Impact of Postpartum Hospital Length-of-Stay on Infant Gut Microbiota: A Comprehensive Analysis of Vaginal and Caesarean birth

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Abstract

Objectives This study aimed to assess the association between postpartum hospital length-of-stay and the composition of gut microbiota at 3 and 12 months of age in different birth modes. Design Prospective cohort of Canadian infants from the Canadian Healthy Infant Longitudinal Development (CHILD) Study born between 2008 and 2012. Setting General community. Sample 1313 infants from three study sites (Edmonton, Vancouver, and Winnipeg) of the CHILD cohort Methods Duration of hospital stay was documented in hospital records. Infants’ gut microbiota was characterized by Illumina 16S rRNA sequencing of fecal samples at 3 and 12 months. Main outcome measures Infant gut microbiota profiles. Results: In the absence of maternal intrapartum antibiotic (IAP) exposure, vaginally delivered infants (VD) with a longer hospital length-of-stay (LOS) had a higher abundance of bacteria in their gut known to cause hospital-acquired infections (HAI), including Enterococcus at 3 months and 12 months and Citrobacter at 3 months of age. Moreover, HAI-causing bacteria Enterobacteriaceae were more abundant in later infancy in postnatal prolonged hospital stayed IAP-exposed caesarean section (CS) infants. Enterococcus or Citrobacter abundance at 3 months significantly mediated the association of LOS with low relative abundance of Bacteroidaceae and a high relative abundance of Enterococcaceae/Bacteroidaceae or Enterobacteriaceae/Bacteroidaceae ratio at 12 months of age in VD infants without IAP exposure. Conclusions LOS after birth is associated with infant gut dysbiosis. Further research is needed to explore the health outcomes of these associations.

Introduction

Birth in hospital is the norm in the industrialized world, where the mother and infant share a room until discharge.¹,² Over the last four decades, a global trend has emerged to reduce hospital length-of-stay (LOS) after birth, driven by cost considerations and the promotion of a “demedicalization” paradigm in childbirth.³ Postpartum infants’ hospital LOS significantly varies depending on the type of birth.⁴ In Canada, infants born through vaginal delivery (VD) are typically discharged within one or two days, while those delivered via caesarean section (CS) typically experience discharge on days two or three.⁵ Amidst conflicting reports on the negative consequences of early postnatal hospital discharge,⁶⁻⁹ the primary concern in instances of prolonged hospitalization centers around the acquisition of hospital-acquired infections (HAIs).¹⁰⁻¹³ Estimated to affect 220,000 individuals in Canada,¹⁴ the pediatric population accounts
for 9% of HAIs, with neonates exhibiting the highest prevalence rates. Studies have identified potential microbial reservoirs in Canadian hospitals, including bacteria like Enterococcaceae, Bacteroidetes, and Lachnospiraceae, as well as pathogens like Methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* (VRE) *Clostridioides difficile*, extended-spectrum beta-lactamase or carbapenemase-producing Enterobacteriaceae.

Starting with the landmark KOALA cohort study, where a longer LOS was associated with *C. difficile* and *Escherichia coli* colonization in one-month-old newborns, there is documented evidence of a hospitalization effect on infant gut microbiota. Furthermore, both the Baby Biome study and the MUIS study have illustrated LOS’s impact on gut microbiota diversity in infancy, particularly within the first month. None of these studies accounted for the LOS as a standalone risk factor, which could potentially enhance the presence of HAI-causing pathogens.

Our goal was to fill this research gap by comprehensively examining the impact of postpartum hospital LOS on gut microbiota well into early as well as later infancy and independent of birth mode. The primary study objective was to investigate the direct and indirect relationships, mediated through microbial pathways, between postpartum hospital LOS and gut microbiota during both early (3 months) and late (12 months) infancy across various birth modes. We hypothesized that HAI-causing pathogens were more abundant in the newborns’ gut who stay longer in the hospital after birth. Given the critical role of exclusive breastfeeding as a determinant of infant gut microbiota, the secondary goal was to assess how breastfeeding modulates LOS-induced dysbiosis.

**Methods**

**Study design**

This study population consisted of 1313 infants whose mothers have enrolled from the Edmonton, Winnipeg, and Vancouver sites of the Canadian Healthy Infant Longitudinal Development (CHILD) cohort ([www.canadianchildstudy.ca](http://www.canadianchildstudy.ca)), excluding home births. Pregnant women were recruited from the general population from 2008-2012. The study was approved by the Human Research Ethics Boards of the Universities of Manitoba, Alberta, and British Columbia. Mode of delivery, breastfeeding status, household pets, siblings, smoking status, maternal IAP, infant’s antibiotic exposure, and infant hospitalization history, including birth hospitalization was obtained from birth chart reviews or standardized questionnaires at birth and at 3 months as well as 12 months post-partum or both. To find out the developmental trajectories of gut microbes from early to late infancy, a total of 2626 fecal samples were collected from 1313 infants at 3-4 months and 12 months of age. According to the standard of care in Canada, birth hospitalization with a length of hospital stays >1 day in VD, >3 days in CS, and infants with hospital admission in the first 3 months and 12 months were categorized into the group with exposure to hospital environment in early life.

**Fecal microbes’ analysis**

Methods of sample collection, DNA extraction and amplification, 16S rRNA sequencing, and taxonomic classification have been described in our previous study. In brief, fresh or previously refrigerated fecal samples were collected by nurses at a scheduled home visit (3 months) or brought to a clinic visit (12 months). Samples were refrigerated during transport and stored at -80°C until analysis. Whole genome DNA was extracted from feces using the QIAamp DNA Stool Mini Kit (Qiagen, Venlo, Netherlands). The bacterial 16S rRNA gene, hypervariable region V4, was amplified by PCR using universal bacterial primers specific for use in IlluminaMiSeq. The protocol for detection of *Clostridioides difficile* colonization by qPCR was described in our previous study.

**Statistical analysis**

The Chi-square test was used to assess the distribution of potential confounders according to early life exposure to hospital environment status. Based on the median value of LOS for each birth mode, LOS was classified. The gut microbial profile of infants hospitalized for >2 days in VD and >3 days in CS after birth were compared to the gut microbes’ profile of shorter hospitalized infants. Microbiota alpha diversity was
assessed using Chao1 indices of species richness and the Simpson and Shannon indices of diversity. Microbial community structures were compared by permutational analysis of variance (PERMANOVA) on the Bray-Curtis dissimilarity index and visualized by principal component analysis. Non-parametric Mann-Whitney U-test and Kruskal-Wallis test compared median richness, diversity, and relative abundance of dominant taxa. Crude p-values were adjusted for multiple comparisons by positive False Discovery Rate (FDR) correction. A p-value of <0.05 was defined as statistically significant, and 95% confidence intervals (CIs) were calculated. In addition, to identify a discriminative biomarker for post-birth hospital LOS, discriminant analysis effect size (LEfSe) was determined with a linear discriminant analysis log score cutoff of 2.31

Stratified analysis was conducted by birth modes and maternal intrapartum antibiotic (IAP) exposures: VD infants without exposure to IAP (VD-no IAP), VD infants with exposure to IAP (VD-IAP) and IAP-exposed CS infants. Microbial measures of diversity and relative abundance were classified into two groups (below vs. above median) to create dichotomous outcome variables. An association between hospital LOS and infant gut microbiota diversity and composition was determined using logistic regression analysis. Models were adjusted for suspected confounders identified in a directed acyclic graph (DAG) model-building approach (Figure S1). Finally, mediation analyses were conducted using the Hayes PROCESS macro in SPSS, version 23.0 (SPSS Inc), to test whether early life hospital LOS (X) is associated with the infant gut microbiota at 12 months of age (Y) through a 3-month microbiota intermediate variable (M). The relative abundance of microbiota was categorized into tertiles. Bootstrapping, a nonparametric resampling procedure (10000 bootstrap resamples), was used to generate 95% CIs in mediation models. Stratified analyses by breastfeeding were conducted to evaluate the potential modifying effect of breastfeeding in the association between LOS and gut microbiota if the interaction was marginally significant (p=<0.1) using logistic regression models.

Results

Study population

Of the 1313 infants in this study population, 632 (63.97%) infants were hospitalized for more than 1 day after birth and 242 (74.46%) for more than 2 days, respectively, in VD and CS groups. The characteristics of the mother-infant pairs according to hospital LOS in each delivery mode were described in Table S1.

Effect of length of hospital stay on fecal microbiota composition

All birth modes combined

Infant gut microbiota community structure was influenced by LOS both in early (p=0.001) and late infancy (p=0.001) (Figure S2). Creating a rank variable for LOS for all birth modes (Table S2 and S3), a higher abundance of Firmicutes and a lower abundance of Bacteroidetes were consistently observed with extended hospital stays (FDR p=0.001, 3 months; FDR p=0.05, 12 months). An increased abundance of HAI-related microbes such as Enterococcus, Clostridium, Enterobacteriaceae, and its genus Citrobacter was also associated with prolonged hospital stays both in early and late infancy.

Vaginally delivered infants without maternal IAP exposure (VD-noIAP)

Early infancy

At the phylum level, Firmicutes were over-represented (median: 0.14 vs. 0.19), and Bacteroidetes were under-represented (median: 0.44 vs. 0.35) among infants with hospital stay for more than one day (Figure 1, Table S4). In comparison to shorter hospitalized, longer hospitalized infants had higher abundances of the HAI-related bacteria Enterococcus (p=0.01, FDR p=0.17) from Firmicutes phylum and Citrobacter (p=<0.01, FDR p=0.11) from Proteobacteria phylum as well as lower abundances of Bacteroides (p=0.07, FDR p=0.41) from Bacteroidetes phylum. Both LEfSe analysis and logistic regression model confirmed the higher abundance of Enterococcus in longer hospitalized infants (Figure 2(a), Figure 3(a)).

The logistic regression models further confirmed the effect of prolonged hospitalization on increased abundance of Citrobacter, Enterobacteriaceae/Bacteroidaceae and Enterococcaceae/Bacteroidaceae (Figure...
Moreover, longer hospitalized infants were more likely to have a high relative abundance family Actinomyces (aOR 1.53, 1.12-2.09), Enterococccaeae (aOR 1.53, 1.12-2.08), unclassified family and genus of Lactobacillales order (aOR 1.79, 1.28-2.54, p=<0.001), Veillonellaceae and its genus Veillonella (aOR 1.66, 1.22-2.28), unclassified genus of Clostridiaceae (aOR 1.50, 1.10-2.05), Erysipelotrichaceae (aOR 1.47, 1.08-2.00) and a low relative abundance of Bacteriodaceae, its genus Bacteroides (aOR 0.75, 0.55-1.03), Prevotellaceae and its genus Prevotella (aOR 0.69, 0.50-0.95), Alcaligenacae and its genus Sutterella (aOR 0.63, 0.44-0.89) in the infant gut (Table S10).

Late infancy

At 12 months of age, longer hospitalized infants had chronically low relative abundances of Bacteroidaceae and its genus Bacteroides (aOR 0.75, 0.55-1.03), and high abundances of Erysipelotrichaceae, unclassified genus of Erysipelotrichaceae (aOR 1.82, 1.33-2.49), Enterococccaeae/Bacteroidaceae (aOR 1.42, 1.04-1.94), Enterococcus (aOR 1.39, 1.01-1.92) and Veillonella (aOR 1.57, 1.15-2.15) (Figure 3(a), Table S5 and S10). Moreover, increased abundance of HAI-related opportunistic pathogen C. difficile was detected with extended hospital stay (p=0.04).

Vaginally delivered infants with maternal IAP exposure (VD-IAP)

Early infancy

Bacteroidetes phylum was in low abundance with longer hospital stay (Table S6). At 3 months, LEfSE analysis showed Enterococcus abundance in longer hospitalized infants’ gut which diminished after adjustment for gestational age in the logistic regression model (Figure 2(b), Figure 3(b)). Infants had a roughly 50% reduction in Prevotellaceae, its genus Prevotella (aOR 0.49, 0.28-0.87) from Bacteroidetes phylum, and Pasteurellaceae (aOR 0.55, 0.32-0.95), its genus Haemophilus (aOR 0.53, 0.30-0.90) from Proteobacteria phylum, and a more than 2-fold increase in abundance of the genus Ruminococcus (aOR 2.41, 1.23-5.11) from Ruminococcaceae family (Figure 3(b), Table S11).

Late infancy

Prolonged hospitalized infants were associated with a reduced abundance of Porphyromonadaceae, its genus Parabacteroides, unclassified family and genus of Lactobacillales order, Faecalibacterium, Alcaligenacae, its genus Sutterella; Verrucomicrobiaceae and its genus Akkermansia and increased abundance of Blautia, Tissierellaceae as well as Trabulsiella (Table S6 and S11).

Caesarean delivered infants (CS)

Early infancy

Phylum Actinobacteria and its genus Bifidobacterium were in lower abundance at 3 months (Figure 1, Table S8). With longer hospital stay, we observed a 2-fold increase of Dorea (aOR 2.02, 1.13-3.78), approximately 52% and 46% reduction of Prevotella (aOR 0.48, 0.28-0.83) from Bacteroidetes phylum and Gemellaceae (aOR 0.54, 0.32-0.92) from Firmicutes phylum (Figure 3(c), Table S12).

Late infancy

At 12 months, Firmicutes was high in abundance whereas Bacteroidetes was in low abundance with extended LOS (Table S9). We observed a 40 to 50% reduction of Bacteroidaceae, genus Bacteroides, genus Parabacteroides, Rikenellaceae, its genus unclassified Rikenellaceae in CS infants with an extended hospital stay. We also observed a 1.5-2-fold increase of Bifidobacteiraceae, its genus Bifidobacterium from Actinobacteria phylum and Enterococcaceae, Lachnospiraceae from Firmicutes phylum in prolonged hospitalized infants. In the Proteobacteria phylum, an approximately 2-fold increase in abundance of Enterobacteriaceae, genus unclassified Enterobacteriaceae, Citrobacter, and deficient were observed in Alcaligenacae and its genus Sutterella with extended hospitalized infants (Table S12).

Hypothetical pathway (mediation analysis)
The association between prolonged hospitalisation after birth and Bacteroidaceae abundance as well as Enterococcaceae/Bacteroidaceae (EC/B) abundance at 12 months in VD-no IAP infants was found to be mediated by the abundance of the HAI-related bacteria Enterococcus at 3 months. Moreover, indirect association for fecal abundance of Citrobacter at 3 months was significant in the association between LOS and Bacteroidaceae or Enterobacteriaceae/ Bacteroidaceae (E/B) at 12 months. Indirect association (mediated through Enterococcaceae or Veillonellaceae at 3 months) between LOS and fecal Erysipelotrichaceae at 12 months were also significant in the same population (Figure 4, Table S13); no association was found in IAP-exposed VD or CS infants.

Modification of LOS effects on infant gut microbiota by breastfeeding status

Over 44.32% of the VD infants were exposed to exclusive breastfeeding from early life hospital stay to the neonatal period (3-4 months) in contrast to 28.04% of the CS infants during the same period. Breastfeeding exclusivity modifies LOS effects on a few bacteria, as explained in Table S14. However, breastfeeding status did not modify the association between LOS and HAI-associated bacteria Enterococcus or Citrobacter in VD-noIAP infants. A sensitivity analysis by excluding infants exclusively breastfed after hospitalization until 3 months from the non-exclusively breastfed group did not alter the presence of LOS-induced HAI-related bacteria (Table S16).

Discussion

Main findings

In this longitudinal cohort study of 1313 Canadian infants, 64% of infants were hospitalized for 2-3 days after vaginal birth, and 75% of them for 3 days after caesarean delivery. The LOS percentage observed here is slightly greater than that reported in the retrospective cohort study of Canadian neonatal LOS conducted by Metcalfe et al. from 2008 to 2010.5 Under the scenario of vaginal birth with no maternal IAP, prolonging newborn length-of-stay was associated with enrichment of Enterococcus in early infancy and a higher abundance of Enterococcus in relation to Bacteroidaceae 12 months later. Moreover, the opportunistic pathogens Citrobacter and C. difficile were detected to a greater extent in early and later infancy, respectively. When Enterococcus and Citrobacter were more abundant in early infancy, this statistically mediated observed associations between length-of-stay and depletion of Bacteroidaceae in later infancy in VD-no IAP infants. The main influence of IAP (with VD or CS) on top of a longer length-of-stay was a 2-fold likelihood of enrichment with Enterobacteriaceae and its genus Trabusiella in later infancy. As commonly-reported, the Bacteroidaceae were depleted in CS versus vaginal delivery but prolonged hospitalization further reduced bifidobacteria in CS-delivered infants. Exclusive breastfeeding mitigated the impact of hospital length-of-stay to a limited extent. In particular, the emergence of HAI-related pathogens with longer hospitalization remained unaffected by early-life breastfeeding practices.

Study strengths and limitations

The use of high-throughput deep sequencing to describe the developmental trajectories of gut microbes in a large general population birth cohort, based on fecal samples obtained at two crucial time intervals in early and late infancy, was the strength of our study. The large sample size permitted us to restrict analyses to vaginally-born infants with no exposure to maternal IAP and thus, test the impact of an extended hospitalization post-birth that was not due to CS or IAP. It also enabled adjustment for confounding factors and tests of interaction with breastfeeding status. On the other hand, the use of 16S rRNA sequencing in this work might have hampered our understanding of the functional characteristics of gut microbes, their low abundance members, and the role of the nonbacterial microbial population.

Interpretation

Our longitudinal study is a pioneering endeavor aimed at investigating the comprehensive disparities in how extended hospitalization following birth affects gut microbiota, with a specific focus on taxa responsible for HAIs, during both early and later stages of infancy among infants delivered vaginally versus via caesarean section, an area less explored by previous research.24-27 We found compositional changes with an extended
hospital stay after antibiotic-free, vaginal birth, such as enrichment with Enterococcus and Citrobacter spp, or C. difficile in later infancy, which point to a ‘hospital effect’ on the gut microbiota of infants. Of note, this type of dysbiosis has predominantly been reported among preterm infants hospitalized in a neonatal intensive care unit and treated with antibiotics. Although enterococci comprise the gut microbiota of healthy humans, they are a major cause of HAI resistance to antimicrobial agents. Prolonged hospitalization and exposure to VRE from colonized patients, through spread by healthcare workers, has led to VRE infection outbreaks. C. difficile infection, which is becoming more prevalent in children due to antibiotic treatment and longer hospital length-of-stays, is an emerging threat to human health. In our study, greater abundance of Enterococcus and Citrobacter at 3 months mediated enrichment of the enterococcal and enterobacterial families relative to Bacteriodaceae 12 months later. Prolonged hospitalization after CS delivery was associated with greater abundance of Enterobacteriaceae in late infancy. These documented changes to gut microbiota are a feature of IAP exposure in other infant populations, leading to the emergence of antibiotics resistance genes in infants and concerns over resistance of the Enterobacteriaceae to last resort antibiotics like carbapenem. An elevated ratio of Enterobacteriaceae to Bacteroidaceae abundance in infant gut microbiota has also been associated with atopic and food sensitization. At 3 months, Bacteroides were depleted in VD infants and Bifidobacterium in CS infants with a longer hospital stay post birth. Already depleted in CS compared to VD, prolonged hospitalization post CS substantially reduced the odds of expected Bacteroides abundance 12 months later (aOR 0.51, 95%CI: 0.29-0.84). Depletion of Bacteroidetes gut microbiota is characteristic in maternal IAP-exposed newborns. In our study, Bacteroidaceae were marginally depleted with extended hospitalization in the absence of maternal IAP. As noted, the early expansion of Enterococcus and Citrobacter seen with a longer length-of-stay led to the persistent depletion of Bacteroidaceae. Disrupted maternal transmission of Bacteroides during vaginal delivery is found to co-occur with the emergence of HAI microbes, like Enterococcus, and a lowered abundance of bifidobacteria with the emergence of antibiotic-resistant genes. Genus Prevotella of the Bacteroidetes was also depleted with an extended length-of-stay and still further, if this was combined with maternal IAP or CS delivery. Similarly, a lowered abundance of Prevotella has been reported in hospitalized, antibiotic-treated infants versus those in the community, and in relation to depletion of bifidobacteria.

We found pathways of the Firmicutes microbes linking early and late infancy changes in gut microbiota subsequent to a hospital ‘effect.’ Among VD infants with no IAP exposure, a higher abundance of Enterococcaceae or Veillonellaceae in early infancy was an intermediate (or mediator) of a positive association between length-of-stay and abundance of Erysipelotrichaceae in later infancy. The butyrate-producing Erysipelotrichaceae of the Firmicutes are found to be more plentiful in the gut of formula-supplemented infants and those exposed to maternal IAP. In our study, the latter was not a factor in the restricted analyses but lack of exclusive breastfeeding may have played a role. Having said this, we found few interactions between length-of-stay and breastfeeding status in LOS associations with gut microbial abundance.

Conclusion

Our study draws attention to an underappreciated side effect of extended postpartum hospital stays newborn gut microbes dysbiosis. Additional study is required to confirm these results in different populations and assess their implications for newborn health.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Author contribution
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Ethics approval and consent to participate

Written informed consent was obtained from parents at enrollment. This study was approved by the ethics board at the University of Alberta.

Supplementary material

Additional supporting information will be found in the following figures and tables:

Figure S1. Directed Acyclic Graph (DAG) is showing the association between post-birth hospital length-of-stay (LOS) and infant gut microbiota. A minimal adjustment is necessary to estimate total effect of LOS on gut microbiota are infant delivery mode, with or without exposure to maternal intrapartum antibiotic (IAP), and infants’ gestational age during birth

Figure S2. Comparison of gut microbiota community structures (beta diversity) at genus level at 3 and 12 months, according to length of hospital stay

Table S1. Population characteristics by duration of hospital stay (vaginal and caesarean section)

Table S2. Relative abundance of dominant phyla, families and genus in fecal microbiota of infants at 3 months of age according to the duration of hospital stay (n=1313)

Table S3. Relative abundance of dominant phyla, families and genus in fecal microbiota of infants at 12 months of age according to duration of hospital stay (n=1313)

Table S4. Relative abundance of dominant phyla, families, and genus in fecal microbiota infants at 3 months of age (according to duration of hospital stay, vaginal delivery without IAP exposure, n=682)

Table S5. Relative abundance of dominant phyla, families, and genus in fecal microbiota infants at 12 months of age (according to duration of hospital stay, vaginal delivery without IAP exposure’ n=682)

Table S6. Relative abundance of dominant phyla, families, and genus in fecal microbiota infants at 3 months of age (according to duration of hospital stay, vaginal delivery with IAP exposure n=294 )

Table S7. Relative abundance of dominant phyla, families, and genus in fecal microbiota infants at 12 months of age (according to duration of hospital stay, vaginal delivery with IAP exposure n=294)

Table S8. Relative abundance of dominant phyla, families and genus in fecal microbiota of infants at 3-4 months of age (according to duration of hospital stay, cesarean delivery (n=325)
Table S9. Relative abundance of dominant phyla, families, and genus in fecal microbiota of infants at 12 months of age (according to duration of hospital stay, cesarean delivery (n=325))

Table S10. Crude and likelihood ratio of abundance of key gut microbiota measures at 3 months and 12 months according to length of hospital stay (Vaginal delivery without intrapartum antibiotic prophylaxis)

Table S11. Crude and likelihood ratio of abundance of key gut microbiota measures at 3 months and 12 months according to length of hospital stay (Vaginal delivery with intrapartum antibiotic prophylaxis)

Table S12. Crude and likelihood ratio of abundance of key gut microbiota measures at 3 months and 12 months according to length of hospital stay (Caesarean-section delivery, N=325)

Table S13. Indirect association between hospital Length-of-stay and infant gut microbiota at 12 months of age mediated through infant gut microbiota at 3 months of age in different birth modes and Intrapartum antibiotic exposures.

Table S14. Adjusted likelihood ratio of abundance of key gut microbiota measures at 3 months and 12 months according to length of hospital stay and breast-feeding status.

Table S15. Crude and likelihood ratio of abundance of key gut microbiota measures at 3 months and 12 months according to length of hospital stay in VD-noIAP infants (gestational age ≥37 weeks)

Table S16. Crude and likelihood ratio of abundance of key gut microbiota measures at 3 months and 12 months according to length of hospital stay in VD-noIAP infants (removing exclusive breastfeeding infants after hospitalization)

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1 Parry DC. “We wanted a birth experience, not a medical experience”: exploring Canadian women’s use of midwifery. Health Care Women Int. 2008; 29 (8-9):784-806.


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