Beneficial Microbes, 2020; 11(3): 235-244

Wageningen Academic Publishers

# Effects of *Lactobacillus fermentum* CECT5716 Lc40 on infant growth and health: a randomised clinical trial in nursing women

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Received: 30 October 2019 / Accepted: 30 January 2020
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**RESEARCH ARTICLE**

## Abstract

The breast milk microbiota has been described as a source of bacteria for infant gut colonisation. We studied the effect of *Lactobacillus fermentum* CECT5716 (Lc40) on growth and infection incidence of the infants, when the probiotic is administrated to the mothers. Moreover, whether such effects might depend on the interaction between the mother or infant microbiota and the probiotic administration. A total of 291 mother-infant pairs were studied for 16 weeks in a randomised double-blinded placebo-controlled multicentre trial. The Lc40 group (n=139) received 1 capsule/day containing $3\times10^9$ cfu Lc40; the control group (n=152) received 1 placebo (maltodextrin) capsule/day. A positive and significant correlation of the *Staphylococcus* load between breast milk and infant faeces was only observed in control group. Additionally, the weight z-score of the infants whose mothers had higher values of *Lactobacillus* in their breast milk were significantly higher for the Lc40 group. We observed a significant lower incidence of conjunctivitis in the infants whose mothers received Lc40. A higher load of *Staphylococcus* in infant faeces significantly increased the risk of respiratory infections. Such incidence, under an absent or low *Staphylococcus* load in the faeces, was significantly 36 times higher in the infants in the control group than in the infants in the Lc40 group. However, the protective effect of Lc40 was gradually reduced as the *Staphylococcus* load of the milk increased. The administration of Lc40 to nursing women might influence infant growth and health but it seems to depend on its interactions with mother or infant microbiota. Registered in the US Library of Medicine (www.clinicaltrials.gov): NCT02203877.

**Keywords:** probiotic, *Lactobacillus*, microbiota, infant, breastfeeding

## 1. Introduction

The World Health Organization (WHO) recommends exclusive breastfeeding during the first six months of life (WHO, 2011), since breast milk provides the optimal nutrition for the infant early stage (Prell and Koletzko, 2016). Indeed, breast milk is an important factor in the initiation, development, and composition of the neonatal gut microbiota (Bäckhed *et al.,* 2015; Martín *et al.,* 2005; Pannaraj *et al.,* 2017). A body of evidence shows that breastfeeding confers protection against respiratory and gastrointestinal tract infections and allergic diseases in addition to reducing the risk of several chronic diseases (American Academy of Pediatrics, 2012). In particular, the breast milk of healthy women can be considered a source of safe and potentially probiotic lactic acid bacteria that

may play an important role in the prevention of neonatal infectious diseases (Martín *et al.,* 2003). Moreover, the interest to elucidate how the human microbiota regulates infant growth during the early stage of life is consistently rising (Robertson *et al.*, 2019).

In this context, breast-fed infants have been shown to have a less diverse intestinal microbiota than formula-fed infants, but exhibit a dominance of the protective gut bacteria that utilise the complex sugars in human milk and interact more with host cells (O'Sullivan *et al.,* 2015; Wopereis *et al.,* 2014). Nevertheless, the origin and biological role of milk bacteria have not yet been unveiled (Fernández *et al.,* 2013), and little is known about the vertical transfer of breast milk microbes from mother to infant (Jost *et al.,* 2014). Furthermore, breast milk seems to transfer a good source of maternal *Lactobacillus* (Martín *et al.,* 2003), as well as other species, such as *Bifidobacterium* and *Enterococcus* (Watkins *et al.,* 2017), to the infant gut. The fact that this biological fluid provides a continuous efflux of lactic acid bacteria to the infant's gut for several weeks after birth may, at least partially, play an important role in the vertical mother-to-child transmission of the first bacterial coloniser(s) (Ahrné *et al.,* 2005; Favier *et al.,* 2002; Martín *et al.,* 2003).

Moreover, probiotic supplementation for women during pregnancy and lactation might modulate breast milk microbial composition, with immune benefits being transferred to their infants (Hashemi *et al.,* 2016; Rautava *et al.,* 2012). In this sense, an improved microbial milk composition might have important consequences for the health of the new-born (Di Mauro *et al.,* 2013). The early stages of life represent a more opportunistic period of human life where the gut microbiota may be more prone to changes by interventions involving probiotics, prebiotics, or combinations of these (Milani *et al.,* 2017). In fact, the effects of probiotics on paediatric diseases include benefits against allergies, gastrointestinal infections, colic (Hashemi *et al.,* 2016) and obesity (Bergmann *et al.,* 2014). It is important to note that the health benefits conferred by probiotic bacteria are strain specific (Baldassarre *et al.,* 2016). Within the probiotic strains isolated from breast milk, *Lactobacillus fermentum* CECT5716 (Lc40) is a probiotic strain originally isolated from four-day postpartum breast human milk (Martín *et al.,* 2005), and the safety and probiotic potential of this strain were demonstrated in animal models (Lara-Villoslada *et al.,* 2009; Olivares *et al.,* 2007) and in human studies that included infants from birth to 12 months of age (Gil-Campos *et al.,* 2012; Maldonado *et al.*, 2012). A randomised clinical trial (RCT) revealed that the administration of Lc40 via formula to infants for six months led to 46%, 27%, and 30% reductions in the incidence rates of gastrointestinal infections, upper respiratory tract infections, and the total number of infections, respectively (Maldonado *et al.,* 2012). Additionally, Gil-Campos *et al.* (2012) observed a 71% reduction in the incidence rate of gastrointestinal infections after a similar intervention with this probiotic strain from birth to six months of life. Furthermore, on a previous work, we conducted an RCT in nursing women to evaluate the effect of Lc40 on the incidence of mastitis. The results showed a significant reduction in the incidence of mastitis together with a reduction in the breast milk *Staphylococcus* load (Hurtado *et al.,* 2017). Here, we perform a secondary study which is part of our previous RCT, with the aim to analysis the effects of Lc40 on growth and infections incidence of the infants, when the probiotic is administrated to the mothers. Moreover, whether such effects might depend on the interaction between the mother or infant microbiota and the probiotic administration.

## 2. Materials and methods

### Study design

A randomised double-blinded placebo-controlled multicentre trial was performed by the per-protocol approach. The inclusion and exclusion criteria, the sample size calculation and the details regarding the study design have been previously published (Hurtado *et al.,* 2017). Written informed consent was obtained from the women prior to their participation. The study was performed in accordance with the Declaration of Helsinki, and the protocol was approved by the Regional Ethics Committee of the Sistema Andaluz de Salud based in Seville (Spain) for the hospitals in the Andalusia region and by the local Ethics Committees of the rest of hospitals (ID code: P032). The trial was registered in the US Library of Medicine (www.clinicaltrials.gov) with the number NCT02203877 on 30 July 2014 (URL: https://clinicaltrials.gov/ct2/show/NCT02203877?term=NCT02203877&rank=1). The Consolidated Standards of Reporting Trials statement (CONSORT) was considered in the reporting of the study design and results, as well as in the abstract and flow diagram.

### Intervention and participants

Women were recruited and assigned to one of two experimental groups, according to a randomisation method performed by R software version 2.12.2. The Lc40 group received the probiotic strain *Lactobacillus fermentum* CECT5716 Lc40 (using a matrix composed by maltodextrin for its preservation) for 16 weeks after childbirth at a dose of $3\times10^9$ cfu/day. The control group received a placebo of maltodextrin. Lc40 was provided by Biosearch Life (Granada, Spain). The capsules for both intervention groups were prepared by Biofabri S.L. (Pontevedra, Spain). Women consumed one capsule per day; both treatments were identical in appearance and were packaged in plastic tubes labelled in plain white with a code number that referred to the manufacturing batch. The standardised conditions for capsule conservation

have been previously described (Hurtado *et al.,* 2017). Mothers' and infants' anthropometry markers, such as body weight (kg) and height (cm) were measured via standardised procedures. Mothers whose infants did not receive milk formula comprised the exclusively breast-fed group. For infants, the z-scores of weight, length, and head circumference for age were calculated based on the WHO Child Growth Standards (WHO, 2006).

At baseline, the medical history (previous engorgement or mastitis, dyslipidaemia, hypertension, diabetes, allergy antibiotics, allergy history) of the women, information related to educational level (primary/secondary school, vocational training, university), smoking habits (yes/no), previous parity, contact with animals (farm animals, domestic animals, both kind of animals, no animals), and type of birth (C-section/natural) were reported.

### Outcome measures

#### *Breast milk microbiota*

To determine the concentration of total bacteria in breast milk, samples were pre-treated. An appropriate dilution of samples in buffered peptonised water (bioMérieux SA, Marcy de Marcy l'Etoile, France) was spread in quadruplicate onto plates of plate count agar (PCA) and Wilkins-Chalgren anaerobe agar (Oxoid, Basingstoke, UK). The cultures were incubated in aerobic (PCA) and anaerobic conditions (AnaeroGen; Oxoid) at 37 °C for 48 h. After incubation, the colonies grown on the culture media were counted, and the number of viable microorganisms/ml of milk (cfu/ml) was calculated for aerobic and anaerobic species. *Staphylococcus*, *Streptococcus*, and *Lactobacillus* counts were measured by quantitative PCR following the method previously described (Maldonado-Lobón *et al.,* 2015). Furthermore, the entire procedure of the Lc40 detection is provided in the Supplementary Methods S1.

#### *Faecal infant microbiota*

Nucleic acid isolation from the faecal samples was performed following the protocol of the EZNA Stool DNA kit (Omega Bio-Tek, Norcross, GA, USA). The microbial population was analysed by quantifying six bacterial groups: *Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium* spp., *Bacteroides* spp., *Escherichia coli* and *Staphylococcus* spp. The procedure of the molecular quantification for each bacterial group is detailed in the Supplementary Methods S1.

#### *Infant disease*

The diagnosis of infectious diseases was performed by the paediatrician based on specific symptoms and standardised definitions. Gastrointestinal infection was defined as loose or watery stool at least three times per day with or without a fever or vomiting (WHO, 2017), and respiratory tract infections were defined as the presence of abundant mucus and/or cough for two or more consecutive days with or without a fever or the presence of wheezing and/or crepitant sounds with or without fever. Infantile colic was defined as continuous crying that lasted for a period of more than three hours, occurring more than three days per week and continuing for more than three weeks (Wessel *et al.,* 1954). Using a diary and 15-day questionnaires, the parents recorded information regarding the daily number of depositions, the daily amount of formula consumed, unscheduled visits to the doctor, behaviour and gastrointestinal discomfort (Gil-Campos *et al.,* 2012).

### Statistical analysis

The categorical outcomes are described as counts (%) for each treatment group. To check the homogeneity between the groups at baseline, chi-squared tests were applied. Continuous outcomes are expressed as the mean ± standard deviation (SD) or as the median (interquartile range (IR)) for variables with a no normal distribution. Normality was tested using Q-Q plots and histograms. When appropriate, data were logarithm transformed (log10) to improve normality. For continuous variables, differences in the intervention groups at baseline and at the end of the trial were assessed by applying Student's t-test for unpaired samples or the Mann-Whitney U-test (for infant length) if the variables were not normally distributed. Moreover, to assess the differences at the study within the treatment groups, Student's t-test for paired samples was applied, or Wilcoxon test was used if the variables were not normally distributed. For correlations between breast milk and infant faeces microbiota, Pearson's coefficient was assessed, or Spearman's coefficient (for *E. coli*) was assessed in cases of no normal distribution.

Furthermore, we analysed the effect of the treatment throughout the study by multivariate models adjusted for the relevant covariates of the study. Covariates selection was performed following a combination of manually introduction variables using backward and forward method to not overfitting the model, checking for possible confounders and significant interactions. The variables were excluded from the model, when it was not significant, it did not interact with any of the other included in the model. Hence, the fixed effects included in all the models were time, treatment and interactions with time, sex, mother's smoking habit, C-section, contact with animals, bacterial loads from mother (baseline and final), bacterial loads from baby and diagnosis of mastitis (measure as number of total events by the end of the study). An intra-subject random effect was included in the model to consider the intra subject variability. For the infant anthropometry measure outcomes, a linear regression model was performed.

Regarding the infant's infections (respiratory infection, intestinal infection, conjunctivitis, urine infection and colic), the total number of events from the study period was counted, and the incidence rates were obtained. For the outcome responses based on counts of events (infection incidence), generalised linear models (GLMs) were applied. The incidence rate ratios (IRRs) were computed as measures of the effect when the number of events was used as the dependent variable. To analyse the modulation of the infant infections by microbiota composition, Poisson regression models with the log link function were fitted and including the intervention groups (control vs probiotic) as interaction terms. The IRRs with 95% confidence intervals (CIs) and *P*-values are shown as the outputs of the analysis. However, a logistic regression model and the odd ratios (ORs) were computed as a measure of the effect on intestinal infections, since the maximum number of events was 1.

The tests were performed using a two-sided 5% significance level. Statistical analyses were conducted using R (www.r-project.org), version 3.4 by a blinded statistician.

## 3. Results

### Participants

Six hundred twenty-five women were recruited for the study. The women were randomised into the treatment or control groups (322 in the control group and 303 in the Lc40 group). As a per-protocol study, the 291 women-infant pairs that completed the 16-week intervention were analysed. A CONSORT flow diagram of the participants in the study is shown in Figure 1. Mother and infant characteristics at baseline are summarised in Table 1. No significant differences were detected in the baseline characteristics of the women and infants between the two groups (Table 1).

### Changes in the faecal microbiota composition of the infants

No differences were observed between the groups at baseline. The faecal microbiota of the infants during the first 16 weeks of breastfeeding showed significant increases in *Lactobacillus*, *Bifidobacterium*, *Clostridium* and *E. coli* populations in both groups and an increase in *Bacteroides* only after the probiotic intervention, with no significant differences between the groups (Table 2).

### Correlation between breast milk and infant faecal microbiota

A positive weak significant correlation was found between the *Lactobacillus* load in breast milk and the *Lactobacillus* load in faecal samples (r=0.241; *P*=0.042) (Supplementary Table S1). The *Staphylococcus* load in breast milk was positively correlated with the *Staphylococcus* (r=0.190; *P*=0.013) and *Bacteroides* (r=0.174; *P*=0.034) loads in infant faeces. Finally, a higher load of *Streptococcus* in breast milk was correlated with a lower load of *E. coli* (r=-0.190; *P*=0.024).

The correlation between the breast milk microbiota and the infant faecal microbiota was different according to the experimental groups. Thus, a positive correlation of

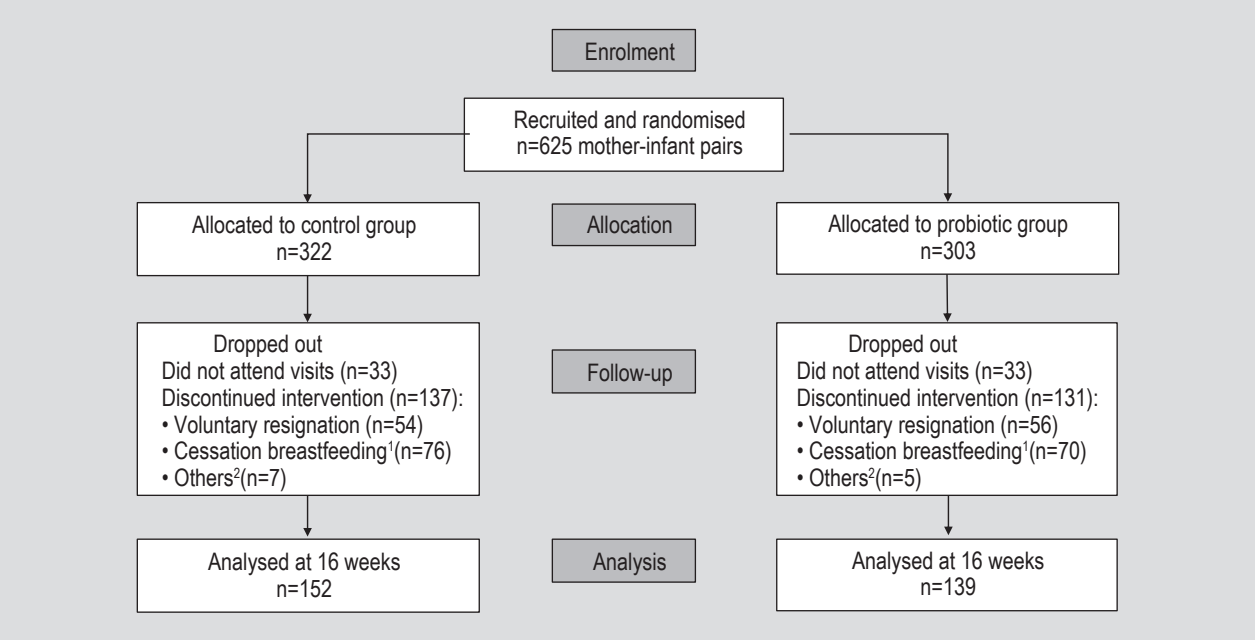


**Figure 1. CONSORT flow diagram of participants. (1) Causes of cessation breastfeeding: mother's decision, perception of insufficient milk, mastitis. (2) Other causes: gastrointestinal problems in infants, maternal rash (Hurtado *et al.*, 2017).**

**Table 1. Study population characteristics.[1]**

| | | Control (n=152) | Lc40 (n=139) | *P*-value[2] |
|---|---|---|---|---|
| Mother characteristics | Height at pre-pregnancy (m) | 1.6±0.1 | 1.6±0.1 | 0.775 |
| | Weight at pre-pregnancy (kg) | 64.9±12.4 | 65±12.6 | 0.953 |
| | BMI at pre-pregnancy (kg/m$^2$) | 24.5±4.9 | 24.4±4.3 | 0.632 |
| | Age at delivery (years) | 33±4.4 | 33±4.5 | 0.612 |
| | Gestational age (weeks) | 39.5±3.5 | 39.7±3.6 | 0.588 |
| | C-section (yes) | 42 (27.6) | 45 (32.4) | 0.331 |
| | Smoking habits (yes) | 10 (7) | 9 (6.8) | 1.000 |
| | Educational level (primary/secondary school; vocational training; university) | 29 (20.6); 36 (25.5); 76 (53.9) | 21 (16.2); 37 (28.5); 72 (55.4) | 0.645 |
| Infant characteristics | Infant sex (male; female) | 84 (55.3); 68 (44.7) | 70 (51.5); 66 (48.5) | 0.555 |
| | Infant age (days) | 2.1±1.2 | 2.2±1.4 | 0.558 |
| | Hours until first contact with the breast | 2.8±5.4 | 3.8±5.8 | 0.05 |
| | Difficulty sucking during breastfeeding (yes) | 42 (28.2) | 30 (22.2) | 0.276 |
| | Mixed lactation (yes) | 152 (40.8) | 139 (46.8) | 0.301 |
| | Length[#] (cm) | 50 (3) | 50 (3) | 0.698 |
| | Length z-score | 0.17±0.97 | 0.40±1.24 | 0.089 |
| | Weight (g) | 3335±428 | 3341±454 | 0.903 |
| | Weight z-score | 0.06±0.89 | 0.09±0.90 | 0.729 |
| | BMI (kg/m$^2$) | 13.2±1.2 | 13±1.4 | 0.452 |
| Breast milk microbiota (log 10 cfu/g) | | | | |
| Measured by quantitative PCR | | | | |
| | *Lactobacillus* | 3.1±0.4 | 3.3±0.7 | 0.061 |
| | *Staphylococcus* | 4.5±0.8 | 4.4±0.7 | 0.736 |
| | *Streptococcus* | 5.1±0.8 | 5.3±1 | 0.442 |
| Measured in quadruplicate onto plates of plate count agar | | | | |
| | Aerobes | 4±1.2 | 4.1±1 | 0.513 |
| Measured in Wilkins-Chalgren anaerobe agar | | | | |
| | Anaerobes | 4.1±1.1 | 4.2±1.1 | 0.411 |

[1] Data are expressed as the mean ± standard deviation or as the median (interquartile range) for continuous variables and counts (%) for categorical variables. BMI = body mass index; Lc40 = *Lactobacillus fermentum* CECT5716.

[2] No significant differences at baseline were observed between the experimental groups (Chi-squared test for the distribution of frequencies and T-test for unpaired samples ([#] Mann-Whitney-U test)).

**Table 2. Comparison of the infant microbiota analysed from faecal samples of the control group and the *Lactobacillus fermentum* CECT5716 (Lc40) group.[1]**

| Bacterial genera | Control | | $T_0$ vs $T_4$ *P*-value[2] | Lc40 | | $T_0$ vs $T_4$ *P*-value[2] | Control vs probiotic *P*-value[3] |
|---|---|---|---|---|---|---|---|
| | $T_0$ | $T_4$ | | $T_0$ | $T_4$ | | |
| *Lactobacillus* | 7.1±1.1 | 7.8±1 | **<0.001** | 7±1.2 | 7.8±0.9 | **<0.001** | 0.752 |
| *Staphylococcus* | 6.2±1.7 | 5.9±0.8 | 0.126 | 6±1.9 | 6.1±0.9 | 0.838 | 0.092 |
| *Clostridium* | 2.5±1.6 | 6±1.2 | **<0.001** | 2.4±1.5 | 6.1±0.9 | **<0.001** | 0.526 |
| *Bacteroides* | 6.6±2.2 | 6.8±2.1 | 0.238 | 6.2±1.8 | 6.8±2.1 | **0.006** | 0.81 |
| *Bifidobacterium* | 5.9±2.0 | 10.3±1.4 | **<0.001** | 6±2.1 | 10.1±1.7 | **<0.001** | 0.523 |
| *Escherichia coli* | 6.4 (4.1) | 10.2 (1.1) | **<0.001*** | 6.4 (4.4) | 10 (1.2) | **<0.001*** | 0.067[#] |

[1] Data are expressed in log 10 cfu/g as the mean ± standard deviation or as the median (interquartile range) for variables with a no normal distribution. Significant differences in bold (*P*<0.05).

[2] Differences between baseline and the end of the intervention within the groups were analysed by T-tests for paired samples (* Wilcoxon test).

[3] Differences between intervention groups at the end of intervention were assessed by T-tests for unpaired samples ([#] Mann-Whitney-U test).

*Staphylococcus* was observed in the control group (r=0.317; *P*=0.003) but not in the Lc40 group (r=0.112; *P*=0.300). In contrast, the correlation of the milk *Staphylococcus* content with the faecal *Bacteroides* content was significant for the Lc40 group (r=0.234; *P*=0.048) but not for the control group (r=0.069; *P*=0.555). Similarly, the negative correlation between *Streptococcus* and *E. coli* was significant in the Lc40 group (r=-0.268; *P*=0.022, Spearman test) but not in the control group (r=-0.102; *P*=0.405).

### Correlation between the bacterial groups in infant faeces

The correlations among the faecal bacterial loads are presented in Supplementary Table S2. A significant positive correlation between *Clostridium* and *Lactobacillus* was found (r=0.155; *P*=0.025). Moreover, positive correlations of *Lactobacillus* with *Bacteroides* (r=0.146; *P*=0.025) and *Bifidobacterium* (r=0.304; *P*<0.001) were detected. In contrast, we observed a negative correlation between *Bifidobacterium* and *Clostridium* (r=-0.195; *P*=0.005).

The analysis stratified by intervention group showed a positive correlation between *Lactobacillus* and *Bacteroides* (r=0.230; *P*=0.017) and *Bifidobacterium* (r=0.334; *P*<0.001) in the Lc40 group. Similarly, in the control group, a positive relationship was found between *Lactobacillus* and *Bifidobacterium* (r=0.281; *P*=0.001) and *Staphylococcus* (r=0.203; *P*=0.014). Negative correlations of *Clostridium* with *Staphylococcus* (r=-0.226; *P*=0.026) and *Bifidobacterium* (r=-0.254; *P*=0.011) were observed in the Lc40 group but not in the control group (between *Clostridium* and *Staphylococcus*: *P*=0.445, between *Clostridium* and *Bifidobacterium*: *P*=0.099).

### Infants growth

The results of the infant growth at 16 weeks of age are presented in Table 3. No differences between groups were observed in any anthropometric parameter. Concerning the effect of the breast milk microbiota and infant faecal microbiota on the growth of infants, a significant interaction effect of breast milk *Lactobacillus* load and intervention group was observed for the weight z-score: The weight z-score of infants whose mothers had higher values of *Lactobacillus* in their breast milk increased significantly in the Lc40 group (for each increasing unit of *Lactobacillus* on a log10 scale, the weight z-score increased by 0.639 (β-coefficient) for the Lc40 group; 95%CI: 0.085-1.193; *P*=0.025). However, in the absence of *Lactobacillus* or when low values were present, the weight z-scores of the control group were significantly higher (β: 2.164; 95%CI: 0.053-4.276; *P*=0.045). No significant effect was observed for length z-score in any intervention group or according to infant sex, and no other microbial species were correlated with any anthropometric parameter (data available upon request).

### Infants health

Among the infant diseases studied, we observed a lower incidence of conjunctivitis in the infants whose mothers received Lc40 (IRR: 4.993; 95%CI: 1.090-46.362; *P*=0.023) (Table 4).

### Modulation of infant infections by microbiota composition

Based on the Poisson models, a higher load of *Staphylococcus* in infant faeces increased the risk of respiratory infections (IRR: 1.330; 95%CI: 1.006-1.744; *P*=0.043). Specifically, an interaction between the faecal *Staphylococcus* load and the intervention group was found to be significant in relation to the incidence of respiratory infections. Hence, when an absent or low load of *Staphylococcus* was present in the faeces, the incidence of respiratory infections in the infants in the control group was 36 times higher than in the infants in the Lc40 group (IRR: 36.17; 95%CI: 3.262-390.863; *P*=0.003). However, the protective effect of Lc40 gradually reduced as the *Staphylococcus* load increased in the milk (IRR: 0.473; 95%CI: 0.240-0.931; *P*=0.030). The incidence of respiratory infections was similarly affected by the *Staphylococcus* load in infant faeces in the intervention group (IRR: 0.570; 95%CI: 0.387-0.845; *P*=0.005). Moreover, a trend toward a lower risk of respiratory infections was

**Table 3. Anthropometric values of the infants at 16 weeks.[1]**

| | Control | Lc40[2] | *P*-value[3] |
|---|---|---|---|
| Length (cm)[#] | 63 (3) | 64 (3) | 0.498 |
| Length z-score | 0.07±1.2 | 0.22±1.5 | 0.385 |
| Weight (kg) | 6.7±0.8 | 6.8±0.8 | 0.750 |
| Weight z-score | -0.14±1.0 | -0.06±1.0 | 0.507 |

[1] Data are expressed as the mean ± standard deviation or as the median (interquartile range).

[2] Lc40 = *Lactobacillus fermentum* CECT5716.

[3] Differences between intervention groups at the end of intervention were assessed by T-tests for unpaired samples ([#] Mann-Whitney-U test).

**Table 4. Events of infant infections per group and effect of the intervention at 16 weeks.[1,2]**

| Infant infections | | Events | IR | SE | 95%CI | Control vs Lc40 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | IRR (control/probiotic) | SE | 95%CI | *P*-value IRR |
| Respiratory infection | Control | 86 | 0.57 | 0.061 | 0.452-0.699 | 1.132 | 1.18 | 0.815-1.577 | 0.536 |
| | Lc40 | 69 | 0.500 | 0.060 | 0.389-0.633 | | | | |
| Gastrointestinal infection | Control | 5 | 0.03 | 0.015 | 0.011-0.077 | 1.513 | 2.08 | 0.294-9.744 | 0.573 |
| | Lc40 | 3 | 0.022 | 0.013 | 0.004-0.064 | | | | |
| Conjunctivitis | Control | 11 | 0.07 | 0.022 | 0.036-0.130 | 4.993 | 2.16 | 1.090-46.362 | **0.023** |
| | Lc40 | 2 | 0.014 | 0.010 | 0.002-0.052 | | | | |
| Urine infection | Control | 2 | 0.01 | 0.009 | 0.002-0.048 | 0.303 | 2.26 | 0.030-1.692 | 0.126 |
| | Lc40 | 6 | 0.043 | 0.018 | 0.016-0.095 | | | | |
| Colic | Control | 8 | 0.05 | 0.019 | 0.023-0.104 | 1.816 | 1.85 | 0.486-8.240 | 0.332 |
| | Lc40 | 4 | 0.029 | 0.014 | 0.08-0.074 | | | | |

[1] Data are expressed as number of infection events (dependent variable). The IRRs were computed as measures of the effect based on generalised linear models. Significant differences in IRR between intervention groups in bold ($P<0.05$).
[2] CI = confidence interval; IR = incidence rate; IRR = incidence rate ratio; SE = standard error; Lc40 = *Lactobacillus fermentum* CECT5716.

observed when *L. fermentum* Lc40 was detected in the breast milk (IRR: 0.68; 95%CI: 0.44-1.01; $P=0.068$). No differences in bacterial load in breast milk or infant faeces were related to conjunctivitis or urinary tract infections (data available upon request). Colic was significantly more likely to occur in infants whose mother had higher levels of anaerobes in their milk regardless of the intervention group (IRR: 1.777; 95%CI: 1.015-3.156; $P=0.044$).

Finally, according to the logistic regression, intestinal infections were significantly less likely to occur in infants with higher values of *Clostridium* (OR: 0.463; 95%CI: 0.212-0.922; $P=0.032$), regardless of the intervention group. On the other hand, infants with higher levels of *Staphylococcus* in their faeces tended to have a higher incidence of intestinal infections (OR: 2.302; 95%CI: 0.939-5.65; $P=0.065$).

## 4. Discussion

The fact that infants begin to develop their microbiota during the early months of life has led to the development of proposals regarding how to improve breast milk microbial composition, opening the possibility of modulating the breast-fed infant gut microbiota. Although it could be promising, the effect of maternal supplementation with probiotics on the infant gut microbiota, as well as on the growth and health of the infant is not well studied. Bacterial interference, in which microorganisms such as probiotics can be used to inhibit the development of pathogens (Markowiak and Śliżewska, 2017), could offer a promising alternative to treat infections. In this study, we analysed 291 breast milk and infant faecal samples collected at 16 weeks of lactation from an RCT in which Lc40 was administered to the mothers (Hurtado *et al.,* 2017). Although no differences were found at the end of the intervention between the control and Lc40 groups in relation to the composition of the infant faecal microbiota, differential correlations were detected between the mothers' and infants' microbiotas according to the intervention group. The most striking finding was the positive correlation between the *Staphylococcus* load in breast milk and infant faeces observed only in the control group. Therefore, Lc40 administration might counteract the increase in *Staphylococcus* in infant faeces promoted by the high load in breast milk. As previously published, mothers receiving Lc40 in our study showed a significantly lower load of *Staphylococcus* in their breast milk (Hurtado *et al.,* 2017). Here, it was observed that a higher *Staphylococcus* load in infant faeces is related to a higher incidence of respiratory infections, and a similar tendency was found for gastrointestinal infections. Respiratory infections are one of the most common infections in childhood and are still a prominent cause of infant death in many areas (Anders *et al.,* 2015). Interestingly, the group receiving Lc40 showed a lower risk of respiratory infections depending on the *Staphylococcus* load in the milk and infant faeces. Therefore, it seems that Lc40 treatment might counteract the negative effect of the *Staphylococcus* load on respiratory infections. This result is in agreement with the observed trend in the present study of a lower risk of respiratory infection in infants born to women presenting Lc40 in their breast milk. A previous RCT revealed that administering Lc40 via formula to infants for six months led to a 27% reduction in the incidence rate of upper respiratory tract infections (Maldonado *et al.,* 2012). Accordingly, the capability of Lc40 to reduce the presence of *Staphylococcus* in breast milk has

been demonstrated in mothers participating in the present study (Hurtado *et al.,* 2017) but also in two previous studies (Arroyo *et al.,* 2010; Maldonado-Lobón *et al.,* 2015).

Additionally, we observed a lower incidence of conjunctivitis in the infants of the mothers in the Lc40 group than in the infants of the mothers in the control group. While a viral aetiology is responsible for the majority of adult cases, bacteria are the primary cause of conjunctivitis in infants (Azari and Barney, 2013), with *Staphylococcus* and *Streptococcus* spp. being the most common causal agents (e.g. *Streprococcus pneumoniae*, *Staphylococcus aureus*) (Mantaring *et al.,* 2018; Patel *et al.,* 2007). Previously, Lc40 was shown to affect the load of both bacterial genera in breast milk (Arroyo *et al.,* 2010), which might be related to a possible interaction of the probiotic strain with the immune system (Olivares *et al.,* 2007). However, the lack of immunological response measures in the infants who participated in the current study makes it impossible to confirm this hypothesis. It is important to highlight the effect of Lc40 administration on infant growth. The weight z-score of infants whose mothers had higher values of *Lactobacillus* in their breast milk increased significantly in the Lc40 group, while in the control group, the increased *Lactobacillus* load in the breast milk had no effect. This finding offers evidence about the effects of this probiotic even when administered to mothers. Lc40 might enhance a possible effect exerted by the *Lactobacillus* strains. Recently, it has been observed that the combined supplementation with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* to the mothers led to greater weight gain and height gain in their infants at 12 months of age (Mantaring *et al.,* 2018). However, a single effect of each probiotic was not observed in the composition of the microbiota of the mothers and infants. The effects of probiotic strains on the growth of infants have been observed for some strains (Härtel *et al.,* 2017; Szajewska and Chmielewska, 2013; Vendt *et al.,* 2006). In the case of Lc40, it was shown that the direct administration of the strain to formula-fed infants might induce differences in the length of infants; the infants receiving Lc40 had slightly higher values (Gil-Campos *et al.,* 2012). It has been proposed that the activity of the bacteria on mucosal physiology might influence the absorption of nutrients, as well as metabolic and endocrine functions (Gil-Campos *et al.,* 2012; Härtel *et al.,* 2017). This activity on mucosal physiology would be favoured by the colonisation of Lc40 in the infant gut. However, although it was described that Lc40 was detected in the breast milk of 23.6% of the women in the Lc40 group and in 14.6% of the women in the control group (Hurtado *et al.,* 2017), data about Lc40 colonisation in infants were not obtained.

We are aware of some limitations in the current study. (1) Techniques such as high throughput sequencing would have allowed to obtain more information in relation to the total bacterial diversity and to determine which bacterial genera were the most abundant in both mother and infant microbiotas. (2) Assessing the Lc40 presence in the infants' faeces would have been advantageous to identify if the Lc40 is capable of colonising the nursing babies' guts as a result of its administration to the mothers. (3) As a secondary study, we acknowledge that the statistical power is lower than for the main objective of the RCT (reduction of mastitis incidence). Nevertheless, the current study offers the first findings regarding the indirect effects of *L. fermentum* CECT5716 Lc40 on infant growth and health through its administration in nursing mothers. Further studies are required to elucidate the mechanism of Lc40 through its supplementation and to continue exploring its possible benefits in the health status of nursing infants.

## 5. Conclusions

The maternal administration of *L. fermentum* CECT5716 Lc40, a probiotic strain originally isolated from breast human milk, during the first 16 weeks of lactation could condition the correlation between breast milk and infant gut microbiota. The probiotic intervention in nursing women influenced the weight z-score of infants according to the load of *Lactobacillus* in the breast milk microbiota. Moreover, Lc40 decreased the incidence of conjunctivitis in the infants. The protective effect of Lc40 against respiratory infections in infants depends on the milk and infant faeces *Staphylococcus* load. More evidence is needed to clarify the possible effects that the administration of Lc40 to nursing women could exert on infant growth and health.

## Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/BM2019.0180.

**Methods S1.** More detailed methodology.

**Table S1.** Correlations between breast milk and infant faecal microbiota for all microbial populations.

**Table S2.** Correlations between infant faecal microbiota for all microbial populations.

**CONSORT 2010 checklist** of information to include when reporting a randomised trial.

## Acknowledgements

We acknowledge the Andalusian Government and the European Regional Development Fund under Andalusia's 2007-2013 Global Innovation-Technology-Enterprise Grant. MALDI-TOF analyses were possible with the help and collaboration of Dr. Federico García and the staff of the Department of Microbiology of the San Cecilio University

Hospital, Granada, Spain. We would also like to thank Llenalia García Fernández (SEPLIN, Soluciones Estadísticas – Granada, Spain) for collaborating in the statistical analysis of the data in this study.

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## Conflict of interest

BPV has been employee of Biosearch Life. JAML, MPDR, OB, JF, MO are employees of Biosearch Life. The authors declare no other conflict of interest.

## References

Ahrné, S., Lönnermark, E., Wold, A.E., Åberg, N., Hesselmar, B., Saalman, R., Strannegård, I.L., Molin, G. and Adlerberth, I., 2005. Lactobacilli in the intestinal microbiota of Swedish infants. Microbes and Infection 7: 1256-1262. https://doi.org/10.1016/j.micinf.2005.04.011

American Academy of Pediatrics, 2012. Breastfeeding and the use of human milk. Pediatrics 129: e827-e841. https://doi.org/10.1542/peds.2011-3552

Anders, K.L., Nguyen, H.L., Nguyen, N.M., Van Thuy, N.T., Hong Van, N.T., Hieu, N.T., Hong Tham, N.T., Thanh Ha, P.T., Lien, L.B., Vinh Chau, N. Van, Ty Hang, V.T., Van Doorn, H.R. and Simmons, C.P., 2015. Epidemiology and virology of acute respiratory infections during the first year of life: a birth cohort study in Vietnam. Pediatric Infectious Disease Journal 34: 361-70. https://doi.org/10.1097/INF.0000000000000643

Arroyo, R., Martín, V., Maldonado, A., Jiménez, E., Fernández, L. and Rodríguez, J.M., 2010. Treatment of infectious mastitis during lactation: antibiotics versus oral administration of lactobacilli isolated from breast milk. Clinical Infectious Diseases 50: 1551-1558. https://doi.org/10.1086/652763

Azari, A.A. and Barney, N.P., 2013. Conjunctivitis: a systematic review of diagnosis and treatment. JAMA 310: 1721-1729. https://doi.org/10.1001/jama.2013.280318

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M.T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y.S., Kotowska, D., Colding, C., Tremaroli, V., Yin, Y., Bergman, S., Xu, X., Madsen, L., Kristiansen, K., Dahlgren, J. and Jun, W., 2015. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host and Microbe 17: 690-703. https://doi.org/10.1016/j.chom.2015.04.004

Baldassarre, M.E., Di Mauro, A., Mastromarino, P., Fanelli, M., Martinelli, D., Urbano, F., Capobianco, D. and Laforgia, N., 2016. Administration of a multi-strain probiotic product to women in the perinatal period differentially affects the breast milk cytokine profile and may have beneficial effects on neonatal gastrointestinal functional symptoms. A randomized clinical trial. Nutrients 8: 677 https://doi.org/10.3390/nu8110677

Bergmann, H., Rodríguez, J.M., Salminen, S. and Szajewska, H., 2014. Probiotics in human milk and probiotic supplementation in infant nutrition: a workshop report. British Journal of Nutrition 112: 1119-1128. https://doi.org/10.1017/S0007114514001949

Di Mauro, A., Neu, J., Riezzo, G., Raimondi, F., Martinelli, D., Francavilla, R. and Indrio, F., 2013. Gastrointestinal function development and microbiota. Italian Journal of Pediatrics 39: 15. https://doi.org/10.1186/1824-7288-39-15

Favier, C.F., Vaughan, E.E., De Vos, W.M. and Akkermans, A.D.L., 2002. Molecular monitoring of succession of bacterial communities in human neonates molecular monitoring of succession of bacterial communities in human neonates. Applied and Environmental Microbiology 68: 219-226. https://doi.org/10.1128/AEM.68.1.219

Fernández, L., Langa, S., Martín, V., Maldonado, A., Jiménez, E., Martín, R. and Rodríguez, J.M., 2013. The human milk microbiota: origin and potential roles in health and disease. Pharmacological Research 69: 1-10. https://doi.org/10.1016/j.phrs.2012.09.001

Gil-Campos, M., López, M.Á., Rodriguez-Benítez, M.V., Romero, J., Roncero, I., Linares, M.D., Maldonado, J., López-Huertas, E., Berwind, R., Ritzenthaler, K.L., Navas, V., Sierra, C., Sempere, L., Geerlings, A., Maldonado-Lobón, J.A., Valero, A.D., Lara-Villoslada, F. and Olivares, M., 2012. *Lactobacillus fermentum* CECT 5716 is safe and well tolerated in infants of 1-6 months of age: a randomized controlled trial. Pharmacological Research 65: 231-238. https://doi.org/10.1016/j.phrs.2011.11.016

Härtel, C., Pagel, J., Spiegler, J., Buma, J., Henneke, P., Zemlin, M., Viemann, D., Gille, C., Gehring, S., Frommhold, D., Rupp, J., Herting, E. and Göpel, W., 2017. *Lactobacillus acidophilus*/*Bifidobacterium infantis* probiotics are associated with increased growth of VLBWI among those exposed to antibiotics. Scientific Reports 7: 5633. https://doi.org/10.1038/s41598-017-06161-8

Hashemi, A., Villa, C.R. and Comelli, E.M., 2016. Probiotics in early life: a preventative and treatment approach. Food and Function 7: 1752-1768. https://doi.org/10.1039/c5fo01148e

Hurtado, J.A., Maldonado-Lobón, J.A., Díaz-Ropero, M.P., Flores-Rojas, K., Uberos, J., Leante, J.L., Affumicato, L., Couce, M.L., Garrido, J.M., Olivares, M., Fonollá, J. and the PROLAC Group, 2017. Oral administration to nursing women of *Lactobacillus fermentum* CECT5716 prevents lactational mastitis development: a randomized controlled trial. Breastfeeding Medicine 12: 202-209. https://doi.org/10.1089/bfm.2016.0173

Jost, T., Lacroix, C., Braegger, C.P., Rochat, F. and Chassard, C., 2014. Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. Environmental Microbiology 16: 2891-2904. https://doi.org/10.1111/1462-2920.12238

Lara-Villoslada, F., Sierra, S., Díaz-Ropero, M.P., Rodríguez, J.M., Xaus, J. and Olivares, M., 2009. Safety assessment of *Lactobacillus fermentum* CECT5716, a probiotic strain isolated from human milk. Journal of Dairy Research 76: 216-221. https://doi.org/10.1017/S0022029909004014

Maldonado, J., Cañabate, F., Sempere, L., Vela, F., Sánchez, A.R., Narbona, E., López-Huertas, E., Geerlings, A., Valero, A.D., Olivares, M. and Lara-Villoslada, F., 2012. Human milk probiotic *Lactobacillus fermentum* CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. Journal of Pediatric Gastroenterology and Nutrition 54: 55-61. https://doi.org/10.1097/MPG.0b013e3182333f18

Maldonado-Lobón, J.A., Díaz-López, M.A., Carputo, R., Duarte, P., Díaz-Ropero, M.P., Valero, A.D., Sañudo, A., Sempere, L., Ruiz-López, M.D., Bañuelos, Ó., Fonollá, J. and Olivares Martín, M., 2015. *Lactobacillus fermentum* CECT 5716 reduces *staphylococcus* load in the breastmilk of lactating mothers suffering breast pain: a randomized controlled trial. Breastfeeding Medicine 10: 425-432. https://doi.org/10.1089/bfm.2015.0070

Mantaring, J., Benyacoub, J., Destura, R., Pecquet, S., Vidal, K., Volger, S. and Guinto, V., 2018. Effect of maternal supplement beverage with and without probiotics during pregnancy and lactation on maternal and infant health: a randomized controlled trial in the Philippines. BMC Pregnancy Childbirth 18: 193. https://doi.org/10.1186/s12884-018-1828-8

Markowiak, P. and Śliżewska, K., 2017. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9: 1021. https://doi:10.3390/nu9091021

Martín, R., Langa, S., Reviriego, C., Jimínez, E., Marín, M.L., Xaus, J., Fernández, L. and Rodríguez, J.M., 2003. Human milk is a source of lactic acid bacteria for the infant gut. Journal of Pediatrics 143: 754-758. https://doi.org/10.1016/j.jpeds.2003.09.028

Martín, R., Olivares, M., Marín, M.L., Fernández, L., Xaus, J. and Rodríguez, J.M., 2005. Probiotic potential of 3 lactobacilli strains isolated from breast milk. Journal of Human Lactation 21: 8-17. https://doi.org/10.1177/0890334404272393

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., Belzer, C., Delgado Palacio, S., Arboleya Montes, S., Mancabelli, L., Lugli, G.A., Rodriguez, J.M., Bode, L., De Vos, W., Gueimonde, M., Margolles, A., Van Sinderen, D. and Ventura, M., 2017. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiology and Molecular Biology Reviews 81: e00036-17. https://doi.org/10.1128/MMBR.00036-17

O'Sullivan, A., Farver, M. and Smilowitz, J.T., 2015. The influence of early infant-feeding practices on the intestinal microbiome and body composition in infants. Nutrition and Metabolic Insights 8: NMI.S29530. https://doi.org/10.4137/NMI.S29530

Olivares, M., Díaz-Ropero, M.P., Sierra, S., Lara-Villoslada, F., Fonollá, J., Navas, M., Rodríguez, J.M. and Xaus, J., 2007. Oral intake of *Lactobacillus fermentum* CECT5716 enhances the effects of influenza vaccination. Nutrition 23: 254-260. https://doi.org/10.1016/j.nut.2007.01.004

Pannaraj, P.S., Li, F., Cerini, C., Bender, J.M., Yang, S., Rollie, A., Adisetiyo, H., Zabih, S., Lincez, P.J., Bittinger, K., Bailey, A., Bushman, F.D., Sleasman, J.W. and Aldrovandi, G.M., 2017. Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. JAMA Pediatrics 171: 647-654. https://doi.org/10.1001/jamapediatrics.2017.0378

Patel, P.B., Diaz, M.C.G., Bennett, J.E. and Attia, M.W., 2007. Clinical features of bacterial conjunctivitis in children. Academic Emergency Medicine 14: 1-5. https://doi.org/10.1197/j.aem.2006.08.006

Prell, C. and Koletzko, B., 2016. Breastfeeding and complementary feeding. Deutsches Ärzteblatt International 113: 435-444. https://doi.org/10.3238/arztebl.2016.0435

Rautava, S., Luoto, R., Salminen, S. and Isolauri, E., 2012. Microbial contact during pregnancy, intestinal colonization and human disease. Nature Reviews Gastroenterology & Hepatology 9: 565-576. https://doi.org/10.1038/nrgastro.2012.144

Robertson, R.C., Manges, A.R., Finlay, B.B. and Prendergast, A.J., 2019. The human microbiome and child growth – first 1000 days and beyond. Trends in Microbiology 27: 131-147. https://doi.org/10.1016/j.tim.2018.09.008

Szajewska, H. and Chmielewska, A., 2013. Growth of infants fed formula supplemented with *Bifidobacterium lactis* Bb12 or *Lactobacillus* GG: a systematic review of randomized controlled trials. BMC Pediatrics 13: 185. https://doi.org/10.1186/1471-2431-13-185

Vendt, N., Grünberg, H., Tuure, T., Malminiemi, O., Wuolijoki, E., Tillmann, V., Sepp, E. and Korpela, R., 2006. Growth during the first 6 months of life in infants using formula enriched with *Lactobacillus rhamnosus* GG: double-blind, randomized trial. Journal of Human Nutrition and Dietetics 19: 51-58. https://doi.org/10.1111/j.1365-277X.2006.00660.x

Watkins, C., Stanton, C., Ryan, C.A. and Ross, R.P., 2017. Microbial therapeutics designed for infant health. Frontiers in Nutrition 4: 48. https://doi.org/10.3389/fnut.2017.00048

Wessel, M.A., Cobb, J.C., Jackson, E.B., Harris, G.S. and Detwiler, A.C., 1954. Paroxysmal fussing in infancy, sometimes called colic. Pediatrics 14: 421-435.

Wopereis, H., Oozeer, R., Knipping, K., Belzer, C. and Knol, J., 2014. The first thousand days – intestinal microbiology of early life: establishing a symbiosis. Pediatric Allergy and Immunology 25: 428-438. https://doi.org/10.1111/pai.12232

World Health Organization (WHO), 2006. The WHO child growth standards. Available at: https://www.who.int/childgrowth/en/

World Health Organization (WHO), 2011. Exclusive breastfeeding for six months best for babies everywhere. Available at: https://www.who.int/mediacentre/news/statements/2011/breastfeeding_20110115/en/

World Health Organization (WHO), 2017. Diarrhoeal disease. Available at: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease