isolate from colonized HCs. An additional isolate after 12 months of carriage was also analysed from infants colonized for >1 year. bla_{CTX-M-15} was detected by PCR and DNA sequencing as previously described. Clonal relatedness was examined by PFGE of XbaI-digested (New England BioLabs, Ipswich, UK) genomic DNA using the Chef-DR™ III System (Bio-Rad, Oslo, Norway) as described previously. PFGE patterns were analysed by BioNumerics version 6.6 software (Applied Maths NV, St-Martens-Latem, Belgium) and the results were interpreted according to the criteria of Tenover et al.

**Clinical data**

Data concerning date of birth, gender, gestational age, birth weight, mode of delivery (caesarean section or vaginal delivery), length of hospital stay, mode of nutrition (total parenteral nutrition or breast-milk feeding) and antimicrobial treatment during hospitalization were obtained from the hospital records.

**Statistical analysis**

For each infant, the time of faecal carriage of CTX-M-15 K. pneumoniae was calculated as the average of the time from discharge until the last CTX-M-15 K. pneumoniae-positive sample and the time from discharge until the first CTX-M-15 K. pneumoniae-negative sample. For colonized HCs, the time of faecal carriage was calculated as the average of the time from the first CTX-M-15 K. pneumoniae-positive sample until the last positive sample and the time from the first positive sample until the first negative sample. In subjects without complete follow-up, the timepoint of the first missing sample was considered as the time of the first negative or last positive sample in infants and HCs, respectively. Kaplan–Meier survival analysis was performed to calculate the median time of faecal CTX-M-15 K. pneumoniae carriage in all infants and colonized HCs. Missing subjects were included as censored observations. Carriage times are reported as median with IQR. The log rank test was used to compare the carriage times between infants and parents. For testing the effect of various variables (risk factors) on time of carriage in infants, log rank tests, including log rank test for trend, univariate Cox regression and DNA sequencing as previously described. Clonal relatedness was examined by PFGE of XbaI-digested (New England BioLabs, Ipswich, UK) genomic DNA using the Chef-DR™ III System (Bio-Rad, Oslo, Norway) as described previously. PFGE patterns were analysed by BioNumerics version 6.6 software (Applied Maths NV, St-Martens-Latem, Belgium) and the results were interpreted according to the criteria of Tenover et al.

**Definitions**

Index cases were infants colonized with CTX-M-15 K. pneumoniae during the NICU outbreak. HCs were family members (parents and siblings) living in the same household as index cases. Three or more consecutive negative faecal samples were defined as CTX-M-15 K. pneumoniae elimination in colonized infants. Identification of one or more CTX-M-15 K. pneumoniae-positive rectal samples from HCs was defined as intra-household transmission.

**Detection of ESBL-producing Enterobacteriaceae**

The intended sampling schedule included a monthly sample from the infants and their HCs during the first 12 months and thereafter a sample every third month for another 2 years. Both faecal samples from the infants and rectal samples from HCs were collected with cotton swabs. Sampling was performed by the parents and the swabs were transported in Stuart’s medium. Samples were inoculated on modified MacConkey agar with a 30 μg cefotaxime tablet (Rosco Diagnostics AS, Taastrup, Denmark) and on Chrom ID ESBL agar (bioMérieux, Marcy l’Étoile, France). Swabs were also cultured in brain heart infusion (BHI) broth (Becton Dickinson, Sparks, MD, USA) supplemented with cefotaxime (1 mg/L) directly after plating. Agar plates and broth samples were incubated for 24–48 h at 35°C under aerobic conditions. Any putative ESBL-producing strain (one colony of each phenotype) growing on the Chrom ID ESBL agar was subcultured on modified MacConkey agar for further identification. Colonies growing within the cefotaxime inhibition zone on the primary modified MacConkey agar were subcultured if no growth was detected on the Chrom ID ESBL agar after 48 h of incubation. Bacterial growth outside the cefotaxime inhibition zone was used as a control for a valid sample. If no growth of ESBL-producing bacteria was suspected on either of the two plates after 48 h of incubation, the corresponding BHI broth sample was re-inoculated on a Chrom ID ESBL plate. Antimicrobial susceptibility testing was performed by agar disc diffusion (Becton Dickinson) on all putative ESBL-producing isolates. An ESBL phenotype was confirmed by the double-disc approximation test using cefotaxime, cefazidine and clavulanic acid. Interpretations were according to clinical breakpoints recommended by the Norwegian Working Group on Antibiotics (NWGA) 2009–11, in line with the clinical breakpoints of EUCAST. The first ESBL-producing isolate from each infant and HC was identified to the species level using Vitek 2 ID-GN (bioMérieux). K. pneumoniae ATCC 700603 (ESBL-positive) and E. coli ATCC 25922 (ESBL-negative) were used as control strains. All ESBL-producing K. pneumoniae isolates were stored at −70°C for molecular analysis.

**Molecular characterization**

Molecular characteristics of the first ESBL-producing K. pneumoniae isolate from each of the colonized infants have been described recently. Briefly, 56 infants were colonized by a major pulsotype I (ST17), whereas two infants were colonized by a distinct pulsotype II (ST485). ST17 and ST485 are unrelated clones, but they were both CTX-M-15 producers. In the present study, similar molecular analysis was performed on the last faecal isolate of ESBL-producing K. pneumoniae recovered from all colonized infants and on the first and last isolate from colonized HCs. An additional isolate after 12 months of carriage was also analysed from infants colonized for >1 year. bla_{CTX-M-15} was detected by PCR and DNA sequencing as previously described. Clonal relatedness was examined by PFGE of XbaI-digested (New England BioLabs, Ipswich, UK) genomic DNA using the Chef-DR™ III System (Bio-Rad, Oslo, Norway) as described previously. PFGE patterns were analysed by BioNumerics version 6.6 software (Applied Maths NV, St-Martens-Latem, Belgium) and the results were interpreted according to the criteria of Tenover et al.
Kaplan–Meier survival analysis illustrating CTX-M-15 K. pneumoniae carriage in infants

Figure 1. Kaplan–Meier survival analysis illustrating CTX-M-15 K. pneumoniae faecal carriage times in 51 infants. CTX-M-15 K. pneumoniae elimination was defined as the endpoint. Censored subjects are indicated by vertical tick-marks on the survival curve.

Results

Participants

A total of 52/57 (91%) invited infants from 48 households and 70 HCs from 33 households were enrolled. Only one infant was excluded because fewer than three follow-up samples were provided. Ten HCs were excluded as they stopped providing samples prior to CTX-M-15 K. pneumoniae elimination in their respective index case. Consequently, screening results from 51 infants (25 male and 26 female) from 47 households and 60 HCs (28 mothers, 25 fathers and 7 siblings) from 28 households (1–4 HCs per index case) were included. In total, 605 faecal and 667 rectal samples were collected from the infants and their HCs, respectively.

Faecal ESBL carriage in infants

The overall survival time data as a marker for CTX-M-15 K. pneumoniae faecal carriage length in infants, of both pulsotype I and II, are illustrated in Figure 1. The median carriage length in 51 infants was 12.5 months (IQR 9.0–17.5). The longest time of carriage observed was 23.5 months. The median follow-up period of the 51 infants was 23 months (IQR 16–26) and the median number of faecal samples per infant was 12 (IQR 8–16). Forty-one infants (80%) were followed up until CTX-M-15 K. pneumoniae elimination was defined as the endpoint. Censored values, , 0.05 were considered statistically significant. PASW version 18 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Household transmission

In total, CTX-M-15 K. pneumoniae isolates of pulsotypes I and II were detected in rectal samples from 12/60 (20%) HCs: 8/28 (29%) mothers, 3/25 (12%) fathers and 1/7 (14%) siblings in 9/28 (32%) households (no difference between groups). The median length of CTX-M-15 K. pneumoniae carriage in the 11 colonized parents was 25 months (IQR 1.0–5.0), which was considerably shorter compared with the carriage length in colonized infants (P<0.001). The median number of positive samples in the colonized HCs was 2 (IQR 1.0–3.5). The median follow-up period of all 60 HCs was 23 months (IQR 15–27) and the median number of samples per HC was 11 (IQR 8–15).

Characterization of ESBL-producing K. pneumoniae isolates

Phenotypic analysis was performed on all ESBL-producing K. pneumoniae isolates collected from infants (n=387) and their HCs (n=34) during 29 months of follow-up. All isolates expressed the typical CTX-M-15 phenotype and co-resistances, indicating persistence in infants and transmission to HCs of both the pulsotype I and the pulsotype II strain during follow-up. Molecular analysis was performed on a selection of ESBL-producing K. pneumoniae isolates (n=92) from 51 infants and from 12 colonized HCs. One infant and five ESBL-positive HCs had no ESBL-positive follow-up sample. blacTX-M-15 was confirmed by PCR and DNA sequencing in all isolates. The major pulsotype I was confirmed by XbaI PFGE in follow-up samples from 49 infants and 11 HCs. The unrelated minor pulsotype II was confirmed in samples from two infants and one corresponding HC. The PFGE patterns of the last isolates from all infants were indistinguishable from or closely related to (a difference of fewer than three bands) their first isolate. CTX-M-15 K. pneumoniae isolated from HCs had indistinguishable or closely related PFGE patterns compared with isolates from their respective infant.

Risk factors for long-term faecal carriage

Seven of the 51 infants had not been admitted to the NICU, but were colonized during their stay in the maternity ward. Two infants had most of their NICU stay in another hospital. Consequently, clinical data from 42 colonized infants, hospitalized in our NICU, were considered in the risk analysis. The majority of the infants were born pre-term [gestational age (GA) <37 weeks] or very pre-term (GA <32 weeks) (Table 1). Median gestational age was 34 weeks (IQR 30–37), median birth weight was 2055 g (IQR 1226–2969) and the median number of hospital days was 19 (IQR 10–61). All infants who were
Cox regression analysis (Table 1). The log rank test gave the factors for long-term faecal carriage in infants by univariate analyses during the first days or weeks of life were identified as risk factors for long-term faecal carriage of CTX-M-15-producing *K. pneumoniae* in the final model. Treatment with antibiotics and caesarean section remained statistically significant risk factors for long-term carriage in infants in the stepwise multivariate Cox regression modelling. GA groups were found for all variables. Three variables were included in the final model. Treatment with antibiotics and caesarean section remained statistically significant risk factors for long-term faecal carriage of CTX-M-15 *K. pneumoniae* in infants in the final model (Table 1).

### Discussion

Several new observations were recorded in this prospective study. A median CTX-M-15 *K. pneumoniae* carriage length in infants of 1 year after hospital discharge was observed. Stable antimicrobial susceptibility patterns and indistinguishable or closely related PFGE patterns from the first and the last isolate of the infants confirmed intestinal persistence of the CTX-M-15 *K. pneumoniae* strains during this period. We identified delivery by caesarean section and treatment with antibiotics during the NICU stay as significant risk factors for prolonged faecal carriage of CTX-M-15 *K. pneumoniae* in infants. Transmission of CTX-M-15 *K. pneumoniae* to HCs was observed in one-third of the households during follow-up.

We defined elimination of CTX-M-15 *K. pneumoniae* as three or more consecutive negative faecal samples, as also suggested in a recent Swedish study.19 However, two other follow-up studies have reported duration of carriage only until the first negative sample.22,23 The majority of infants in our study had several additional negative samples after elimination of CTX-M-15 *K. pneumoniae*. Two infants had one positive sample following two negative samples. Hence, the overall results support the chosen definition of CTX-M-15 *K. pneumoniae* elimination.

There are some reports on the duration of human faecal carriage of ESBL-producing *Enterobacteriaceae* in the community setting. Long-term faecal carriage of ESBL-producing *E. coli* for 41–59 months was observed in 5/39 (13%) adult patients (median age 66 years) after a nosocomial outbreak in Sweden.19 Of 41 patients (median age 38 years; range 1–83) with travellers’ diarrhoea who were also carriers of ESBL-producing *E. coli*, 10 (24%) were ESBL carriers after 3–8 months and 3 were still carriers after 3 years.24 Moreover, another Swedish study reported faecal carriage of an ESBL-producing *E. coli* or *K. pneumoniae* strain in 17/61 (28%) patients (median age 61 years) for 12 months after ESBL-related infections.22 A recent study from Slovenia showed that 17/33 (52%) patients (mean age 60 years) were still faecal carriers of ESBL-producing *K. pneumoniae* or *E. coli* 6 months after ESBL colonization or infection.23 Finally, a French study reported a mean duration of faecal *Enterobacteriaceae* ESBL carriage of 9 months in 22 children adopted from Mali.20

To the best of our knowledge, this is the first study of faecal ESBL carriage length in infants after hospital discharge. We observed ESBL carriage for up to 2 years, which is longer than most of the observations in older children and adults, with or without recent hospitalization. Healthy adults and older children have an established gut microbiota which provides a barrier for colonization by microbes from the environment. The fetal intestine is normally sterile and the intestinal bacterial colonization process starts as soon as the infant is born.30 However, infants hospitalized in NICUs may be exposed to several factors (pre-term delivery, caesarean section, parenteral nutrition and antibiotic treatment) that may alter the development of their early intestinal microflora and make them vulnerable to long-term colonization by bacteria from the NICU environment.30–34 A recent study of the gut microbiota of 85 vaginally delivered, treated with antibiotics during hospitalization received ampicillin and gentamicin intravenously. The median duration of therapy was 5 days (IQR 3–7). In addition, four of these infants were treated with meropenem during the NICU stay due to suspected septicaemia with the outbreak strain. Further clinical information is shown in Table 1. GA <32 weeks and treatment with antibiotics during the first days or weeks of life were identified as risk factors for long-term faecal carriage in infants by univariate Cox regression analysis (Table 1). The log rank test gave the same results (data not shown). Proportional hazards between groups were found for all variables. Three variables were included in the stepwise multivariate Cox regression modelling. GA <32 weeks was strongly related to treatment with antibiotics in the multivariate analyses (data not shown), and disappeared in the final model. Treatment with antibiotics and caesarean section remained statistically significant risk factors for long-term faecal carriage of CTX-M-15 *K. pneumoniae* in infants in the final model (Table 1).

### Table 1. Cox regression analysis of risk factors for long-term faecal colonization with CTX-M-15-producing *K. pneumoniae* in infants (n = 42) after NICU hospitalization

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number (%)</th>
<th><strong>Univariate analysis</strong></th>
<th><strong>Final multivariate model</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Male gender</td>
<td>22 (52)</td>
<td>0.7 (0.3–1.4)</td>
<td>0.30</td>
</tr>
<tr>
<td>GA &lt;37 weeks</td>
<td>33 (79)</td>
<td>1.3 (0.6–3.1)</td>
<td>0.53</td>
</tr>
<tr>
<td>GA &lt;32 weeks</td>
<td>14 (33)</td>
<td>2.4 (1.1–5.4)</td>
<td>0.03</td>
</tr>
<tr>
<td>Treatment with antibiotics</td>
<td>33 (79)</td>
<td>3.1 (1.2–8.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>24 (57)</td>
<td>1.8 (0.8–3.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>Total parenteral nutrition</td>
<td>16 (38)</td>
<td>1.2 (0.6–2.6)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

**a**Variables are binary and coded 0 = no and 1 = yes.

**b**Forward and backward likelihood ratio analyses gave the same results.

**c**Infants treated with antibiotics during their NICU stay were all treated with ampicillin and gentamicin intravenously. In addition, four infants were treated with meropenem.
healthy term infants showed a strong association between the microbiota at days 4 and 120, suggesting that the composition of the early intestinal microbiota influences the later microbiota.\textsuperscript{33} Hence, it seems biologically plausible that infants colonized by ESBL-producing Enterobacteriaceae during NICU hospitalization are at risk of long-term faecal carriage due to altered gut colonization during their first weeks of life.

Delivery by caesarean section and treatment with antibiotics during NICU hospitalization were significant risk factors for long-term faecal carriage of CTX-M-15 K. pneumoniae. Treatment with antibiotics and caesarean section are both factors previously found to influence the composition and development of the intestinal microflora during the first days of life.\textsuperscript{30–34} Mode of delivery is the first factor with an effect on early intestinal colonization.\textsuperscript{30} Infants born vaginally are colonized by the maternal vaginal and faecal flora during delivery, whereas those born by caesarean section are exposed to bacteria from the hospital environment.\textsuperscript{30} Several studies report lower counts of Bacteroides spp. and bifidobacteria in infants delivered by caesarean section compared with vaginally delivered infants at 3 days to 6 months of age.\textsuperscript{35–37} Hence, infants born by caesarean section may be at risk of long-term faecal carriage of ESBL-producing Enterobacteriaceae when colonized early in life, due to disturbed development of a protective intestinal microbiota. Early antibiotic treatment has also been associated with delayed intestinal colonization of beneficial bacteria.\textsuperscript{32,34,38,39} A recent Japanese study showed that oral treatment with cefalexin during the first 4 days of life influenced the development of the intestinal microflora, as indicated by delayed bifidobacteria colonization and high counts of Enterobacteriaceae.\textsuperscript{39} Thus, antibiotic treatment during the first days of life may provide a favourable gastrointestinal niche promoting long-term faecal carriage of ESBL-producing Enterobacteriaceae. We have recently described overall prematurity (GA <37 weeks) and treatment with antibiotics (ampicillin and gentamicin) as independent risk factors for becoming colonized with CTX-M-15 K. pneumoniae during the outbreak in our NICU.\textsuperscript{60} However, the findings of this study do not support low GA as an independent risk factor for long-term carriage of CTX-M-15 K. pneumoniae.

A few studies have investigated intra-household transmission of ESBL-producing Enterobacteriaceae. Faecal colonization was observed in 27.4\% (20/73) of HCs in a study from Spain using adults with community-acquired urinary tract infections caused by ESBL-producing E. coli as index cases.\textsuperscript{17} A similar Spanish study reported ESBL colonization in 16.7\% (9/54) of HCs.\textsuperscript{16} These rates are in line with the observed intra-household transmission rate in our study. However, in our study the index cases were infants and the parents were aware of the carrier state of their infant as they were continuously informed about the screening results. Participating families were asked to live a normal life, but to practise good hand hygiene as a preventive measure. These factors may have reduced the transmission rates in our study. Thus, it is possible that colonized infants represent a higher risk of intra-household transmission compared with adult carriers. The median ESBL carrier duration in colonized parents was considerably shorter than in the infants, which might indicate that the risk of long-term carriage of an ESBL-producing K. pneumoniae strain is higher in infants than in healthy adults and older children. However, our carriage data in adults are limited and further studies are needed to investigate the duration of faecal carriage in different groups of the population in the community setting.

In conclusion, our results demonstrate that infants may be faecal carriers of ESBL-producing K. pneumoniae for 1–2 years after being colonized during hospitalization in early infancy. Delivery by caesarean section and antibiotic treatment may be risk factors for prolonged carriage. Long-term faecal ESBL carriage in infants represents a significant reservoir for intra-household spread of ESBL-producing K. pneumoniae.

Acknowledgements
This work was presented in part as a selected oral presentation at the 20th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2010 (O041), and as a poster at the Twenty-eighth Annual Meeting of the Scandinavian Society for Antimicrobial Chemotherapy, Reykjavik, Iceland, 2011 (P-19).

We thank: all of the participating families; the technicians at the Department of Medical Microbiology, Stavanger University Hospital, especially Ragnhild Ornholt, Anita Løvås Brekken, Kirsti Gummmedal, Louise Kindingstad, Mona Øyle Lütcherath and Eva Bernhoff, for their excellent work; Jan Terje Kvåløy, University of Stavanger, for guiding us through the statistics; and the Norwegian Reference Center for Detection of Antimicrobial Resistance, University Hospital of North Norway, for their support.

Funding
This work was supported by grants from Stavanger University Hospital, NORM (Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway) (grant number 09 15) and The Western Norway Regional Health Authority (grant number 911640 to I. H. L.).

Transparency declarations
None to declare.

References


Titelman E, Iversen A, Kais M et al. Duration of faecal carriage of ESBL-producing *E. coli* and *K. pneumoniae* following first-time clinical infection. *Clin Microbial Infect* 2012; **18** Suppl 1: 459.


