INTRODUCTION

Clostridium difficile is an intestinal pathogen typically associated with dysbalanced gut microbiota.1

More specifically, it has been observed that interactions between C. difficile and microbiota are bidirectional.1,2 (Fig. 1) C. difficile vegetative cells or conditioned media influenced the diversity and composition of fecal microbiota. Changes in microbiota composition were specific and similar to those observed in patients with C. difficile infection (CDI), suggesting that dysbiosis initially caused by e.g. antibiotics and predisposing to CDI, is to some extent maintained by C. difficile during and after the infection.

In the case of microbiota effects on C. difficile we have shown that growth is strain dependent, while all strains showed higher sporulation frequency in the co-culture with dysbalanced fecal microbiota.4,5

Adult dysbalanced microbiota showed different changes as adult healthy microbiota in previous experiments. The aim of our study presented here was to compare the impact of C. difficile vegetative cells and C. difficile conditioned medium on gut microbiota of infants under 2 years of age in vitro model.

Fig. 1: Possible interactions between gut microbiota and C. difficile.

MATERIALS AND METHODS

Fecal emulation was prepared by pooling C. difficile negative fecal samples of four healthy infants (< 2 years). Six C. difficile toxigenic strains, belonging to ribotypes 027 (n=2), 176 (n=2) and 078 (n=2), were selected to preparespent media for subsequent fecal microbiota culturing. Simultaneously, fecal microbiota was cultured with C. difficile vegetative cells (= 2 x 10^6 CFU/ml). Samples were taken after 72 hours incubation period and screened for total cell count and spore count of C. difficile in co-cultures. After centrifugation pellets were used for total bacterial DNA extraction. Bacterial community composition was determined by pair-end sequencing on Illumina MiSeq platform, targeting V3-V4 hypervariable region of the 16S rRNA gene. MiSeq output data was analyzed with statistical tool included in the mother software (version 1.38.1).

RESULTS

Five out of six tested strains grew significantly better in growth medium only than in co-cultures with infant microbiota (Fig. 2a). All six strains formed higher percentage of spores in co-culture with infant microbiota (20 - 57%), while in control cultures spore percentage was lower than 8% (Fig. 2b).

Cultivation of infant fecal microbiota in the presence of vegetative cells or in the presence of conditioned medium decreased the bacterial diversity (Fig. 3) and significant differences were observed for genera within Bacteroidetes, Firmicutes and Proteobacteria phyla (Fig. 4).

CONCLUSIONS

The results indicate that C. difficile is able to affect the gut microbiota changes. Cultures similar to those observed in children infected with C. difficile 1 in addition, the high amount of C. difficile CFUs present in co-cultures with microbiota could explain the high rate of asymptomatic carriage observed in infants.1,6

REFERENCES


