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ORIGINAL ARTICLE

Extended-spectrum β-lactamase-producing Enterobacteriaceae among pregnant women in Norway: prevalence and maternal–neonatal transmission

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OBJECTIVE: To study (i) the prevalence and risk factors for carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) in pregnant women, (ii) the maternal–neonatal transmission rate of ESBL-E at birth and (iii) the prevalence of ESBL-E in expressed breast milk of colonized mothers.

STUDY DESIGN: In this cross-sectional, population-based study with case follow-up on maternal–neonatal transmission of ESBL-E, women were screened for rectal ESBL-E colonization at 36 weeks of pregnancy and delivery. Possible risk factors for colonization were studied by logistic regression. Infants of ESBL-E-positive mothers were screened for ESBL-E during their first weeks of life. ESBL-encoding genes were detected by PCR and clonal relatedness was investigated by pulsed-field gel electrophoreses.

RESULTS: In total, 26 out of 901 (2.9%) women were colonized by ESBL-producing *Escherichia coli* at 36 weeks of pregnancy. One of the women carried an additional ESBL *Klebsiella pneumoniae* strain. Adjusted for traveling, African or Asian nationality was a risk factor for colonization; OR = 5.62 (2.21, 14.27) (LR-p = 0.003). Fourteen women remained ESBL-E carriers at delivery. ESBL-E strains indistinguishable from the strains isolated from their respective mothers were detected in 5 (35.7%) infants during their first days of life (median day 3; range = 2 to 8). A total of 146 expressed milk samples were cultured from 25 out of 26 colonized mothers, all were ESBL-E negative.

CONCLUSIONS: The prevalence of ESBL-E carriage among pregnant women was low in our region, but the high maternal–neonatal transmission rate suggests that colonized mothers represent a substantial risk for infant colonization.

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INTRODUCTION

Extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) are responsible for an increasing number of both early- and late-onset sepsis and nosocomial outbreaks in neonatal intensive care units (NICUs), causing excess morbidity, mortality and cost.[1–4] Community fecal carriage rates of ESBL-E are increasing worldwide and in some regions exceed 60%.[5] Colonized mothers may transmit ESBL-E to their infants at birth, but to what extent vertical transmission contributes to colonization or infection in the infant is not known.[6–9] The intestinal tract of infants colonized by ESBL-E may serve as a reservoir and represent a risk for maternity ward and more importantly NICU outbreaks if standard infection control precautions fail.[10,11] Knowledge of the colonization burden in pregnant women and the extent of maternal–neonatal transmission are important for preventive strategies, including active surveillance and isolation of colonized patients.[9,11,12]

In this study, we describe the prevalence and risk factors for ESBL-E carriage in a large cohort of pregnant women in our region, the maternal–neonatal transmission rate at birth and ESBL-E prevalence in expressed breast milk of ESBL colonized mothers.

MATERIALS AND METHODS

Study design and setting

Stavanger University Hospital is the only hospital in the region serving a population of ~ 350 000 and has 5000 deliveries annually. The hospital has a tertiary level NICU with ~ 500 admissions a year, and treats all infants from gestational week 23 except those in need of surgery. Intermittent kangarooing is practiced in the NICU. Approximately two-thirds of pregnant women giving birth at the hospital attend an optional pre-delivery consultation at the hospital at 36 weeks of pregnancy. During 6 months in 2012, pregnant women were consecutively approached at these consultations and invited to participate in the study. There were no exclusion criteria. Enrollment was limited by the capacity of the midwife on duty, and no attempts were made to recruit women who did not attend before delivery at the hospital. Enrolled participants provided the first rectal samples at 36 weeks of pregnancy, which were screened for the two most clinically relevant and prevalent ESBL-producing *Enterobacteriaceae*, *Escherichia coli* and *Klebsiella pneumoniae*. Women colonized with ESBL-E at 36 weeks of pregnancy were also screened upon admission for delivery. Repetitive fecal/rectal samples were collected on days 0, 2, 4, 6, 8, 10 and at 1 to 5 months of age from infants of mothers colonized with ESBL-E at 36 weeks of pregnancy. Breast milk samples from the ESBL-E colonized mothers were provided at the equivalent time intervals. The study was approved by the Regional Committee for Medical and Health Research

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Ethics, Western Norway (2011/139A), and written informed consents from participating women were obtained.

Definitions

We applied the ESBL definition and classification suggested by Giske et al.[13] ESBL-E colonization in pregnant women was defined as detection of ESBL$_A$ (CTX-M, TEM or SHV) or ESBL$_{M-C}$ (plasmid-mediated AmpC) producing E. coli and/or K. pneumoniae from one or more rectal samples. Vertical transmission was defined as detection of indistinguishable or clonally closely related ESBL$_A$ or ESBL$_{M-C}$-producing E. coli and/or K. pneumoniae isolates in infants and their respective mothers.

Sampling and culture conditions

Rectal and fecal samples from pregnant women (mothers) and infants, respectively, and breast milk samples, were collected with cotton swabs. Sampling was performed by the pregnant women (mothers) or by health-care workers and the swabs were transported in Stuart's medium. Swab samples were first plated on Chrom ID ESBL agar (bioMèrieux, Marcy l'Etoile, France) and then on an 'in house' AmpC agar (lactose agar containing cefpodoxim 2 mg l^{-1} and cefoxitin 8 mg l^{-1}) for ESBL$_A$ and ESBL$_{M-C}$ screening, respectively. Finally, all samples were inoculated on lactose agar without antibiotics. If there was no growth on the plain lactose agar, the sample was considered invalid and excluded. All plates were incubated for 24 to 48 h at 35 °C under aerobic conditions.

Phenotypic ESBL screening

Any putative ESBL$_A$- or ESBL$_{M-C}$-producing E. coli or K. pneumoniae isolate growing on the Chrom ID ESBL agar and/or the 'in house' AmpC agar were identified to the species level by Vitek2 (bioMèrieux) or MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility testing was performed using Vitek2 (bioMèrieux). An ESBL$_A$ phenotype was confirmed by the combined disk method using cefotaxime/cefotaxime +clavulanate and ceftazidime/ceftazidime+clavulanate discs (Oxoid, Basingstoke, UK). An ESBL$_{M-C}$ phenotype was confirmed by the AmpC MIC Test Strip (containing cefotetan/cefotetan+cloxacillin; Liofilchem, Roseto degli Abruzzi, Italy). Interpretations of antimicrobial susceptibility testing results and confirmatory tests were according to clinical break-points and methods for ESBL detection as recommended by the EUCAST 2012.[14] All ESBL-producing E. coli or K. pneumoniae isolates recovered from the women and their infants were frozen at −70 °C for subsequent molecular analysis. E. coli ATCC 25922 (ESBL-negative), E. coli K2-64 (producing CTX-M-9), E. coli K5-51 (CTX-M-15), K. pneumoniae ATCC 700603 (SHV-18), E. coli K5-20 (CMY-2) and E. coli K15-8 (chromosomal AmpC) were used as control strains.

Detection of ESBL-encoding genes

Isolates were screened for the presence of CTX-M-encoding genes by a real-time PCR assay covering the five groups of CTX-M genes, CTX-M group 1, 2, 8, 9 and 25.[15] SHV- and TEM-encoding genes were detected by conventional PCR[16] and amplicons in non-CTX-M-producing isolates were sequenced for subtyping. ESBL$_{M-C}$-encoding genes were detected and sub-classified by conventional PCR using primers described previously.[17]

Bacterial strain typing

Clonal relatedness between ESBL-E isolates recovered from mothers and their respective infants was investigated by pulsed-field gel electrophoresis of XbaI-digested (Promega, Madison, WI, USA) genomic DNA using the Chef-DR® III System (Bio-Rad, Oslo, Norway) according to the standard PulseNet USA protocol for E. coli [18] with the following run conditions: 14 °C, 120° fixed angle, 200 V fixed voltage (6 V cm^{-1}) and pulse time intervals from 1 to 20 s for 21 h. The interpretation of the results was based on the criteria suggested by Tenover et al.[19]

Clinical data

Information regarding the women's nationality, hospitalization, use of antibiotics and travel outside Scandinavia in the past 12 months was obtained from questionnaires at 36 weeks of pregnancy (Supplementary Information). Data on infants were retrieved from their medical records:

date of birth, birth weight, gestational age, gender, mode of delivery (cesarean section or vaginal), time between rupture of the membranes and birth, and admittance to the NICU (yes/no).

Statistical analysis

Based on an expected ESBL-E carriage rate of ~ 3%,[20] we aimed for a sample size of ~ 1000 participants to be able to detect clinically relevant risk factors for ESBL-E colonization in the study population. Possible risk factors for being colonized were selected after a review of the literature and included age, hospitalization, travel to high-endemic countries, exposure to antibiotics and nationality. Each explanatory variable were first analyzed in simple regression models. All variables were further included in forward and backward stepwise multiple logistic regression analyses. The final model included variables significant at the 5% level as well as traveling. Interaction was tested for in the final model. The overall likelihood ratio P-value (LR-p) was used in all analyses. Comparisons between groups were performed by Fisher exact test. All tests were two-tailed and P-values ≤ 0.05 were considered statistically significant. SPSS for Windows (IBM SPSS Statistics for Windows, Version 21.0., IBM, Armonk, NY, USA) was used for all analyses.

RESULTS

ESBL-E carriage among pregnant women

During the 6 months study period, 901 pregnant women were enrolled from ~ 2100 births that took place. In total, 26 out of 901 (2.9%) women were found colonized by ESBL-E at 36 weeks of pregnancy. Of these, 23 out of 26 (88.5%) carried ESBL$_A$-producing E. coli and 3 out of 26 (11.5%) carried ESBL$_{M-C}$-producing E. coli. One of the women, who carried CTX-M-group 9-producing E. coli, also carried an additional CTX-M-group 9-producing K. pneumoniae strain (Table 1). In total, 21 out of 24 ESBL$_A$-producing isolates were co-resistant to trimethoprim-sulfamethoxazole, gentamicin and/or ciprofloxacin. The remaining

Table 1. Characteristics of ESBL-producing isolates recovered from 26 pregnant women at 36 weeks of pregnancy

Patient	Isolate	ESBL-group/type	Co-resistance
1	E. coli ESBL$_A$	CTX-M-group 9	SXT
2	E. coli ESBL$_A$	CTX-M-group 9	SXT
3	E. coli ESBL$_A$	CTX-M-group 1	SXT
4	E. coli ESBL$_{M-C}$	CIT-subgroup	None
5	E. coli ESBL$_A$	CTX-M-group 1	SXT/CIP
6	E. coli ESBL$_{M-C}$	CIT-subgroup	None
7	E. coli ESBL$_{M-C}$	CIT-subgroup	None
8	E. coli ESBL$_A$	CTX-M-group 9	SXT/CIP
9	E. coli ESBL$_A$	CTX-M-group 1	SXT/CIP/GEN
10	E. coli ESBL$_A$	CTX-M-group 1	SXT/GEN
11	E. coli ESBL$_A$	CTX-M-group 1	SXT
12	E. coli ESBL$_A$	SHV-12	SXT
13	E. coli ESBL$_A$	CTX-M-group 9	SXT
14	E. coli ESBL$_A$	CTX-M-group 1	SXT
15	E. coli ESBL$_A$	CTX-M-group 9	SXT
16	E. coli ESBL$_A$	CTX-M-group 1	SXT
16	K. pneumoniae ESBL$_A$	CTX-M-group 9	SXT
17	E. coli ESBL$_A$	CTX-M-group 9	SXT
18	E. coli ESBL$_A$	CTX-M-group 1	None
19	E. coli ESBL$_A$	CTX-M-group 1	SXT/CIP/GEN
20	E. coli ESBL$_A$	CTX-M-group 9	SXT/CIP
21	E. coli ESBL$_A$	CTX-M-group 1	None
22	E. coli ESBL$_A$	CTX-M-group 1	SXT/CIP
23	E. coli ESBL$_A$	CTX-M-group 1	SXT/CIP/GEN
24	E. coli ESBL$_A$	CTX-M-group 1	None
25	E. coli ESBL$_A$	CTX-M-group 9	CIP/GEN
26	E. coli ESBL$_A$	CTX-M-group 1	SXT/GEN

Abbreviations: CIP, ciprofloxacin; ESBL, extended-spectrum β-lactamase; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole.

Table 2. Multiple backward logistic regression analyses of risk factors for being colonized by extended-spectrum beta-lactamase-producing Enterobacteriaceae at 36 weeks of pregnancy at the Stavanger University Hospital

	Unadjusted model					Fully adjusted model, n = 821				Final model[a], n = 898			
	n	c	OR	95% CI	LR-p	n	OR	95% CI	LR-p	n	OR	95% CI	LR-p
Age of mother	901	26	0.99	(0.92, 1.07)	0.805	821	0.99	(0.91, 1.08)	0.851				
Travel the last 6 months[b]	898	26			0.228				0.743				0.430
no travel outside Scandinavia	529	14	1	(Reference)		485	1	(Reference)		529	1	(Reference)	
to western countries	335	9	1.02	(0.44, 2.37)		305	1.13	(0.45, 2.85)		335	1.28	(0.53, 3.09)	
to Asia or Africa	34	3	3.56	(0.97, 13.04)		31	1.93	(0.39, 9.46)		34	2.56	(0.66, 9.93)	
Use of antibiotics	828	23			0.270	619			0.156				
during the last 12 months	204	8	1.66	(0.69, 3.97)		202	1.96	(0.80, 4.84)					
Hospitalization	891	26			0.292	742			0.237				
during the last 12 months	81	1	0.39	(0.52, 2.94)		79	0.34	(0.04, 2.69)					
Nationality	901	26			0.002				0.023				0.003
Norwegian	675	15	1	(Reference)		611	1	(Reference)		672	1	(Reference)	
Non-Norwegian, non-Asian or African	159	3	0.85	(0.24, 2.96)		149	0.91	(0.25, 3.31)		159	0.80	(0.23, 2.84)	
Asian or African	67	8	5.97	(2.43, 14.65)		61	4.77	(1.71, 13.00)		67	5.62	(2.21, 14.27)	

Abbreviations: c, colonized; CI, confidence interval; LR-p, likelihood ratio P-value; n, number analyzed; OR, odds ratio. [a]Forward and backward stepwise analyses attained the same results. [b]The results were principally the same when analyzed for 3 or 6 months.
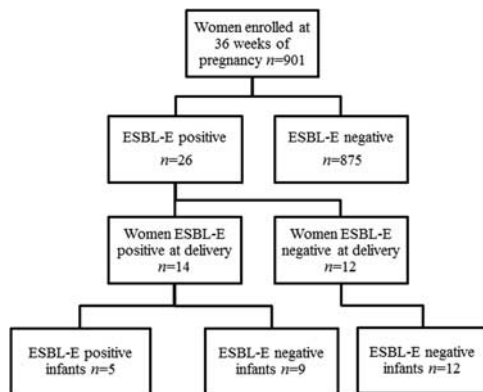
Figure 1. Flow chart of ESBL-E screening of pregnant women and respective neonates eligible for the study.

three $ESBL_A$- and the three $ESBL_{M-C}$-producing isolates had none of the above-mentioned co-resistances (Table 1). All isolates were sensitive to meropenem.

Screening of the 26 ESBL-E-positive women was repeated upon admission for delivery, median 26 days (range = 7 to 41) after the initial screening. Fourteen (53.8%) were still ESBL-E positive. None of the colonized women became ESBL-E infected.

Risk factors for colonization in pregnancy

The number of participants exposed to different risk factors and the number being colonized in each group are given in Table 2. For traveling and nationality, Asia and Africa were analyzed together due to the low number of cases. Colonization was observed in 15 out of 675 (2.2%) Norwegians, 3 out of 159 (1.9%) non-Norwegians of Western nationality and 8 out of 67 (11.9%) of African or Asian nationality ($P < 0.001$).

The results of the unadjusted and adjusted regression analyses for being colonized at week 36 of pregnancy are shown in Table 2. African or Asian nationality was a risk factor for being colonized, also in the final model when corrected for traveling during the last 6 months. There was no interaction between Asian or African nationality and traveling outside Scandinavia.
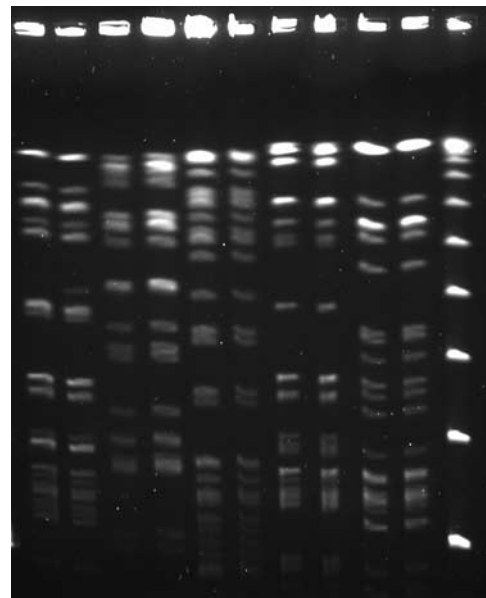
Figure 2. XbaI-pulsed-field gel electrophoresis-profiles of CTX-M-producing E. coli isolates from five mother–neonate (MN) pairs. Lanes 1 and 2: MN-I; Lane 3 and 4: MN-II; Lanes 5 and 6: MN-III; Lanes 7 and 8: MN-IV; Lanes 9 and 10: MN-V; Lane 11: λ-ladder.

Vertical transmission

The numbers of enrolled participants and screening results are displayed in Figure 1. Infants of the 26 women colonized with ESBL-E at 36 weeks of pregnancy were screened during their first 2 weeks of life, with a median number of fecal/rectal samples of 6 (5 to 7), from a total of 157 samples. Maternal–neonatal transmission of CTX-M-producing E. coli was confirmed by indistinguishable pulsed-field gel electrophoresis patterns in five mother–neonate pairs (Figure 2). The five infants were all born to the 14 mothers who were still ESBL-E positive at delivery, giving a transmission rate of 35.7%.

The five infants who became colonized by ESBL-E were delivered vaginally at term (range = 38 to 41 weeks of gestation)

with median birth weight 3142 g (3010 to 4430 g). The median time between rupture of membranes and birth was 7 h (2 to 33 h). Two infants were admitted to the NICU, one due to brachial plexus injury following shoulder dystocia and the other due to hypoglycemia. The two infants did not show any clinical signs or symptoms of infection, and were discharged after three and five days, respectively. Intestinal colonization in infants was detected on median day 3 (2 to 8).

A total of 24 out of 26 (92.3%) infants provided follow-up samples after discharge; one colonized and one non-colonized infant were lost to follow-up. Four of the five ESBL-E-positive infants provided a follow-up sample at median 2 months (range = 2 to 3); of these, three were still colonized by ESBL-E. Twenty of the twenty-one ESBL-E-negative infants provided a follow-up sample; all remained negative at median age 2 months (range = 1 to 5).

A total of 25 out of 26 ESBL-E colonized mothers provided 146 breast milk samples during a period of 5 months after delivery, median 6.0 samples (range = 2 to 8). All were negative for ESBL-E.

DISCUSSION

There is a gap in knowledge concerning perinatal transmission of ESBL-E despite the potential implications. This is to our knowledge the first full report describing the maternal–neonatal transmission rate of ESBL-E in an unselected birth cohort.

An ESBL-E prevalence of 2.9% is in keeping with prevalence studies from Scandinavian countries.[20–23] Reported carriage rates of ESBL-E in pregnant women range from 7.3 to 15.4%, and are likely to reflect carriage rates in the general healthy population in different regions.[6,24–26]

African or Asian origin was a risk factor for being colonized by ESBL-E, possibly due to higher carriage rates in these geographical areas. This is in concordance with a UK study analyzing stool samples from out-patients, where they found a statistically significant difference between carriage in the European (8.1%) and the Middle East/South Asian group (22.8%).[27] The impact of household transmission of ESBL-E has been shown in other settings.[28–30] Speaking an Asian language most commonly at home was identified as a risk factor for colonization with ESBL-producing *E. coli* in a community setting in the Germany (OR = 13.4, $P < 0.001$).[31] Our results were adjusted for traveling to Africa or Asia. The possibility of an ESBL-E reservoir in one or more family members, with subsequent intra-household transmission, may have contributed to the increased carriage rates in women of African or Asian nationality.

A number of publications have described significant increase in ESBL-E carriage rates after travel to India, Asia, Africa and the Middle East.[23,32–36] Travel to Africa or Asia was not a significant risk factor for colonization in this study, however, due to low power this cannot be ruled out. The rate of intestinal ESBL-E carriage at week 36 of pregnancy may have been influenced by clearance of ESBL-E during the past weeks, as women in later stages of pregnancy are not permitted on most international flights. The duration of ESBL-E carriage after travel has in some studies been reported to last only for a few weeks or months.[37,38] In one study, only 5 out of 21 of healthy adults who became colonized during travel to high-endemic areas were intestinal ESBL-E carriers 6 months later.[33]

A high percentage of pregnant women had received antibiotics the past 12 months, most commonly oral narrow-spectrum penicillin. The use of antibiotics was not a risk factor for ESBL-E colonization in pregnant women in the present study. However, we only had access to information on exposure to antibiotics the past 12 months, as opposed to other studies identifying exposure to antibiotics the past 3 months as a risk factor for ESBL-E colonization.[39–41] Persistence of resistant strains after exposure to antibiotics may in some cases be transient, lasting only a few

weeks.[42] Furthermore, the overall exposure of the population to antibiotics, and not just individual intake, is previously reported to correlate with ESBL-E carriage rates in the community.[5]

We found a high maternal–neonatal ESBL-E transmission rate at birth. Despite the low number of cases in this study, the finding suggests that ESBL carriage in pregnancy constitute a substantial risk for infant colonization. Maternal–neonatal transmission has been documented previously in very low birth weight infants and their mothers; identifying the mother as the most important risk factor for ESBL-E colonization in the infants.[6] In contrast to other studies,[6–8] a strength of the present study is that pregnant women colonized by ESBL-E were identified before delivery, excluding neonatal–maternal transmission. In a preliminary report from 2014, maternal–neonatal transmission was shown in 7 out of 39 (18%) mother–neonate pairs (vagino-rectal and rectal samples, respectively).[26] With increasing prevalence rates in the communities worldwide, ESBL-E in women giving birth is likely to represent an important route of introduction of ESBL-E to maternity wards or NICUs.

The gut microbiota is established in the post-natal period, and in particular, infants born vaginally normally establish a gut microbiota similar to that of their mother's.[43] ESBL-producing bacteria were not identified in rectal samples collected from infants on the day of birth. Others have recovered ESBL-E by screening samples collected from gastric fluid and the outer ear within 3 h after birth,[44] or in rectal samples collected within the first 24 h of life.[26] In our study, intestinal ESBL-E colonization was detected on day 3. This difference may be due to disparity in timing of the sampling within the first 24 h or methodological differences.

Intestinal carriage persisted in some of the colonized infants at 2 months of age. Although we did not follow the infants until ESBL-E clearance, they may have represented a source for intra-household and community spread. We recently reported that median carriage length in infants colonized by ESBL-producing *K. pneumoniae* during a NICU outbreak was 12.5 months after hospital discharge (51 infants), and some remained carriers for up to 2 years. Intra-household transmission was furthermore documented in one-third of the households.[28] This is in line with another study where infants remained ESBL-E carriers for up to 12 months, after colonization during their NICU stay.[45]

To our knowledge, no current guidelines recommend routine screening for ESBL-E carriers in maternity wards or NICUs. Systematic screening is costly, and there is a paucity of studies on the efficacy of ESBL-E screening in reducing cross-transmission in non-outbreak situations.[5] In the present study, a high proportion (12/26; 46%) of pregnant women found to be colonized by ESBL-E at 36 weeks of pregnancy were ESBL negative when re-screened on admittance for delivery, and none of their infants were colonized by ESBL-E. This observation suggests that ESBL-E screening at delivery is a timelier and more practical approach if screening among pregnant women were to be considered. Screening NICU infants has recently been studied in a low-prevalence setting, using a once-a-week screening protocol. The study showed a reduction in time between admission and detection of ESBL carriage of 8 days ($P < 0.05$), a decrease in secondary cases from 44% to 9% ($P < 0.05$) and clinical infections from 4 to 0 cases ($P < 0.05$).[12] A reduction from 24 to 11% in ESBL-producing *K. pneumoniae* colonization rates with once-a-week screening and subsequent cohorting of infants with NICU stays >7 days was reported in high-prevalence settings.[11]

The study has some limitations. We do not know the exact number of women who either declined participation or were not approached. A selection error among participants cannot be ruled out, although this is not considered as probable. We only screened for ESBL$_A$- and ESBL$_M$-producing *E. coli* and *K. pneumoniae*. However, these represent the majority of clinical ESBL-E isolates in Norway, and although we may have missed other ESBL-E it is

not probable that this has resulted in a significant under-estimation of the ESBL-E prevalence. Owing to the low ESBL-E prevalence, it was not possible to detect weak risk factors for colonization in pregnancy, risk factors for vertical transmission or the impact of colonization on neonatal outcomes.

In conclusion, this study shows that Norway still has a low prevalence of ESBL-E carriage in an unselected population of pregnant women. However, the prevalence was higher in women of African or Asian nationality. We found a high maternal–neonatal transmission rate at birth, which in an era of increasing global carriage rates of ESBL-E may represent a substantial risk for infant colonization and NICU outbreaks. Transmission through expressed breast milk does not seem a common route of transmission. These observations should be taken into consideration in the revision of NICU infection control practices.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Journal of Perinatology website (http://www.nature.com/jp).