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Impact of postpartum hospital length of stay on infant gut microbiota: a comprehensive analysis of vaginal and caesarean birth

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SUMMARY

Background: The primary concern with prolonged hospitalization following birth is the risk of acquiring hospital-acquired infections (HAIs) caused by opportunistic bacteria, which can alter the early establishment of gut microbiota.
Objective: To assess the association between postpartum hospital length of stay (LOS) and the composition of gut microbiota at 3 and 12 months of age according to birth mode.
Methods: In total, 1313 Canadian infants from the CHILD Cohort Study were involved in this study. Prolonged LOS was defined as ≥2 days following vaginal delivery (VD) and ≥3 days following caesarean section (CS). The gut microbiota of infants was characterized by Illumina 16S rRNA sequencing of faecal samples at 3–4 months and 12 months of age.
Findings: Following prolonged LOS, VD infants with no exposure to intrapartum antibiotics had a higher abundance of bacteria known to cause HAIs in their gut, including *Enterococcus* spp. at 3 and 12 months, *Citrobacter* spp. at 3 months, and *Clostridioides difficile* at 12 months. Abundance of *Enterococcus* spp. or *Citrobacter* spp. at 3 months significantly mediated the association between LOS and low abundance of Bacteroidaceae, or higher Enterococcaeae/Bacteriodaceae or Enterobacterales/Bacteroidaceae abundance ratios at 12 months of age in VD infants without intrapartum antibiotic exposure. HAI-causing Enterobacterales were also more abundant in later infancy in infants with prolonged LOS following CS. In the absence of exclusive breastfeeding at 3 months or any

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breastfeeding at 12 months, Porphyromonadaceae (of Bacteroidota) were depleted in CS infants with prolonged LOS.

Conclusions: Prolonged hospital stay after birth is associated with infant gut dysbiosis.

Introduction

Hospital birth 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]. Infant LOS in hospital is longer after caesarean section (CS) delivery than after vaginal delivery (VD) [4]. In Canada, a greater percentage of newborns born vaginally and following CS are being discharged 1 day later and 2 days later, respectively, than in the early 2000s [5].

Amidst conflicting reports on the negative consequences of early postnatal hospital discharge [6—9], the primary concern in instances of prolonged LOS centres around the acquisition of hospital-acquired infections (HAIs) [10—13]. Estimated to affect 220,000 individuals in Canada [14], the paediatric population accounts for 9% of HAIs, with neonates exhibiting the highest prevalence rates [15]. Studies have identified potential microbial reservoirs in Canadian hospitals, including bacteria such as Enterococcaceae, Bacteroidota (previously known as Bacteroidetes) and Lachnospiraceae, as well as pathogens such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococcus (VRE), *Clostridioides difficile*, and extended-spectrum beta-lactamase or carbapenemase-producing Enterobacterales [16—23].

Starting with the landmark KOALA cohort study, where prolonged LOS was associated with *C. difficile* and *Escherichia coli* colonization in 1-month-old newborns, there is documented evidence of an effect of LOS on infant gut microbiota [24]. Furthermore, both the Baby Biome study and the MUIS study have illustrated the effect of LOS on the diversity of gut microbiota in infancy, particularly within the first month [25,26]. None of these studies accounted for LOS as a standalone risk factor, which could potentially enhance the presence of HAI-causing pathogens.

The aim of this study was to fill this research gap by undertaking a comprehensive evaluation of the impact of postpartum LOS on gut microbiota in early, as well as later, infancy, independent of birth mode. The primary study objective was to investigate the direct and indirect relationships, mediated through microbial pathways, between postpartum LOS and gut microbiota in early (3 months) and late (12 months) infancy across various birth modes. It was hypothesized that HAI-causing pathogens were more abundant in the gut of newborns with prolonged LOS after birth. Given the critical role of exclusive breastfeeding as a determinant of infant gut microbiota [27], the secondary goal was to assess how breastfeeding modulates LOS-induced dysbiosis.

Methods

Study design

The study population consisted of 1313 infants and mothers enrolled in the Edmonton, Winnipeg and Vancouver sites of the Canadian Healthy Infant Longitudinal Development (CHILD) Cohort Study (www.canadianchildstudy.ca), excluding home births (Supplementary Figure A1). Pregnant women were recruited from the general population between 2009 and 2012. Mode of delivery, breastfeeding status (exclusive breastfeeding until 3 months and any breastfeeding at 12 months), household pets, siblings, smoking status, maternal intrapartum antibiotic prophylaxis (IAP), infant antibiotic exposure, and infant hospitalization history were obtained from birth chart reviews or standardized questionnaires at the 3- and 12-month postnatal visits. To capture developmental trajectories of gut microbes from early to late infancy, 2626 faecal samples were collected from 1313 infants at 3—4 months (herein stated as 3 months) and 12 months of age.

Faecal microbe analysis

In a previous study, the authors detailed the procedures for sample collection, DNA extraction and amplification, 16S rRNA sequencing, and taxonomy classification [28]. Nurses collected faecal samples, either fresh or refrigerated, during a pre-arranged home or clinic visit. Before analysis, samples were refrigerated during transportation, then kept at -80 °C. Whole genome DNA was extracted from faeces using the QIAamp DNA Stool Mini Kit (Qiagen, Venlo, Netherlands). The bacterial 16S rRNA gene, hypervariable region V4, was amplified by polymerase chain reaction (PCR) using universal bacterial primers specific for use in Illumina MiSeq. The protocol for detection of *C. difficile* colonization by quantitative PCR was described in the authors' previous study [29].

Patient and public involvement

This was a secondary analysis of data from the CHILD cohort study. Infants and their families were not involved in setting research questions, outcome measures or the design of the study.

Statistical analysis

Chi-squared test was used to assess the distribution of potential confounders according to early life exposure to hospital environment status. Prolonged LOS was defined according to current norms for postpartum hospital stay in Canada: $\geq$2 days after VD and $\geq$3 days after CS birth [5]. The gut microbial profile of infants with prolonged LOS was compared with that of

infants hospitalized for days below the defined cut-off values. 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 were used to compare 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 <0.05 was considered to indicate significance, and 95% confidence intervals (CIs) were calculated. In addition, to identify a discriminative biomarker for postpartum LOS, discriminant analysis effect size (LEfSe) was determined with a linear discriminant analysis log score cut-off of 2 [30].

Stratified analysis was conducted by birth mode and maternal IAP exposure: VD infants without exposure to IAP (VD-no IAP), IAP-exposed VD infants (VD-IAP) and IAP-exposed CS infants. An association between LOS and taxon relative abundance (above versus below the median) was determined using logistic regression analysis. A directed acyclic graph (DAG) was employed to identify suspected confounders, which were subsequently adjusted in logistic regression models [31]. DAGs offer advantages over traditional confounder selection methods, including visual representation of causal relationships between exposure and outcome, identification of biases in early study design, and summarization of complex inter-relationships between variables based on existing knowledge in the research area [32]. The minimal adjustment confounders identified by the DAG included birth mode, maternal IAP and gestational age (Supplementary Figure A2). As data were already stratified by birth mode and maternal IAP, adjustment was made for gestational age alone in logistic regression models. Finally, mediation analyses were conducted using the Hayes PROCESS macro in SPSS Version 23.0 (IBM Corp., Armonk, NY, USA) to test whether prolonged LOS (X) is associated with infant gut microbiota at age 12 months (Y, taxon relative abundance above or equal to the median, taxon abundance ratio, *C. difficile* colonization status) through a microbiota intermediate at 3 months (M, taxon abundance tertile variable). Bootstrapping, a non-parametric resampling procedure (10,000 bootstrap resamples), was used to generate 95% CIs in mediation models. To assess a possible moderating effect of breastfeeding in the association between LOS and taxon relative abundance, the interaction between breastfeeding and LOS was tested. If the interaction term was marginally significant ($P\leq0.1$), stratified analyses by breastfeeding were performed using logistic regression models.

Two sensitivity analyses were performed with the CS- and VD-no IAP groups. First, the few late preterm infants ($N=10/682$) in the study were removed, or adjustment was made for this variable in regression models. Secondly, the exclusive breastfeeding variable was modified by deleting infants who were initially fed formula in the hospital, and then exclusively breastfed.

Results

Study population

Of the 1313 infants in the study population, 632 (64%) were hospitalized for $\geq$2 days after birth and 242 (74.5%) for $\geq$3 days, respectively, in the VD and CS groups. The characteristics of the mother–infant pairs according to LOS for each delivery mode are described in Table I. Among the VD infants ($N=988$), there were significant differences between the two LOS groups in maternal IAP, maternal age and infants' gestational age at birth, breastfeeding status at 3 months, presence of siblings, and study centre. In the CS group ($N=325$), differences between shorter and prolonged LOS were observed for breastfeeding status at 3 months, gestational age, presence of siblings, and study centre.

Effect of LOS on faecal microbiota composition

All birth modes combined

Infant gut microbiota community (beta) diversity was influenced by LOS in both early ($P=0.001$) and late ($P=0.001$) infancy (Supplementary Figure A3). Creating a rank variable for increasing LOS for all birth modes (Supplementary Tables A1 and A2), a higher abundance of Firmicutes and a lower abundance of Bacteroidota were observed consistently with prolonged LOS (FDR $P\leq0.001$ at 3 months; FDR $P\leq0.05$ at 12 months). With increasing LOS, Chao1 total species richness declined at 12 months but not at 3 months. Increased abundance of HAI-related microbes such as *Enterococcus* spp., *Clostridioides* spp., and Enterobacterales and its genus *Citrobacter* was also associated with prolonged LOS in both early and late infancy.

VD infants without maternal IAP exposure

Early infancy. At 3 months, Firmicutes were over-represented (median 0.14 vs 0.19) and Bacteroidota were under-represented (median 0.44 vs 0.35) in the gut microbiota of VD-no IAP infants following LOS $\geq$2 days (Figure 1 and Supplementary Table A3). VD-no IAP infants with prolonged LOS had higher abundance of HAI-related bacteria, *Enterococcus* spp. ($P=0.01$, FDR $P=0.17$) of phylum Firmicutes, and *Citrobacter* spp. ($P\leq0.01$, FDR $P=0.11$) of phylum Proteobacteria, and lower abundance of *Bacteroides* spp. ($P=0.07$, FDR $P=0.41$) of phylum Bacteroidota. LEfSe analyses and multivariable logistic regression [adjusted odds ratio (aOR) 1.41, 95% CI 1.04–1.93] confirmed the higher abundance of *Enterococcus* spp. in infants with prolonged LOS (Figure 2a, Supplementary Figure A4a). These results remained the same when term infants alone were considered (Supplementary Table A15).

Logistic regression models further confirmed the influence of prolonged LOS on gut microbiota in terms of the higher relative abundance of *Citrobacter* spp., and higher abundance ratios for Enterobacterales/Bacteroidaceae and Enterococcaceae/Bacteroidaceae (Figure 2a, Supplementary Table A9). Infants with prolonged LOS were also more likely to have a higher abundance of *Actinomyces* spp. (aOR 1.53, 95% CI 1.12–2.09), Enterococcaceae (aOR 1.53, 95% CI 1.12–2.08), an unclassified family and genus of Lactobacillales (aOR 1.79, 95% CI 1.28–2.54), Veillonellaceae and its genus *Veillonella* (aOR 1.66, 95% CI 1.22–2.28), an unclassified genus of Clostridiaceae (aOR 1.50, 95% CI 1.10–2.05), and Erysipelotrichaceae (aOR 1.47, 95% CI 1.08–2.00). On the other hand, lower abundance of Bacteriodaceae and its genus *Bacteroides* (aOR 0.75, 95% CI 0.55–1.03), Prevotellaceae and its genus *Prevotella* (aOR 0.69, 95% CI 0.50–0.95), and Alcaligenacae and its genus *Sutterella* (aOR 0.63, 95% CI 0.44–0.89) was found in the gut

Table I
Population characteristics by duration of hospital stay after birth (vaginal and caesarean section)

Vaginal delivery (total; *N*=988)	Admission (≥2 days) *N*=632 (63.96%)	*P*-value	Caesarean section (total; *N*=325)	Admission (≥3 days) *N*=242 (74.46%)	*P*-value
Infant gender (*N*=988)			Infant gender (*N*=325)		
Male (*N*=515)	336 (65.2)		Male (*N*=193)	145 (75.1)	
Female (*N*=473)	296 (62.6)	0.38	Female (*N*=132)	97 (73.5)	0.73
Gestational age (39.33±0.04)	39.18±0.06	**<0.001**	Gestational age (38.94±0.08)	38.84±0.10	0.05
Maternal intrapartum antibiotic prophylaxis (*N*=978)			Maternal intrapartum antibiotic prophylaxis (*N*=325)		
No (*N*=682)	410 (60.1)		No (*N*=0)	-	
Yes (*N*=296)	220 (74.3)	**<0.001**	Yes (*N*=325)	242 (74.5)	-
Newborn antibiotic use (*N*=941)			Newborn antibiotic use (*N*=325)		
No (*N*=895)	72 (8.04)		No (*N*=274)	24 (8.76)	
Yes (*N*=46)	5 (10.87)	0.41	Yes (*N*=28)	2 (7.14)	1.00
Exclusively breastfed from hospital to 3 months (*N*=968)			Exclusively breastfed from hospital to 3 months (*N*=321)		
No (*N*=539)	374 (69.39)		No (*N*=231)	181 (78.35)	
Yes (*N*=429)	250 (58.28)	**<0.001**	Yes (*N*=90)	59 (65.56)	**0.02**
Any breastfeeding at 12 months (*N*=968)			Any breastfeeding at 12 months (*N*=315)		
No (*N*=529)	369 (69.75)		No (*N*=186)	137 (73.66)	
Yes (*N*=439)	250 (56.95)	**<0.001**	Yes (*N*=129)	95 (73.64)	0.99
Older siblings (*N*=988)			Older siblings (*N*=325)		
No (*N*=507)	377 (38.16)		No (*N*=184)	51 (27.7)	
Yes (*N*=480)	255 (25.81)	**<0.001**	Yes (*N*=141)	90 (63.8)	**<0.001**
Household pets (*N*=932)			Household pets (*N*=309)		
No (*N*=444)	269 (28.86)		No (*N*=144)	105 (72.9)	
Yes (*N*=488)	323 (34.66)	0.07	Yes (*N*=165)	124 (75.1)	0.65
Smoking during pregnancy (*N*=967)			Smoking during pregnancy (*N*=318)		
No (*N*=925)	581 (60.08)		No (*N*=307)	227 (73.9)	
Yes (*N*=42)	32 (3.31)	0.07	Yes (*N*=11)	8 (72)	0.97
Maternal education — university degree and above (*N*=958)			Maternal education — university degree and above (*N*=316)		
No (*N*=377)	249 (66.1)		No (*N*=126)	90 (71.4)	
Yes (*N*=581)	360 (62)	0.19	Yes (*N*=190)	143 (75.3)	0.44
Study centre (*N*=988)			Study centre (*N*=325)		
Edmonton (*N*=234)	201 (85.9)		Edmonton (*N*=76)	76 (100)	
Vancouver (*N*=312)	139 (44.6)		Vancouver (*N*=125)	83 (66.4)	
Winnipeg (*N*=442)	292 (66.1)	**<0.001**	Winnipeg (*N*=124)	83 (66.9)	**<0.001**
Maternal age (31.79±4.59)	31.375±4.62	**<0.001**	Maternal age (33.31±4.63)	33.26±4.61	0.77
Maternal ethnicity (*N*=980)			Maternal ethnicity (*N*=325)		
Asian (*N*=109)	77 (70.6)		Asian (*N*=45)	37 (82)	
First nation (*N*=52)	33 (63.5)		First nation (*N*=19)	16 (84.2)	
White (*N*=777)	483 (62.1)		White (*N*=247)	178 (72.1)	
Other (*N*=42)	32 (76.2)	0.11	Other (*N*=14)	11 (78.5)	0.35
Solid food introduction at 3 months (*N*=980)			Solid food introduction at 3 months (*N*=323)		
No (*N*=954)	606 (63.52)		No (*N*=313)	232 (74.12)	
Yes (26)	19 (73.08)	0.32	Yes (10)	8 (80)	0.68

Data are *N* (%) or mean±standard deviation.
Bold indicates significance ($P \leq 0.05$).

microbiota of infants with prolonged LOS (Supplementary Table A9).

Late infancy. At 12 months of age, prolonged LOS was associated with depletion of Bacteroidaceae and its genus *Bacteroides* (aOR 0.75, 95% CI 0.55–1.03), with higher abundance of Erysipelotrichaceae and its unclassified genus (aOR 1.82, 95% CI 1.33–2.49), *Enterococcus* spp. (aOR 1.39, 95% CI 1.01–1.92) and *Veillonella* spp. (aOR 1.57, 95% CI 1.15–2.15), and a higher Enterococcaceae to Bacteroidaceae

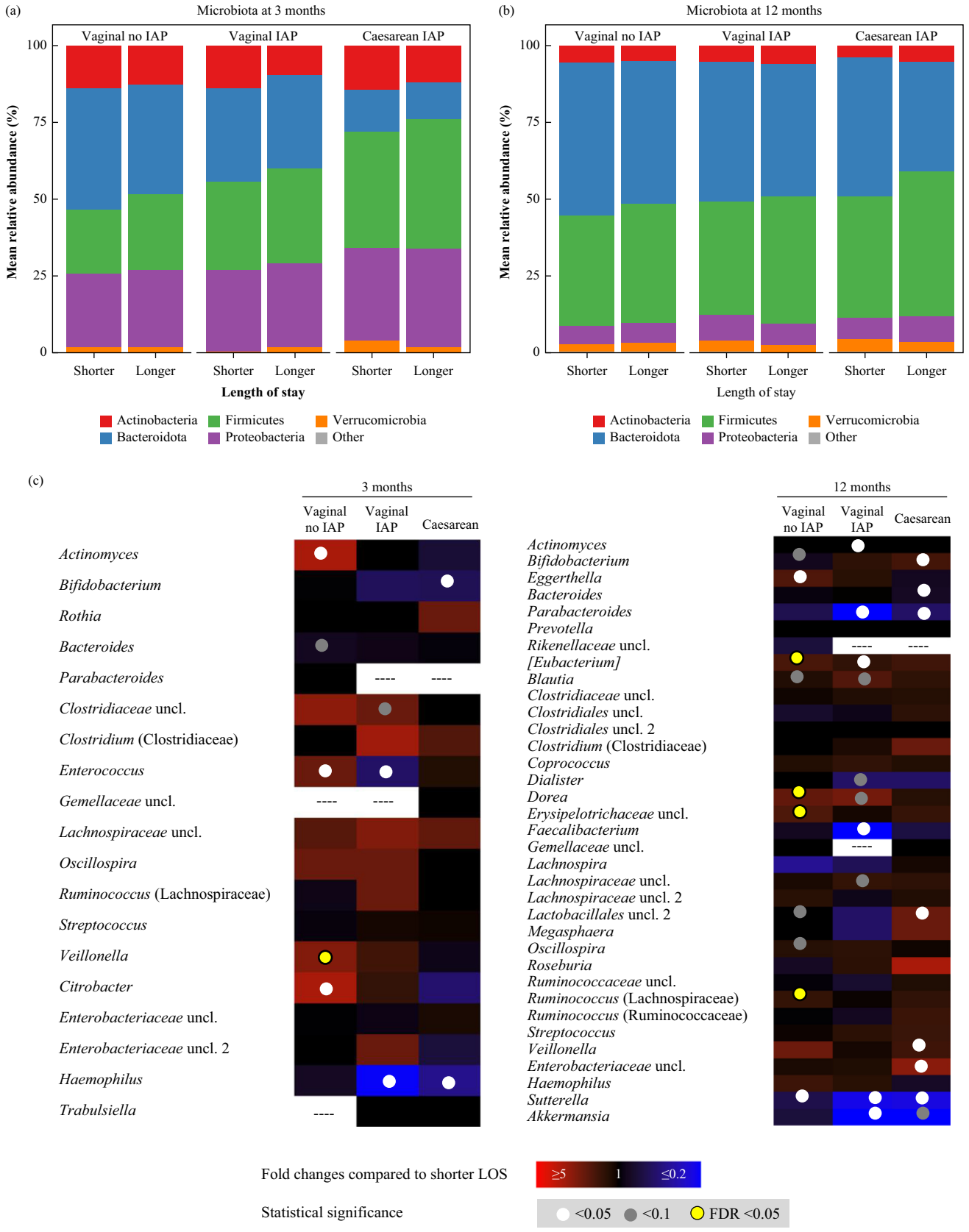

Figure 1. Infant gut microbiota composition at 3 and 12 months by hospital length of stay (LOS) postpartum, birth mode, and maternal intrapartum antibiotic prophylaxis (IAP) exposure. (a,b) Mean phylum-level composition at 3 and 12 months, respectively, according to LOS and mode of delivery. (c) Differences in median relative abundance of dominant genera in infants with prolonged LOS (vs infants with shorter LOS)

abundance ratio (aOR 1.42, 95% CI 1.04–1.94) (Figure 2a, Supplementary Tables A4 and A9). Moreover, colonization with the HAI-related opportunistic pathogen, *C. difficile* was more likely in infants with prolonged LOS (aOR 1.42, 95% CI 1.02–1.97).

VD infants with maternal IAP exposure

Early infancy. Following LOS ≥2 days, phylum Bacteroidota was depleted in the gut microbiota of VD-IAP infants at 3 months (Supplementary Table A5), namely through a 50% reduction in abundance of the Prevotellaceae family and its genus *Prevotella* (aOR 0.49, 95% CI 0.28–0.87). Pasteurellacaeae (aOR 0.55, 95% CI 0.32–0.95) and its genus *Haemophilus* (aOR 0.53, 95% CI 0.30–0.90) of phylum Proteobacteria were also reduced, and there was a greater than two-fold higher abundance of the genus *Ruminococcus* (aOR 2.41, 95% CI 1.23–5.11) from family Ruminococcaceae (Figure 2b, Supplementary Table A10). LEfSe analysis showed *Enterococcus* spp. to be less abundant in VS-IAP infants with prolonged LOS, but this association was inconsistent with results from logistic regression models that adjusted for gestational age (Figure 2b, Supplementary Figure A4b).

Late infancy. At 12 months, prolonged LOS of VD-IAP infants was associated with reduced abundance in gut microbiota of Porphyromonadaceae and its genus *Parabacteroides*, an unclassified family and genus of Lactobacillales, *Faecalibacterium* spp., Alcaligenaceae and its genus *Sutterella*, and Verrucomicrobiaceae and its genus *Akkermansia*, but greater abundance of *Blautia* spp., Tissierellaceae and *Trabulsiella* spp. (Supplementary Tables A6 and A10).

CS infants

Early infancy. Phylum Actinobacteria and its genus *Bifidobacterium* were depleted in gut microbiota at 3 months in CS infants with LOS ≥3 days (Figure 1, Supplementary Table A7). A two-fold higher abundance of *Dorea* spp. (aOR 2.02, 95% CI 1.13–3.78), and reductions in *Prevotella* spp. (aOR 0.48, 95% CI 0.28–0.83) from phylum Bacteroidota, and *Gemellaceae* spp. (aOR 0.54, 95% CI 0.32–0.92) from phylum Firmicutes were also observed (Figure 2c, Supplementary Table A11).

Late infancy. At 12 months, in CS infants with prolonged LOS, Bacteroidota were depleted in gut microbiota (Supplementary Table A8). This comprised Bacteroidota members with reduced abundance as follows: Bacteroidaceae (aOR 0.52, 95% CI 0.31–0.86), genus *Bacteroides*, genus *Parabacteroides* (aOR 0.55, 95% CI 0.32–0.93), and Rikenellaceae and genus unclassified *Rikenellaceae* (aOR 0.50, 95% CI 0.29–0.87). In addition, almost two-fold increases in abundance of Bifidobacteriaceae (aOR 1.92, 95% CI 1.15–3.23) and its genus *Bifidobacterium*, and of two families in phylum Firmicutes, Enterococcaceae (aOR 1.71, 95% CI 1.03–2.87) and Lachnospiraceae (aOR 1.74, 95% CI 1.04–2.91), were observed. In phylum Proteobacteria, there was a two-fold higher abundance of Enterobacterales (aOR 2.03, 95% CI 1.22–3.43), genus unclassified Enterobacterales (aOR 2.18, 95% CI 1.31–3.69) and *Citrobacter* spp. (aOR 1.81, 95% CI 1.01–3.39), but depleted abundance of Alcaligenaceae and its genus *Sutterella* in infants with prolonged LOS (Supplementary Table A11).

Hypothetical pathway (mediation analysis)

The association between prolonged LOS after VD-no IAP birth and abundance of Bacteroidaceae, or the Enterococcaceae/Bacteroidaceae abundance ratio at 12 months was found to be mediated by the abundance of HAI-related *Enterococcus* spp. at 3 months. Moreover, the indirect (mediating) effect of the abundance of *Citrobacter* spp. at 3 months was significant in the association between LOS and abundance of Bacteroidaceae or the Enterobacterales/Bacteroidaceae ratio at 12 months. Indirect effects (mediated through the abundance of Enterococcaceae or Veillonellaceae at 3 months) between LOS and faecal Erysipelotrichaceae at 12 months were also significant in the same population (Figure 3, Supplementary Table A12); no gut microbiota mediating paths were found in IAP-exposed VD or CS infants.

Modification of LOS effects on infant gut microbiota by breastfeeding status

Over 44.3% of VD infants were exposed to exclusive breastfeeding from hospitalization to the neonatal period (3 months), in contrast to 28.0% of the CS infants during the same period. Only 45.4% of VD infants and 41% of CS infants received any breast milk at 12 months. Exclusive breastfeeding from birth until 3 months or any breastfeeding until 12 months modified LOS effects, as reported in Supplementary Tables A13 and A14. Consistent with findings in all CS infants, Porphyromonadaceae (of Bacteroidota) became less abundant in later infancy in infants with prolonged LOS in the absence of exclusive breastfeeding at 3 months or any breastfeeding at 12 months. This association was not seen in CS infants breastfed at either age. Also, in the absence of early exclusive breastfeeding, *Akkermansia* spp. and *Epulopisium* spp. were more abundant in later infancy in VD-no IAP and VD-IAP infants with prolonged LOS, respectively. Among infants exclusively breastfed at 3 months, prolonged LOS was associated with the following changes to gut microbiota: enhanced *Haemophilus* spp. at 3 months in VD-no IAP infants, depleted *Prevotella* spp. at 3 months in VD-IAP infants, and enhanced *Coprococcus* spp. and depleted *Bilophilia* spp. at 12 months after CS. Seen with any breastfeeding at 12 months, the families Veillonellaceae and Erysipelotrichaceae were more abundant in VD-no IAP infants with prolonged LOS. Early exclusive breastfeeding or breastfeeding at 12 months did not modify the association between LOS and *C. difficile* in either VD or CS infants in early or late infancy. Breastfeeding status did not modify the association between LOS and HAI-associated bacteria *Enteroccoccus* spp. or *Citrobacter* spp. in VD-no IAP infants. A sensitivity analysis by removing infants exclusively breastfed after hospitalization did not alter the presence of LOS-induced HAI-related bacteria (Supplementary Table A16).

according to maternal IAP exposure and mode of delivery: comparison using Mann–Whitney *U*-test. *P*-values <0.05 and <0.1 were taken to indicate statistical significance and marginal significance, respectively. Dashed line indicates not estimable due to median zero in the reference group. Genera with overall median relative abundance >0 are included. FDR, false discovery rate.

Discussion

In this longitudinal cohort study of 1313 Canadian infants, 64% of VD infants were hospitalized for ≥2 days, and 75% of CS infants were hospitalized for ≥3 days. Despite changing norms for earlier discharge after birth, these LOS statistics are typical for births in Canadian hospitals [5]. Under the scenario of VD with no antibiotic exposure from maternal IAP, prolonged newborn LOS was associated with enhanced *Enterococcus* spp. at 3 months, and a higher abundance of Enterococcaceae in relation to Bacteroidaceae at 12 months. Moreover, the opportunistic pathogens, *Citrobacter* spp. and *C. difficile*, were detected to a greater extent in early and later infancy, respectively. When *Enterococcus* spp. and *Citrobacter* spp. were more abundant in early infancy, significant associations between LOS and depletion of Bacteroidaceae in later infancy were observed in VD-no IAP infants. The main influence of IAP (with VD or CS) in infants with prolonged LOS was a two-fold higher likelihood of enhanced Enterobacteriaceae and its genus *Trabusiella* in later infancy. The emergence of HAI-related pathogens with prolonged LOS remained unaffected by breastfeeding practices in early life. As commonly reported, Bacteroidaceae were depleted in CS infants compared with VD infants, but CS infants with prolonged LOS showed a further reduction in bifidobacteria in early infancy, and subsequently many families of Bacteroidota.

This longitudinal study is a pioneering endeavour that aimed to investigate disparities in how extending LOS following birth affects gut microbiota, with a specific focus on taxa responsible for HAIs. Less explored by previous research [24—26], this was conducted during both early and later stages of infancy following VD and CS. Compositional changes were found with prolonged LOS after antibiotic-free vaginal birth, such as enrichment with *Enterococcus* spp. and *Citrobacter* spp, or *C. difficile* in later infancy, which points to a 'hospital effect' on the gut microbiota of infants. This type of dysbiosis has been reported predominantly among preterm infants hospitalized in neonatal intensive care units and treated with antibiotics [33]. Although enterococci comprise the gut microbiota of healthy humans, they are a major cause of HAI resistance to antimicrobial agents [34,35]. Prolonged LOS and exposure to VRE through spread by healthcare workers has led to outbreaks of VRE infections [25,36]. *C. difficile* infection is increasingly prevalent in children due to antibiotic treatment and prolonged LOS [37]. In the present study, greater abundance of *Enterococcus* spp. and *Citrobacter* spp. in early infancy mediated the enrichment of enterococcal and enterobacterial families relative to Bacteriodaceae 12 months later. In CS infants, prolonged LOS was associated with greater abundance of Enterobacterales in late infancy. These documented changes to gut microbiota are a feature of IAP exposure in other infant populations [38], leading to the emergence of antibiotic resistance genes in infants and concerns over resistance of the Enterobacterales to last-resort antibiotics [39,40]. An elevated ratio of Enterobacterales to Bacteroidaceae in infant gut microbiota has also been associated with atopic and food sensitization [41].

At 3 months of age, *Bacteroides* spp. were depleted in VD infants, and *Bifidobacterium* spp. were depleted in CS infants with prolonged LOS. Already depleted in CS infants compared with VD infants, prolonged LOS in CS infants substantially reduced the odds of expected abundance of *Bacteroides* spp. 12 months later (aOR 0.51, 95% CI 0.29—0.84). Depletion of Bacteroidota gut microbiota is characteristic in maternal IAP-exposed newborns [42—44]. In the present study, Bacteroidaceae were also depleted in VD-no IAP infants with prolonged LOS. As noted, the early abundance of *Enterococcus* spp. and *Citrobacter* spp. seen in infants with prolonged LOS led to the persistent depletion of Bacteroidaceae. Disrupted maternal transmission of *Bacteroides* spp. during VD is found to co-occur with the emergence of HAI microbes, such as *Enterococcus* spp. [25], and reduced abundance of bifidobacteria with the emergence of antibiotic-resistant genes [38,45]. Genus *Prevotella* of the Bacteroidota was also depleted in infants with prolonged LOS, and depleted even further if this was combined with maternal IAP or CS delivery. Similarly, reduced abundance of *Prevotella* spp. has been reported in hospitalized, antibiotic-treated infants compared with those in the community [46], and in relation to depletion of bifidobacteria [47].

This study found pathways of Firmicutes microbes linking early and late infancy changes in gut microbiota subsequent to a hospital 'effect'. Among VD-no IAP infants, a higher abundance of Enterococcaceae or Veillonellaceae in early infancy mediated the positive association between LOS and abundance of Erysipelotrichaceae in later infancy. The butyrate-producing Erysipelotrichaceae of the Firmicutes are found to be more plentiful in later infancy in the gut of mixed-fed infants or those exposed to maternal IAP; information on LOS was not given in these studies [48,49]. As the enrichment with Erysipelotrichaceae was also observed among mixed-fed infants in the present study, but in relation to prolonged LOS without IAP, it is plausible that LOS-related abundance of Enterococcaceae or Veillonellaceae was the main driver. Further, lack of exclusive breastfeeding in early infancy following prolonged LOS after CD appeared to promote depletion of the Porphyromonadaceae family in the Bacteroidota phylum. These findings extend earlier results from the CHILD Cohort Study by identifying the underlying influence of prolonged LOS [48].

The use of high-throughput deep sequencing to describe the developmental trajectories of gut microbes in a large general population birth cohort, based on faecal samples obtained at two crucial time intervals in early and late infancy, was a strength of this study and enhances its external validity. The large sample size enabled the analyses to be restricted to VD-no IAP infants, and thus to test the impact of prolonged LOS 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, use of 16S rRNA sequencing in this work did not permit characterization of gut microbial function or the non-bacterial microbial population. Moreover, the CHILD Cohort Study did not collect information on why infants remained in hospital for a longer period after birth, such as maternal or infant infection, which may alter the infant gut microbiota. Furthermore, infant probiotic usage in the study sample was too low (approximately 13—18 infants) to bias the association between LOS and changes in gut microbiota.

In summary, this study draws attention to the underappreciated influence of prolonged LOS on gut microbe

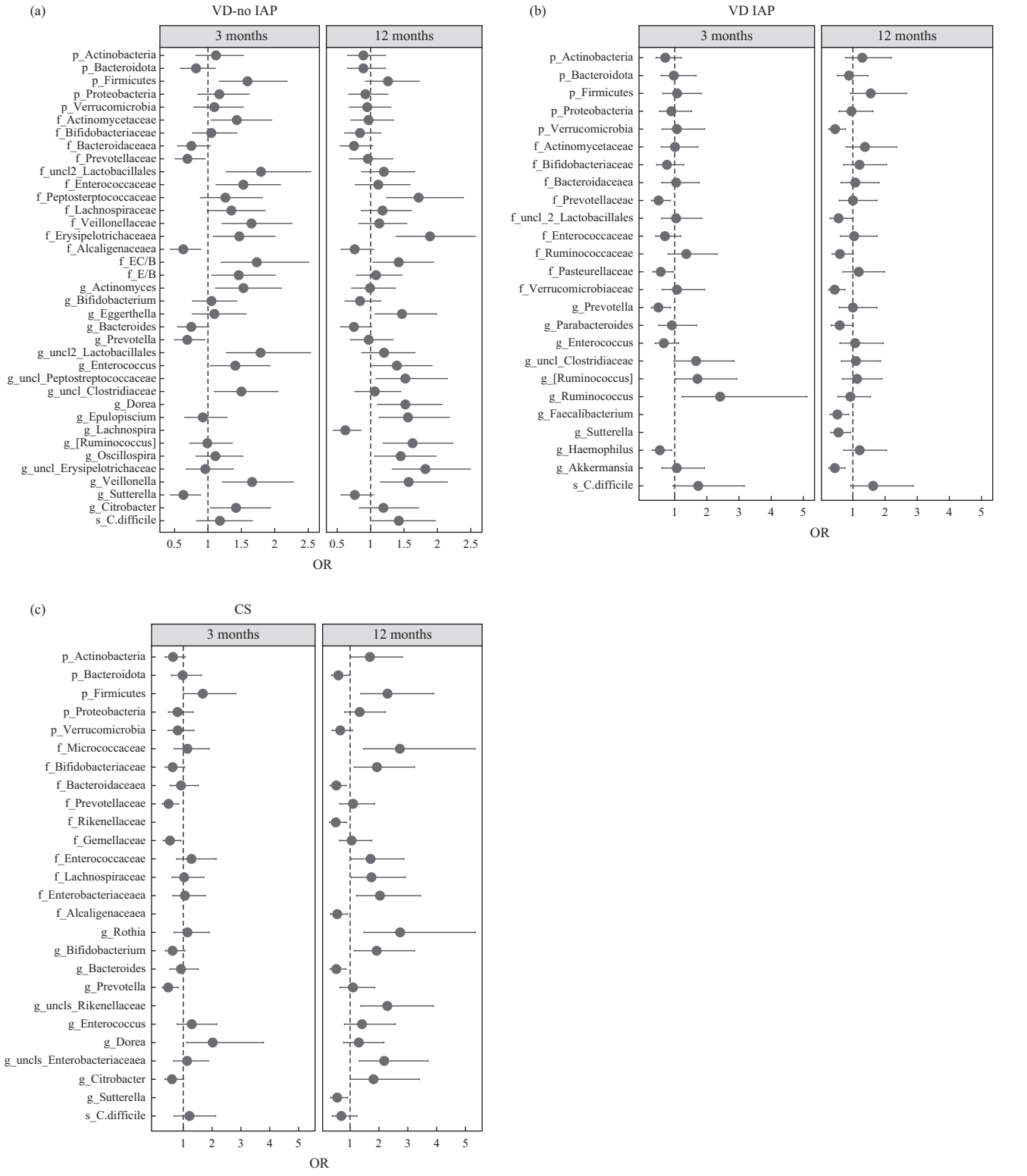

Figure 2. Adjusted likelihood ratio of key gut microbiota measure at 3 months and 12 months according to the duration of hospital stay in vaginally-delivered (VD) infants without maternal intrapartum antibiotic prophylaxis (IAP) exposure (a), with IAP exposure (b), and infants delivered by caesarean section (CS). (c). The models were adjusted for gestational age. p, phylum; f, family; g, genus; s, species; OR, odds ratio; the genus *Ruminococcus* is from the family Lachnospiraceae.

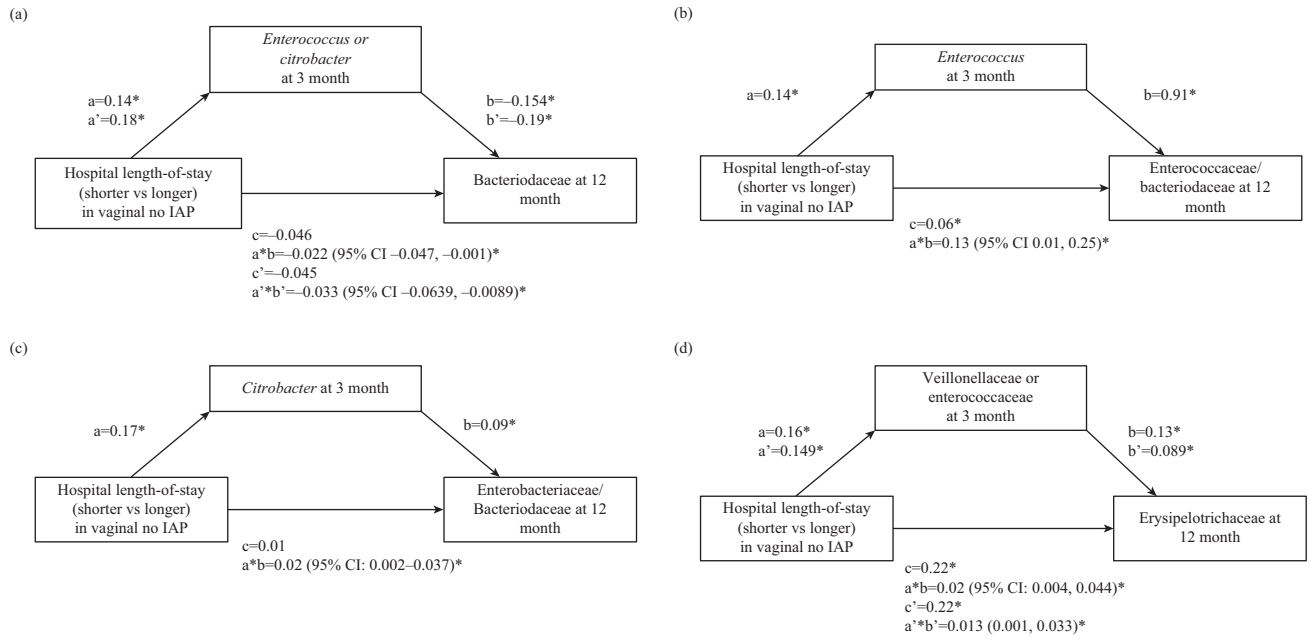

Figure 3. Hypothetical pathway for the association between hospital length of stay (LOS) and infant gut microbiota abundance at 12 months of age in vaginally-delivered infants without maternal intrapartum antibiotic exposure. a or a', association between exposure (LOS) and mediator (*Enterococcus* spp. or *Citrobacter* spp. or Veillonellaceae or Enterococcaceae at 3 months); b or b', association between mediator and outcome (Bacteroidaceae or Enterococcaceae/Bacteroidaceae ratio or Enterobacterales/Bacteroidaceae or Erysipelotrichaceae at 12 months); c or c', direct effect of LOS on bacterial abundance at 12 months after taking into account bacterial abundance at 3 months; a*b or a'*b', indirect effect of LOS on bacterial abundance at 12 months mediated through bacterial abundance at 3 months. *$P<0.05$ was considered to indicate significance.

dysbiosis in newborns. Additional study is required to confirm these results in different populations, and to assess their implications for newborn health.

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Conflict of interest statement

None declared.

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Ethical approval

Written informed consent was obtained from parents at enrolment. This study was performed under Synergy in Microbiota (SyMBIOTA), which was approved by the Human Research Ethics Board of the University of Alberta (Pro00010073).

Author contributions

SB: data curation; formal analysis; methodology; visualization; writing — original draft; writing — review and editing. HMT: conceptualization; data curation; investigation; project administration; supervision; writing — review and editing. JT: writing — review and editing. MH: writing — review and editing. PM: data curation; writing — review and editing. TJM: validation; writing — review and editing. ES: data curation; validation; writing — review and editing. ST: data curation; validation; writing — review and editing. PS: data curation; writing — review and editing. JS: data curation; writing — review and editing. AK: conceptualization; funding acquisition; investigation; project administration; supervision; writing — review and editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2024.10.012.

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