Mother-to-infant transmission of the carcinogenic colibactin-producing bacteria

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Abstract

While colibactin-producing *Escherichia coli* is linked to colorectal oncogenesis, its infection route remains poorly characterized. Here, analysis of fecal samples of infants over the first month of birth for the presence of a colibactin biosynthetic gene revealed that the bacterium may be transmitted from mother to infant through intimate contacts, such as natural childbirth and breastfeeding. This finding suggests the possibility of developing early preventive measures against colorectal cancer.

Background

Colorectal cancer (CRC) is the third most common form of cancer and the second most common cause of cancer mortality in the world\(^1\). It is predicted that by 2030 approximately 2.2 and 1.1 million people will develop CRC and succumb to it\(^2\). To reduce the number of CRC incidences and mortalities, it is vital to identify and mitigate the source of risk factors that contribute to the onset of CRC. Certain strains of *Escherichia coli* that harbor the gene cluster *clb* (also referred to as *pks*) responsible for the biosynthesis of the genotoxin colibactin have been linked to colorectal oncogenesis\(^3\)–\(^10\). Recently, we created fluorescent probes\(^11\) that are turned on specifically by ClbP, a peptidase required to activate the prodrug-like colibactin precursor\(^12,13\). The probe allowed high-throughput screening of *E. coli* isolates from clinical samples, which led to the isolation of the high-colibactin producer *E. coli*-50 that will be useful in studying the properties of colibactin-positive (*clb*) *E. coli* and colibactin\(^11\). While we continue to elucidate the molecular mechanism of oncogenesis by *clb*+ *E. coli* and colibactin, there is still a limited understanding on the infection route of the *E. coli* to the affected individuals. Since identification of the routes of infection would help develop measures to mitigate or prevent the infection, we initiated a screening effort to examine the prevalence of *clb*+ *E. coli* among healthy individuals by analyzing their fecal samples\(^14\).

Furthermore, we extended our screening to include newborns, as it is known that the newborn gut microbiota starts to form upon exposure to the vaginal and maternal skin microbiomes after birth\(^15\). Here we report that the colibactin-producing *E. coli* can be rapidly transmitted from mother to child after birth, suggesting that a respectable number of healthy individuals may become predisposed to high risk of CRC at the very early stage of life.

In a related study, we surveyed 223 healthy adults from the Tokyo metropolitan area in Japan for the presence of *clb*+ *E. coli* in their fecal samples. We found that 60 participants or 26.9% of the participants were positive \(^14\). We extended the study further to investigate the timing at which individuals become infected with *clb*+ *E. coli*. For the current study, we examined 51 infants (25 male, 26 female). From the subjects, one set of feces was collected at birth to a few days after birth, and another set was collected one month after birth. The samples were examined by PCR to detect the presence of *clbB*, the gene for one of the PKS–NRPS hybrid megasynthetases encoded in the colibactin biosynthetic gene cluster. We found that 8 out of 51 newborns or 15.7% of the test samples harbored *clb*+ *E. coli* immediately after birth (lanes 7, 10, 17, 22, 25, 34, 45 and 47 in Fig. 1a, and Fig. 2). On the other hand, 16 of the 51 (31.4%) tested positive for *clb*+ *E. coli* one month after birth (lanes 7, 10, 17, 18, 22, 25, 26, 27, 28, 29, 31, 34, 35,
45, 47 and 49 in Fig. 1b, and Fig. 2), indicating that 8 newborns or 15.7% acquired the clb+ E. coli strain during their first month. We are currently investigating the continued clb+ E. coli infections that occur among healthy individuals.

**Results And Discussion**

The clb-positive rate increased from 15.7–31.4% by the end of the first month, reaching to the equivalent level of 26.9% observed among healthy adults examined recently\(^{14}\). Once infected, it is expected that clb+ E. coli remains within the system of the infected individual persistently. Thus, the adult-like clb-positive rate we found among the one-month-old newborns in this study suggests that healthy individuals become infected with clb+ E. coli very early in their life stage in Japan. These results also indicate that the infants are getting exposed to the source of clb+ E. coli under the ordinary living condition during its first month of life.

Perinatal transmission can happen *in utero*, in the birth canal or through breastfeeding. Because intrauterine transmission of E. coli in healthy pregnancies is considered to be infrequent\(^{16}\), we next investigated the correlation between the method of delivery and the clb-positive ratio among the newborns we studied. Because we expected that the delivery method would only affect the clb-positive ratio among newborns to a few-days-old babies, we did not employ the data collected one month after birth. Regarding the birth canal transmission, clb+ E. coli was detected in seven out of eight or 87.5% of the infants that were born through natural delivery (Table 1). In contrast, only one of eight or 12.5% of the infants that were delivered by Cesarean section was clb-positive (Table 1). Those results indicated that a higher clb-positive ratio was observed among infants that were born through natural childbirth, similar to how the chance of infants acquiring the vaginal flora bacteria, including E. coli, increases by passing through the birth canal. As to the breastfeeding-mediated transmission, we examined the correlation between the infant feeding mode and the clb-positive ratio. We screened the 43 infants who were determined to be clb-negative at birth to a few days after birth. After one month, eight of the 43 become clb-positive, while the remaining 35 (81.4%) were not affected as determined by the PCR analysis of their fecal samples (Table 2). In total, there were 26 and 17 infants who were fed breastmilk alone and a mixture of formula and breastmilk, respectively. Among the eight clb-positive infants, seven were breastfed strictly over the one-month period, whereas only one newborn was fed a mixture of formula and breastmilk over the month. While 26.9% of those given breastmilk alone became clb-positive, only 5.9% of the infants given a mixed feed became clb-positive (Table 2). Lastly, we did not observe any difference in the clb+ E. coli infection ratio between the sexes of the infants either at birth or one month after birth in this study.
Table 1
The correlation between the frequency of infection with clb+ *E. coli* among the infants examined and the delivery methods used for the infants.

| Delivery method      | clb+ | clb− | Total | OR (95% CI) |
|----------------------|------|------|-------|-------------|
| Natural delivery     | 7    | 35   | 42    | 1.6 (0.17 to 14.90) |
| Cesarean section     | 1    | 8    | 9     | 1           |

OR: odds ratio, CI: confidence interval

Table 2
The correlation among the frequency of diagnosis for clb+ vs. clb− and provision of one month of mixed feeding vs. breastfeeding alone among the newborns who tested negative at birth or two to three days after birth.

| Feeding                                    | clb+ | clb− | Total | OR (95% CI) |
|--------------------------------------------|------|------|-------|-------------|
| Breastfeeding alone                        | 7    | 19   | 26    | 5.89 (0.65 to 53.11) |
| Mixed feeding with breastmilk and formula  | 1    | 16   | 17    | 1           |

OR: odds ratio, CI: confidence interval

Conclusions

In summary, the infection ratio of clb+ *E. coli* is 31.4% among the one-month-old infants studied, which is similar to the frequency found among healthy adults\(^\text{14}\). It was also reported that 130 Swedish infants followed from birth to 18 months of age were determined to be 33% clb-positive, very similar to our findings of 31.4%, when their feces were analyzed using a similar PCR method\(^\text{17}\). Our analysis identified that infants born by natural delivery had a higher incidence of being clb-positive than those born by Cesarean section. Similarly, those who were breastfed strictly showed a higher clb-positive frequency than those given a mixed feed. The fact that the mixed feed also contained breastmilk suggests that breastmilk itself was not the source of clb+ *E. coli*. In addition, screening of 58 food materials including tap water also failed to identify clb+ *E. coli* except in a sample of cattle stomach (Table 3). However, the strain found in the sample of cattle stomach belonged to the phylogroup B1, whereas all of the clb+ *E. coli* strains isolated from human subjects thus far belonged to B2 (manuscript in preparation, Y.Y., Y.T., M.S., Y.I., N.M., M.Mutoh., H.I., H.S., K.Wakabayashi and K.Watanabe). Thus, exposure of infants to clb+ *E. coli* was likely not through the food their caretakers consumed. Rather, the most likely route of infection
of the potentially oncogenic \textit{clb} + \textit{E. coli} strain appears to be through direct skin-to-skin contact, or skin-to-mouth contact to be more specific, between the mother and her infant. The same can be said about the method of delivery, where infants born by natural birth would have a substantially higher frequency of direct skin-to-skin contact with its mother than those born by Cesarean section. Taken together, similar to how the gut microbiota is transmitted from mother to infant\textsuperscript{18}, our results strongly imply that \textit{clb} + \textit{E. coli} might be transmitted from mother to newborn from the very early stage of life of the newborn through intimate contacts with the mother. Therefore, by implementing measures that can reduce the transmission of \textit{clb} + \textit{E. coli} from adults to infants, we may be able to lower the incidence of CRC in our population. To this end, we are currently analyzing the fecal samples from the mothers of the infants to fully understand the rates and modes of \textit{clb} + \textit{E. coli} transmission from mothers and her infants.
Table 3
Screening of 58 food materials commonly consumed in Japan for \textit{clb}+ \textit{E. coli} by PCR analysis.

| Vegetable       | PCR | Source          | PCR | Source          | PCR | Source          |
|-----------------|-----|-----------------|-----|-----------------|-----|-----------------|
| bran pickles    | –   | broccoli        | –   | brown beech     | –   | mushroom       |
| burdock root    | –   | cabbage         | –   | celery          | –   |                 |
| cherry tomato   | –   | Chinese cabbage | –   | cloud ear       | –   | mushroom       |
| cucumber        | –   | dried           | –   | enoki           | –   | mushroom       |
| eryngii mushroom| –   | ginger          | –   | Japanese        | –   | ginger         |
| Japanese parsley| –   | Japanese radish | –   | kimchi          | –   |                 |
| Korean lettuce  | –   | lettuce         | –   | lotus root      | –   |                 |
| maitake mushroom| –   | mesclun greens  | –   | olive oil       | –   |                 |
| onion           | –   | parsley         | –   | pea sprouts     | –   |                 |
| pickled Chinese cabbage | – | potherb mustard | – | red leaf lettuce | – |                 |
| salted plum     | –   | shiitake mushroom| – | soybean sprouts | –   |                 |
| spinach         | –   | spring onion    | –   | tomato          | –   |                 |
| white radish sprouts | – |                 |     |                 |     |                 |

| Meat            | PCR | Source          | PCR | Source          | PCR | Source          |
|-----------------|-----|-----------------|-----|-----------------|-----|-----------------|
| beef            | –   | beef / ground meat | –   | cattle / large intestine | –   |                 |
| cattle / liver  | –   | cattle / small intestine | –   | cattle / stomach | +   |                 |
| chicken / ground meat | – | chicken / liver | – | pork / ground meat | –   |                 |

| Seafood         | PCR | Source          | PCR | Source          | PCR | Source          |
|-----------------|-----|-----------------|-----|-----------------|-----|-----------------|
| clam            | –   | crab            | –   | freshwater clam | –   |                 |
| salmon          | –   | shrimp          | –   | tuna            | –   |                 |

| Dairy products  | PCR | Source          | PCR | Source          | PCR | Source          |
|-----------------|-----|-----------------|-----|-----------------|-----|-----------------|
Methods

Participants and sample collection. The subjects were healthy 51 infants from the Tokyo metropolitan area in Japan. Fecal samples were collected at birth to two to three days after birth (at the time of discharge within a few days after birth) and one month after birth (at the time of the one-month checkup). The collected feces were immediately placed in a sealed container and stored in a –20°C freezer until DNA extraction was performed.

DNA extraction from fecal samples. DNA was extracted from the frozen fecal samples with the bead beating method using a GNOME DNA Isolation Kit (MP Biomedicals). DNA quality was assessed with an Agilent 4200 TapeStation (Agilent Technologies). After the final DNA precipitation step, the DNA samples were resuspended in TE buffer and stored at −80 °C before the PCR analysis.

DNA extraction from food materials. Each of the food materials (10–50 g) obtained from grocery stores in Shizuoka, Japan, was added to 20 mL of EC medium (20 g peptone, 5 g lactose, 1.5 g bile salt, 4 g K₂HPO₄, 1.5 g KH₂PO₄ in 1 L H₂O) in a sterilized bag. The mixture was incubated at 44.5 °C for 24 h. The mixture was filtered, and the filtrate was centrifuged at 10,000 g for 6 min at 4 °C. The collected precipitate was suspended in 1 mL of sterile Milli-Q water. The suspension was plated on a MacConkey agar medium and incubated at 37 °C for 16 h. The grown bacteria were cultured and its genomic DNA was extracted by using a DNA isolation kit (QIAGEN). The isolated DNA was resuspended in TE buffer and stored at −80 °C before the PCR analysis.

Confirmation of the presence of the clb gene cluster by PCR. The extracted fecal DNA was subjected to PCR (SapphireAmp Fast PCR Master Mix, Takara) and qualitatively analyzed for clbB (a 9.6-kilobase gene encoding one of the colibactin biosynthetic enzymes) contained in the DNA extract by using a primer set of clbB-F: 5’-TGTTCGTTTTGTGTTTACGCC-3’ and clbB-R: 5’-GTGCGCTGACCATTGAAGATTTCCG-3’. The correlation was analyzed by comparing the presence or absence of the clbB gene with the subject’s birth method, diet content and sex.
Statistical analysis. Each experiment was performed at least three independent times. Representation of data as dot-plots and bar-and-whisker graphs is described in figure legends. The t test for determining the statistically difference between the expected and observed frequencies was calculated using JMP (SAS Institute Inc.) and the NORM.DIST function in Microsoft Excel version 16.16.25.

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Contributions

Y.T., M.S., H.M., Y.Y., N.M., K.Wakabayashi and K.Watanabe conceived and designed the study. J.K., K.H., N.S. and E.S. collected the fecal samples. Y.T. and K.Watanabe designed and performed PCR analysis. Y.I., M.Mutoh, H.I., H.S. and M.Miyachi performed the statistical analysis. All authors analyzed and discussed the results. K.Wakabayashi and K.Watanabe prepared the manuscript.

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Ethics declarations
Ethics approval and consent to participate

A written letter of consent was prepared after obtaining informed consent from each of the parent to participate in this study. The study protocol was approved by the Ethical Committee of the National Institutes of Biomedical Innovation, Health and Nutrition, Japan (Approval No. 199-03).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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