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Differences in intestinal microbiota of infants between vaginal delivery and C-section, in particular alpha-toxigenic and enterotoxigenic *Clostridium perfringens*

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【Purpose】 Establishment of early-life gut microbiota may influence the maturation of immune system and predisposition to several diseases in later years. Mode of delivery and feeding may influence the acquisition of neonatal microbiota. Herein, we examined the intestinal microbiota of six-month old infants, with a particular focus on α-toxigenic and enterotoxigenic *C. perfringens*, since toxigenic *C. perfringens* is a widespread opportunistic pathogens linked with numerous diseases but its intestinal carriage in infants remains underexplored.

【Methods】 The study included healthy infants (*n* 124; M 70; F 54; Age: 161-178 days) enrolled at Gonohashi Obstetrics and Gynecology Hospital, Tokyo. Fecal samples (≈1.0 g) were collected into fecal collection tube containing RNA*later* and an empty tube, and were stored at 4°C until nucleic acid extraction. *C. perfringens* was quantified by qPCR targeting α-toxin and enterotoxin genes. *Clostridium difficile*, and Bifidobacterial subgroups and species were enumerated by qPCR targeting 16S rRNA genes. Other bacterial groups, subgroups and genera were quantified by RT-qPCR.

【Results】 Alpha-toxigenic and enterotoxigenic *C. perfringens* were detected in 36% and 10% infants, respectively, with counts ranging from 10^3-10^8 cells/g feces. Colonization rate of toxigenic *C. perfringens* was higher in cesarean-born infants, as compared to vaginally-born infants. In contrast, the carriage of *Bacteroides fragilis* group was significantly lower in cesarean group. Enterotoxigenic *C. perfringens* was detected in 10% infants but remained undetected in exclusively breast-fed infants. Carriage of *C. perfringens* and *C. difficile* was lower in breast-fed infants, as compared to formula-fed or mix-fed infants. In cesarean group, the carriage of toxigenic *C. perfringens* was lower in breast-fed infants as compared to formula-fed infants. In vaginally-born infants, the counts of *Clostridium coccoides* group, *Prevotella* gen., *Enterococcus* gen. and enterotoxigenic *C. perfringens* were significantly lower in breast-fed group, as compared to mix-fed or formula-fed groups.

【Conclusions】 These results indicate that healthy infants may also carry toxigenic *C. perfringens* at significant levels. Carriage of toxigenic *C. perfringens* is higher in cesarean-born and formula-fed infants, as compared with vaginally-born and breast-fed infants. High carriage of toxigenic *C. perfringens* in healthy infants warrants further investigation on its potential sources and clinical significance.