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Feeding tube practices and the colonisation of the preterm stomach in the first week of life

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ABSTRACT

INTRODUCTION. We aimed to determine if changing nasogastric feeding tubes more often would impact colonisation of the upper gastrointestinal tract of the premature infant.

METHODS. We included 22 neonates born < 32 weeks gestation within 48 hours after birth. The neonates were randomised to have their feeding tubes changed on day seven or daily during the first week of life. We determined the bacterial concentration by the culture method in maternal milk samples, gastric aspirates and feeding tube flushes. Bacteria were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). The primary outcome was the concentration of bacteria in the gastric aspirate from a freshly placed nasogastric tube on day seven of life.

RESULTS. Data from only 11 neonates were eligible for primary outcome analysis. We found no difference in bacterial concentration between the two groups with a mean colony-forming unit count per ml aspirate of 4.62 $\log_{10}$ (standard deviation (SD): ± 3.43) in the intervention group and 2.76 $\log_{10}$ (SD: ± 3.13) in the control group. Data from 19 neonates were eligible for analysis of secondary outcome measures. We found no statistically significant differences in the composition of the bacterial load between the two groups. Infants with a lower gastric pH had lower gastric bacterial counts.

CONCLUSION. Changing the feeding tube daily rather than weekly in the first week of life did not result in reduced bacterial concentration in gastric aspirates. The bacterial load from the feeding tubes was low suggesting that contamination of feeding tubes did not affect early colonisation of the upper gastrointestinal tract.

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Premature infants depend on being fed through a feeding tube and need to receive frequent meals. These feeding tubes are typically replaced as rarely as possible to avoid disturbing the infants. Studies have shown feeding tubes to be contaminated with potentially pathogenic bacteria [1] and to yield large amounts of bacteria when flushed with saline [2]. A biofilm has been shown to evolve within the first 48 hours of use [3, 4].

Early colonisation of the premature gut is influenced by factors such as antibiotic use, mode of delivery and feeding strategies [5]. Due to the immaturity of the preterm neonate's immune system and gut barrier, the neonate is vulnerable to pathogenic bacteria and dysbiosis of the gut. Particularly a lack of bacterial diversity, an

abundance of Gram-negative bacilli and an underrepresentation of obligate anaerobic bacteria have been associated with the development of necrotising enterocolitis (NEC) [6], a severe condition with a high mortality.

A quality-improvement study conducted to reduce NEC in preterm infants indicated that minimising the duration of usage of feeding tubes may potentially play an important role in reducing NEC incidence [7], but exchanging a feeding tube in an infant causes stress [8] and tubes should not be exchanged daily unless this is beneficial. We therefore wanted to explore if changing nasogastric feeding tubes more often would impact colonisation.

METHODS

Study population

We conducted a randomised controlled trial on premature infants born at a tertiary neonatal department; Rigshospitalet, Copenhagen. The inclusion criteria were gestational age (GA) < 32 weeks and parental signed informed consent within 48 hours of birth. The exclusion criteria were no use of tube feeding within 48 hours after birth, major gastrointestinal malformations or admission expected to last less than seven days. Infants participated until they were seven days old.

Power calculation and randomisation

A previous study of duodenal samples from preterm infants showed a mean of 3.81 $\log_{10}$ colony-forming units (CFU)/g Gram-negative organisms on days 8-14 with a standard deviation (SD) of 1.24 $\log_{10}$ CFU/g [9]. We needed 11 infants per group to detect a 1.5-unit reduction in $\log_{10}$ CFU/ml in the gastric aspirates of the intervention group with a power of 80% and a statistical significance level of $p = 0.05$.

Eligible infants were randomised using sealed envelopes placed in a random allocation sequence by a person unaffiliated with the project. For the control group, feeding tubes were changed on day seven, as is common practice in the department, and earlier if the tubes were displaced or clogged. For the intervention group, feeding tubes were scheduled to be changed daily, preferably at precise 24-hour intervals.

Feeding practices

A nasogastric feeding tube is placed immediately after birth and used every two to three hours. Before each meal, gastric content is aspirated for inspection and typically refed. A meal preferably consists of collected raw maternal milk, alternatively pasteurised human donor milk or infant formula, poured into an open syringe and fed to the infant by gravity. From day three, infants born at GA < 30 weeks are given daily supplementary probiotics with a meal through the feeding tubes consisting of two capsules of each 10^9 CFU *Lactobacillus rhamnosus* and 10^8 CFU *Bifidobacterium animalis,* subspecies *lactis.*

Sample collection

A daily gastric aspirate sample of no more than 1 ml was collected from both groups. The sample was collected by aspirating the gastric content into a syringe through the feeding tube. When changing the feeding tube, the sample would preferably be collected immediately through the new feeding tube. The timing of the time of day would differ, but samples were always collected a minimum of two hours after last meal and/or probiotic supplementation. From both groups, we also collected a 0.5 ml maternal milk sample on days three and seven. On day seven, the discarded feeding tube was retrieved and stored in a sealed plastic bag. All other samples were collected in cryotubes. All samples were stored at 4 °C for a maximum of 72 hours and transported to Statens Serum Institut, Copenhagen, for further analyses.

Analysis of samples, bacterial culture

We measured pH values with litmus paper and noted the colour of all gastric aspirate samples. Feeding tubes were flushed with 1 ml of saline and discarded. All types of samples were then cultured directly and by ten-fold-dilution on blood agar plates for 24 hours under aerobic conditions, on blood agar plates for 48-72 hours under anaerobic conditions and on chocolate plates for 48-72 hours under 5% CO_2 conditions. All culture media were purchased from SSI Diagnostica, Hillerød. Unique colonies were counted, replated and recultured for identification with matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF).

Outcomes

The primary outcome of this study was concentration of bacteria (excluding probiotics) in gastric aspirates from *the freshly inserted* nasogastric tube on day seven. Secondary measures were i) concentration of bacteria in all gastric aspirates, ii) description of qualitative differences between bacteria found in the gastric aspirates of intervention and control group on day seven, iii) whether viable probiotic bacteria were detectable in gastric aspirates and at which concentration and iv) description of the pH in gastric aspirates and possible changes during the first week of life.

Statistics

All data were tabulated into Microsoft Excel and analysed using R (version 3.2.3). Statistical significance was set at $p < 0.05$.

Ethics

Ethical approval was given by the Danish Ethical Council with protocol number H-15021673 as required under Danish law. Data were stored according to an agreement with the Data Protection Agency of the Capital Region of Denmark.

Trial registration: ClinicalTrials identification number NCT02830503.

RESULTS

From June 2016 to June 2018, 24 neonates were included in the study (20 individual infants plus two sets of twins). Nineteen of these infants, including the firstborn only from each set of twins, were eligible for further analysis (see **Figure 1** and **Table 1**). In 11 infants, we were able to collect gastric aspirates through clean feeding tubes on day seven to conduct our primary outcome analysis. Per infant, we collected a median of six gastric aspirates (range: 1-7 aspirates, a total of 98 aspirates) and two milk samples (range: 0-2 milk samples, a total of 34 milk samples, hereof 32 own mother's milk and two donor milk samples).

FIGURE 1 Flow diagram.

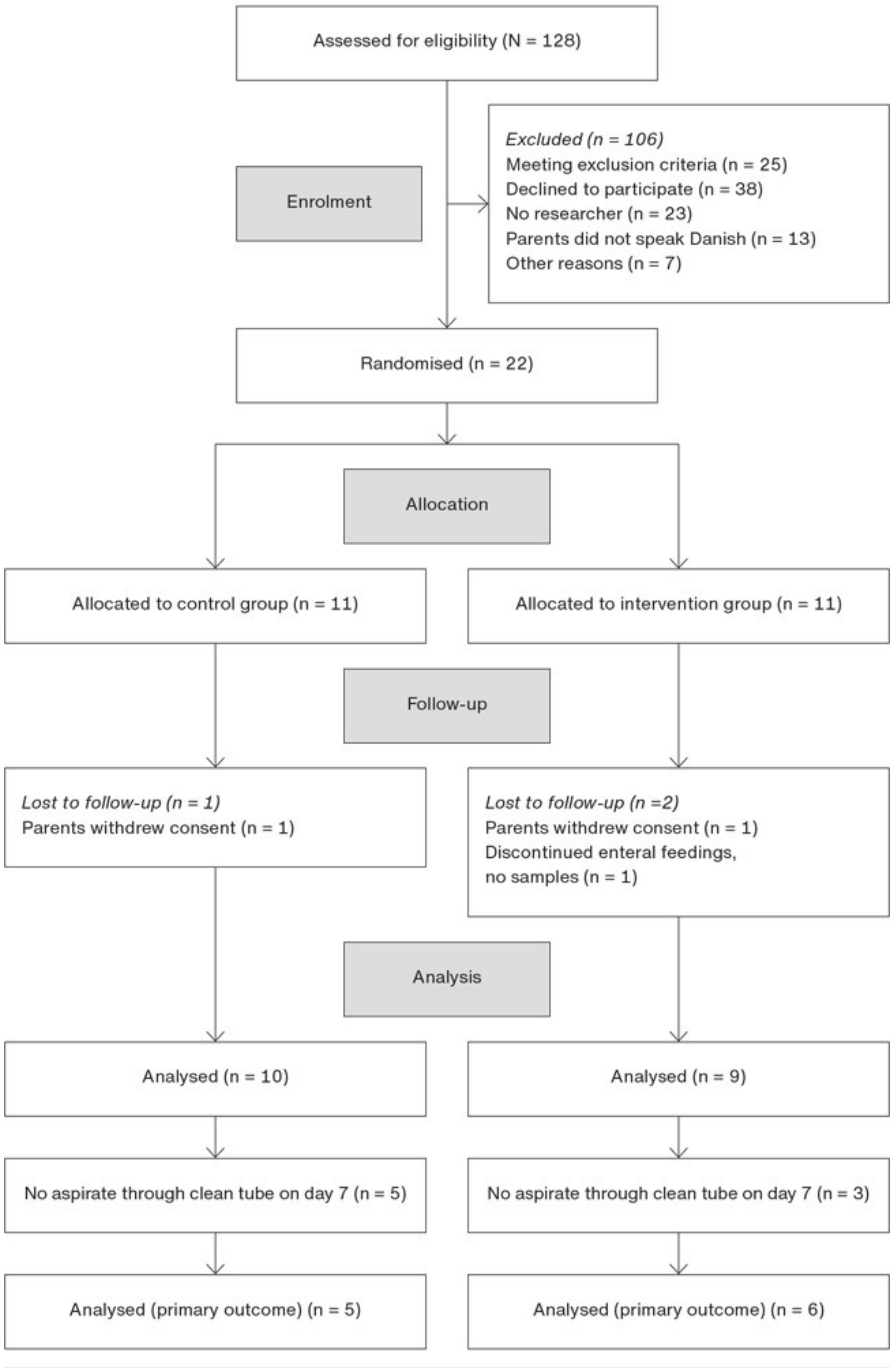

TABLE 1 Clinical data of the infants.

	Control group (N = 10)	Intervention group (N = 9)
At birth		
Weight, median (IQR), g	882 (296)	1,120 (210)
Gestational age, median (IQR), days	196 (17)	195 (7)
Apgar 5 score, median (IQR)	10 (2)	10 (1)
Gender: male, %	40	56
Caesarean section, %	50	56
Multiple birth, %	10	33
PPROM, %	30	56
Chorioamnionitis, %	10	0
Received early antibiotics[a], %	30	89
In the 1st week of life, %		
Enteral proportion of own mothers milk[b], median (IQR)[c]	72 (27)	56 (29)
Received parenteral feeding[d]	80	89
Received supplementary probiotics[d]	70	56
Received antibiotics[e]	50	100
During entire admission, %		
Verified NEC[d]	0	0
Sepsis with positive blood culture[d]	40	0

NEC = necrotizing enterocolitis; IQR = interquartile range; PPROM = preterm prelabour rupture of membranes.
a) Antibiotics on day 0 or 1 of life.
b) Daily mean % of own mother's milk.
c) $p > 0.05$ (Student's t-test).
d) $p > 0.05$ (Fisher's exact test).
e) $p = 0.03$ (Fisher's exact test).

Gastric aspirates

On day seven, gastric aspirates from the fresh feeding tubes had a mean bacterial CFU/ml of 4.62 $\log_{10}$ (SD: ± 3.43) in the intervention group and 2.76 $\log_{10}$ (SD: ± 3.13) in the control group. Hence, we found a mean difference of 1.85 $\log_{10}$ CFU/ml (95% confidence interval (CI): –2.67-6.37, $p = 0.38$, Student's t-test), not including probiotics. **Table 2** summarises the bacterial species found in all aspirates throughout the week. No differences were observed in frequencies between the two groups.

TABLE 2 Gastric aspirate isolates.

	Control group (N = 10)		Intervention group (N = 9)	
	infants, n	log_{10} CFU/ml, mean ± SD	infants, n	log_{10} CFU/ml, mean ± SD
CoNS[a]	8	4.07 ± 1.94	7	4.82 ± 2.06
Staphylococcus epidermidis	4	3.27 ± 1.32	6	3.65 ± 2.07
S. haemolyticus	7	4.75 ± 1.76	7	5.33 ± 1.88
S. hominis	0	-	1	1.84 ± 0.36
S. lugdunensis	0	-	2	4.62 ± 0.08
S. cohnii	1	1.30	0	-
Enterococcus species[a]	3	5.22 ± 2.49	3	5.44 ± 2.24
E. faecium	1	1.30	1	5.61 ± 2.46
E. faecalis	2	6.53 ± 1.19	2	4.80 ± 1.96
Probiotics[a]	5	6.42 ± 1.94	6	6.63 ± 1.09
Bifidobacterium animalis	2	8.03 ± 0.15	1	5.00
Lactobacillus rhamnosus	5	6.24 ± 1.86	6	6.50 ± 1.35
S. aureus[a]	4	5.39 ± 1.61	1	5.55 ± 0.83
Streptococcus oralis[a]	0	-	1	4.27
Corynebacterium amycolatum[a]	0	-	1	5.70
Klebsiella oxytoca[a]	0	-	1	8.54
Bacillus species[a]	3	2.00 ± 0.73	0	-
Proprionibacterium acnes[a]	0	-	1	4.30
Pseudomonas aeruginosa[a]	0	-	1	2.34
Clostridium difficile[a]	0	-	1	9.30
Gemella haemolysans[a]	0	-	1	4.48
Yeast[a]	2	5.73 ± 1.02	0	-

CFU = colony-forming units; CoNS = coagulase-negative staphylococci; SD = standard deviation.
a) $p > 0.05$ (Fisher's exact test).

For 83.3% of the 12 neonates receiving supplementary probiotics, at least one of the probiotic species could be cultured from at least one gastric aspirate. Probiotics could also be cultured from one infant (14.2%) who did not receive probiotics. The association between supplementation and positive probiotic culture was highly significant ($p = 0.006$, Fisher's exact test). Gastric aspirates that yielded *L. rhamnosus* had a mean concentration of this bacteria of 6.38 (SD: ± 1.64) log_{10} CFU/ml, and samples that yielded *B. animalis* had a mean concentration of this bacteria of 7.02 (SD: ± 1.75) log_{10} CFU/ml.

The median pH for all aspirates was four (range: 1.5-7, interquartile range: 1). We found no significant change in pH during the first week of life. Gastric pH had a significant impact on bacterial concentration: samples with pH < 4 had a mean concentration of 0.87 log_{10} CFU/ml, and samples with pH ≥ 4 had a mean concentration of 5.15 log_{10} CFU/ml ($p = 0.014$, Student's t-test). Probiotics were excluded from this analysis.

Feeding tubes

Table 3 summarises the bacterial species found in feeding tube flushes. Feeding tubes from the intervention group yielded a mean CFU/ml of 4.65 $\log_{10}$ (SD: ± 2.37) (excluding probiotics), whereas feeding tubes from the control group yielded a mean of 5.53 $\log_{10}$ (SD: ± 1.62) (p = 0.37, Student's t-test). Hence, a difference of –0.88 CFU/ml (95% CI: –2.93-1.17) was found.

TABLE 3 Feeding tube flush isolates and milk isolates.

	Control group, n (N = 10)	Intervention group, n (N = 7)	Infants, n (%)	$\log_{10}$ CFU/ml, mean ± SD
Feeding tube flush isolates				
CoNS:	10	6	-	-
Staphylococcus epidermidis	8	5	-	-
S. haemolyticus	7	4	-	-
S. capitis	1	0	-	-
Enterococcus species:	2	1	-	-
E. faecium	0	1	-	-
E. faecalis	2	0	-	-
Corynebacterium species:	2	1	-	-
C. amycolatum	1	1	-	-
C. tuberculostearum	1	0	-	-
Probiotics:	4	2	-	-
Bifidobacterium animalis	1	0	-	-
Lactobacillus rhamnosus	4	2	-	-
S. aureus	3	1	-	-
Yeast	2	0	-	-
Milk isolates[a]				
CoNS:	-	-	18 (100)	4.57 ± 1.52
S. epidermidis	-	-	18 (100)	4.45 ± 1.61
S. haemolyticus	-	-	7 (38.9)	4.28 ± 1.18
S. hominis	-	-	1 (5.6)	2.23
S. lugdunensis	-	-	5 (27.8)	3.99 ± 0.63
E. faecalis	-	-	1 (5.6)	4.54
Streptococcus anginosus	-	-	1 (5.6)	5.00
Staphylococcus aureus	-	-	2 (11.1)	3.30 ± 1.13
Bacillus species	-	-	1 (5.6)	1.30
Proprionibacterium acnes	-	-	1 (5.6)	4.00
Haemophilus parainfluenzae	-	-	1 (5.6)	2.00
Pseudomonas aeruginosa	-	-	1 (5.6)	2.09 ± 1.09
Finegoldia magna	-	-	1 (5.6)	4.00

CFU = colony-forming units; CoNS = coagulase-negative staphylococci; SD = standard deviation.
a) All infants with a minimum of 1 milk sample included (n = 18).

Milk samples

Table 3 summarises the bacterial species found in milk samples. All infants who received maternal milk were exposed to coagulase-negative staphylococci (CoNS).

Harms

We observed no severe adverse reactions such as oesophageal perforation, bleeding from the nasal or gastric mucosa or accidental extubation. For one neonate, we experienced difficulty inserting a new feeding tube, possibly due to irritation of the nasal mucosa after frequent feeding tube changes.

DISCUSSION

In this RCT, we found no statistically significant difference in bacterial growth from gastric aspirates when changing the feeding tubes of preterm infants daily instead of weekly. However, this primary outcome was available only in half of the patients, and the interindividual variability was greater than expected. Hence, the 95% CI was wide. Exclusion of the planned minimal clinically relevant difference was not achieved, and we cannot even rule out that there may be up to a 2.67 unit (approx. 500 times) more bacteria in the intervention group. We found no qualitative differences in bacteria found in aspirates; and CoNS, probiotics, *Enterococcus* species and *Staphylococcus aureus* were most common with no difference between groups.

Existing research on the upper gastrointestinal tract of preterm infants is scarce with small studies using molecular analyses. One study found gastric aspirates to be nearly sterile immediately after birth [10], and another study showed that bacteria are low in numbers and unstable in diversity with *Bacteroides* species and *Escherichia coli* as the main colonisers in the first month of life [11].

A study of 122 preterm infants cultured duodenal aspirates; they found CoNS, *Enterococcus* species, *S. aureus* and *E. coli* to be most frequent. They found that the likelihood of colonisation with Gram-negative bacteria increased with time; but already at postnatal age 4-7 days, almost 10% of the samples had Gram-negative bacteria [9]. These findings are in accordance with our results.

Since we lack knowledge about the microbiota and the colonisation process of the upper gastrointestinal tract, we know little about the conditions encountered by probiotic bacteria. We cultured large amounts of probiotics from most infants receiving supplementary probiotics, with means of 6.38 and 7.02 $\log_{10}$ CFU/ml of *L. rhamnosus* and *B. animalis*, respectively, indicating that probiotics are viable in the gastric environment.

An RCT from India was conducted to determine feeding tube contamination and possible clinical consequences. A total of 85 preterm neonates were randomised to have feeding tubes changed every 12, 24 or 48 hours for the first two weeks of life, and the study found an increase in bacterial contamination of feeding tubes for the last two groups [12]. It may be speculated that the lack of difference between the two groups in our study may be due to rapid contamination that stagnates after 24 hours of use. In the Indian study, *Enterobacteriaceae* and *Pseudomonas* were much more common. This is probably so because of differences in spontaneous colonisation between different parts of the world and may warrant care when conclusions are drawn.

The effect of gastric pH on bacterial concentration is mechanistically plausible, but this comparison was not planned.

During the present study, it became clear that we cultured fewer CFU than expected and that the difference between groups was not large. We also observed that parents preferred their infants not to be disturbed. Therefore, we decided not to prolong the study when realising that we could not achieve a satisfactory primary outcome analysis.

The question remains if inserting and removing a feeding tube at each meal may impact the colonisation beneficially. Another benefit would be that the lower oesophageal sphincter would not be held open by a resident feeding tube. Since the bacterial load in aspirates was low, and because the practice would go against the principle of disturbing the infant as little as possible, we will not proceed with further studies. It could be possible and interesting, however, to study further if bacteria growing on feeding tubes do contribute to the development of NEC or late-onset sepsis in the vulnerable preterm infant; diseases which most often occur after the first week of life.

Strengths and limitations

The most important limitation of this trial is the sample size. Unfortunately, we experienced some withdrawal of parental consent, and more neonates than expected had no gastric contents to aspirate on day seven.

We note that although the included neonates were randomised, an imbalance was recorded regarding neonates receiving early antibiotics, but, notably, more antibiotics were given to the infants in the intervention groups, which would be expected to result in a positive rather than a negative bias. We observed more positive blood cultures in the control group than in the intervention group. However, we consider this a random finding as our study was not powered to detect differences in blood stream infections.

Another important limitation is that we provide our patients born before week 30 GA with supplementary probiotics, which alters the gut microbiome [13]. The findings are therefore not necessarily generalisable to neonatal intensive care units that do not employ such practices.

CONCLUSION

Changing the nasogastric feeding tubes daily instead of weekly in a small study did not result in a reduced bacterial concentration in gastric aspirates, but numbers were smaller than planned; and even though we found a slightly higher concentration of bacteria in the aspirates of the intervention group than in the control group, we cannot exclude that the intervention may have had the opposite effect if numbers had been greater.

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Conflicts of interest Potential conflicts of interest have been declared. Disclosure forms provided by the authors are available with the article at ugeskriftet.dk/dmj

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REFERENCES

1. Mehall JR, Kite CA, Saltzman DA et al. Prospective study of the incidence and complications of bacterial contamination of enteral feeding in neonates. J Pediatr Surg. 2002;37(8):1177-82.
2. Petersen SM, Greisen G, Krogfelt KA. Nasogastric feeding tubes from a neonatal department yield high concentrations of potentially pathogenic bacteria - even 1 d after insertion. Pediatr Res. 2016;80(3):395-400.
3. Hurrell E, Kucerova E, Loughlin M et al. Neonatal enteral feeding tubes as loci for colonisation by members of the Enterobacteriaceae. BMC Infect Dis. 2009;9:146.
4. Gómez M, Moles L, Melgar A et al. Early gut colonization of preterm infants. J Pediatr Gastroenterol Nutr. 2016;62(6):893-

900.

5. Westerbeek EAM, van den Berg A, Lafeber HN et al. The intestinal bacterial colonisation in preterm infants: a review of the literature. Clin Nutr. 2006;25(3):361-8.
6. Warner BB, Deych E, Zhou Y et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. Lancet. 2016;387(10031):1928-36.
7. Patel AL, Trivedi S, Bhandari NP et al. Reducing necrotizing enterocolitis in very low birth weight infants using quality-improvement methods. J Perinatol. 2014;34(11):850-7.
8. Kristoffersen L, Skogvoll E, Hafström M. Pain reduction on insertion of a feeding tube in preterm infants: a randomized controlled trial. Pediatrics. 2011;127(6):e1449-e1454.
9. Hoy CM, Wood CM, Hawkey PM et al. Duodenal microflora in very low birth weight neonates and relation to necrotizing enterocolitis. J Clin Microbiol. 2000;68(12):4539-47.
10. Bajorek S, Parker L, Li N et al. Initial microbial community of the neonatal stomach immediately after birth. Gut Microbes. 2019;10(3):289-97.
11. Patel K, Konduru K, Patra AK et al. Trends and determinants of gastric bacterial colonization of preterm neonates in a NICU setting. PLoS One. 2015;10(7):e0114664.
12. Chauhan H, Morbiwala S, Thaddanee R et al. Bacterial contamination of nasogastric feeding tube and development of neonatal sepsis in premature newborns: a prospective observational research at a tertiary care center in Gujarat, India. Int J Contemp Pediatr. 2018;5(4):1462.
13. Plummer EL, Bulach DM, Murray GL et al. Gut microbiota of preterm infants supplemented with probiotics: sub-study of the ProPrems trial. BMC Microbiol. 2018;18(1):184.