Impact of Long-Term Low Dose Antibiotic Prophylaxis on Gut Microbiota in Children

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Abbreviations and Acronyms

CAP = continuous antibiotic prophylaxis

fUTI = febrile urinary tract infection

RIVUR = Randomized Intervention for Children with VesicoUreteral Reflux

TMP-SMX = trimethoprim-sulfamethoxazole

UTI = urinary tract infection

VUR = vesicoureteral reflux

Accepted for publication June 13, 2020.

Supported by JSPS KAKENHI Grant JP18K16750.

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee where the studies were conducted (Kansai Medical University and Nakano Children's Hospital), and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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Purpose: We evaluated the effect of long-term low dose antibiotic prophylaxis on children's gut microbiota.

Materials and Methods: We conducted 16S ribosomal RNA gene sequencing using stool samples from 35 patients younger than 3 years old (median age 5.2 months; male-to-female ratio 17:18) who underwent antibiotic treatment during the acute phase of febrile urinary tract infection. Samples were collected at 5 time points, ie before, during and at 1 to 2, 3 to 4, and 5 to 6 months after febrile urinary tract infection onset and antibiotic treatment. Continuous antibiotic prophylaxis using trimethoprim-sulfamethoxazole was initiated in 23 patients with grade III or higher vesicoureteral reflux and was not administered in 12 patients without reflux.

Results: Within 2 weeks after initiation of treatment for febrile urinary tract infection almost all enteric bacteria belonged to the order Lactobacillales, and gut microbiota diversity decreased compared to the pretreatment level (average Shannon index 2.9 before treatment, 1.4 during treatment). The diversity recovered within 1 to 2 months after febrile urinary tract infection onset in both groups. Diversity was maintained during the study period in both groups ($p=0.43$). A smaller proportion of gut microbiota component belonged to the order Enterobacteriales ($p=0.002$) in the antibiotic prophylaxis group.

Conclusions: Our results revealed that patients receiving continuous antibiotic prophylaxis had normal gut microbiota diversity, indicating that the effect of trimethoprim-sulfamethoxazole on gut microbiota was insignificant. Furthermore, prophylaxis with trimethoprim-sulfamethoxazole might selectively suppress the growth of bacteria belonging to the order Enterobacteriales, such as Escherichia coli and Klebsiella species, which are the main causative bacteria of febrile urinary tract infections.

Key Words: gastrointestinal microbiome, dysbiosis, antibiotic prophylaxis, urinary tract infections, vesico-ureteral reflux

Owing to the emergence of genome sequencing technology, human gut microbiota, which consists of more than 1,000 different species and 100 billion microorganisms,[1] has now been widely recognized as an important organ. Emerging evidence shows that the gut microbiota has a large impact on human health and diseases.[2] Gut microbiota dysbiosis is related to increased risk of gastrointestinal diseases,[3,4] allergic diseases,[5] diabetes,[6] obesity[7] and autism spectrum disorder.[8] There are several factors affecting the development of pediatric gut microbiota, including antibiotic treatment,[9,10] delivery mode and

0022-5347/20/2046-1320/0
THE JOURNAL OF UROLOGY®

https://doi.org/10.1097/JU.0000000000001227
Vol. 204, 1320-1325, December 2020
Printed in U.S.A.

feeding type,[9,11–13] gestational age[14] and regional differences.[15] Among these factors antibiotic use during childhood is reported to be associated with an increased risk of health problems.[4,16,17]

Febrile urinary tract infection during childhood is a common bacterial illness requiring antibiotic treatment, with an incidence of 10% in girls and 3% in boys. As vesicoureteral reflux is a risk factor for recurrent UTIs, which lead to renal scarring and can cause reflux nephropathy and chronic renal failure,[18] children diagnosed with vesicoureteral reflux after a fUTI are commonly treated with continuous antibiotic prophylaxis to reduce the risk of fUTI recurrence. The RIVUR (Randomized Intervention for Children with VesicoUreteral Reflux) trial indicated that although the rate of renal scarring remains the same, the incidence of recurrent fUTI is lower in children with reflux treated with trimethoprim-sulfamethoxazole CAP vs placebo.[19,20]

Long-term low dose antibiotic treatment with TMP-SMX may lead to gut microbiota dysbiosis, although this has not been experimentally tested. Therefore, we investigated the long-term effects of TMP-SMX CAP on infant gut microbiota.

MATERIALS AND METHODS

Participants and Sample Collection

The study included 35 children (17 males) younger than age 3 years (median 5.2 months) diagnosed with and treated for fUTI in the pediatric department of Kansai Medical University Hospital or Nakano Children's Hospital, from whom we collected stool samples between November 2016 and March 2019. The patients underwent antibiotic treatment (ceftriaxone intravenous injection and cefditoren pivoxil oral administration) during the acute phase of fUTI for 14 days. Patients administered antibiotics before admission to either hospital were not included. After the initial treatment all patients underwent voiding cystourethrography and then were divided into 2 groups, ie CAP and nonCAP. Unified dose of TMP-SMX continuous antibiotic prophylaxis (0.2 gm per day) was initiated in cases with grade III or higher VUR (CAP group) but not in cases with grade II or lower VUR (nonCAP group). Drug adherence of more than 90% was confirmed by interviewing the guardians during their monthly hospital visit. No patient in either group experienced fUTI recurrence during the study period.

Stool samples were collected at 5 possible time points, ie before the initial treatment, during the initial treatment, and at 1 to 2, 3 to 4, and 5 to 6 months after fUTI onset. Approximately 0.5 gm stool samples were collected directly from paper diapers using a small sterilized spoon provided with the specimen container and stored at −80C for further analysis.

16S Ribosomal RNA Gene Sequencing

DNA was extracted from stool samples using a NucleoSpin® DNA Stool Kit. Seven hypervariable regions excluding v1 and v5 of the 16S rRNA region were amplified using a 16S™ Metagenomics Kit. After purification library construction was performed using an Ion Plus Fragment Library Kit (Thermo Scientific™) and an Ion Xpress™ Barcode Adapters Kit according to the manufacturer instructions. Barcoded libraries were quantified using a Bioanalyzer™ 2000 and then pooled to a final concentration of 30 pM per target. Emulsion polymerase chain reaction and target enrichment for template preparation were performed using an Ion Chef™ Instrument and Kit according to the manufacturer protocol. Sequencing analysis was performed with an Ion PGM™ Sequencer and Ion 318™ Chip. All obtained sequence data were analyzed using Ion Reporter™ software.

We compared relative abundance and alpha diversity (Shannon index, observed species and Simpson index) between the 2 groups. Shannon and Simpson indices are quantitative measures of bacterial diversity that reflect species richness and evenness. Statistical analysis was performed using the Mann-Whitney U test for comparisons between 2 groups. p Values less than 0.05 were considered statistically significant.

Ethics Statement

All study procedures, including infant stool sample collection, storage and analysis, were approved by the ethics committee of Nakano Children's Hospital (ICE No. 29) and Kansai Medical University (ICE No. 2015631). Written informed consent was obtained from the parents of the patients enrolled in this study.

RESULTS

Patient Characteristics

Among the 35 patients diagnosed with and treated for fUTI 23 were placed in the CAP group (median age 4.8 months, IQR 3.4–12.8) and 12 in the nonCAP group (5.4 months, IQR 4.4–7.2). A total of 90 samples were collected during the study period. There was no significant difference between the groups regarding gender, age, mode of delivery, feeding type, daily intake of probiotics or proportion of cases in which Escherichia coli was detected as a pathogen (see table). Moreover, 11 patients had no VUR and 1 had grade 1, 9 had grade 3 and 14 had grade 4 VUR.

Gut Microbial Diversity

Owing to the initial antibiotic treatment, microbial diversity decreased as the average Shannon index decreased from 2.9 to 1.4 with significant difference ($p < 0.01$; fig. 1; supplementary table, https://www.jurology.com). After the initial antibiotic treatment Shannon index recovered to the pretreatment level within 1 to 2 months in the CAP and nonCAP groups, and was maintained throughout the study period. Comparison of all samples between the CAP and nonCAP groups demonstrated no significant difference in Shannon index or Simpson index (fig. 1; supplementary table, https://www.jurology.com). However, the average number of observed species was significantly higher in the nonCAP vs CAP group

Patient characteristics

	Overall	CAP Group	Non-CAP Group	p Value
No. pts	35	23	12	
No. male (%)	17 (48.6)	14 (60.9)	3 (25.0)	0.097
Mean mos age (IQR)	5.2 (3.8—10.8)	4.8 (3.4—12.8)	5.4 (4.4—7.2)	0.580
No. vaginal delivery (%)	26 (74.3)	19 (82.6)	7 (58.3)	0.249
No. feeding type (%):*				
Breastfed	14 (40.0)	10 (43.5)	4 (33.3)	0.721
Mixed fed	17 (48.6)	11 (47.8)	6 (50.0)	1.00
Formula fed	4 (11.4)	2 (8.7)	2 (16.7)	0.594
No. intake of probiotics/total No. (%)†	24/34 (70.1)	16/22 (72.7)	8/12 (66.7)	0.714
No. E. coli detected (%)	22 (62.9)	14 (60.9)	8 (66.6)	0.736

* Classified in accordance with Japan Pediatric Society recommendations, with more than 80% of breastfed patients being classified as breastfed and more than 80% of formula fed patients being classified as formula fed.
† Intake of supplement of either probiotics, probiotic drink, yogurt, cheese, miso (fermented soybean paste) or natto (fermented soybeans) at least 4 times weekly. Data are missing for 1 patient.

(40.3 vs 30.2, p <0.01; supplementary table, https://www.jurology.com).

Microbial Composition

Although several bacterial orders were observed in each sample, Lactobacillales was the main order observed after the initial treatment. After 1 to 2 months gut microbiota was composed of several orders, which lasted throughout the study period. However, comparison of relative abundance between the CAP and nonCAP groups indicated higher proportion of gut microbiota belonging to the order Enterobacteriales in the nonCAP group (20.0% vs 12.5%, p=0.02; fig. 2; supplementary table, https://www.jurology.com).

DISCUSSION

Our study revealed that the initial 14 days of treatment for children with fUTI by intravenous and oral antibiotics led to a significant change in pediatric gut microbiome with decreased microbial diversity, suggesting dysbiosis. However, diversity recovered within 1 to 2 months and it was maintained throughout 6 months of CAP.

Several studies have shown decreased gut microbiota diversity because of antibiotic use, supporting

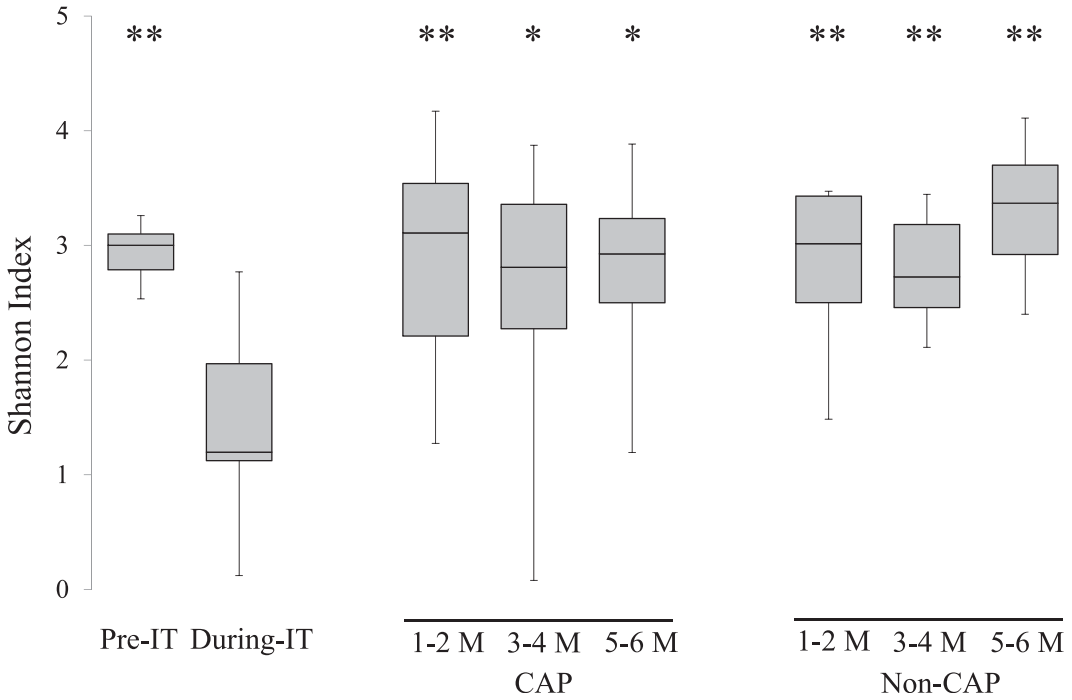

Figure 1. Whisker plot illustrates Shannon index of each time point. Whiskers indicate maximum and minimum values. Graph shows median, upper quartile and lower quartile values. Single a sterisk represents p <0.05 between initial antibiotic treatment (*IT*) and 3 to 4-month (*M*) time point. Double asterisk represents p <0.005 between initial antibiotic treatment and other time points as indicated. There was no significant difference between pre-initial antibiotic treatment and CAP vs nonCAP.

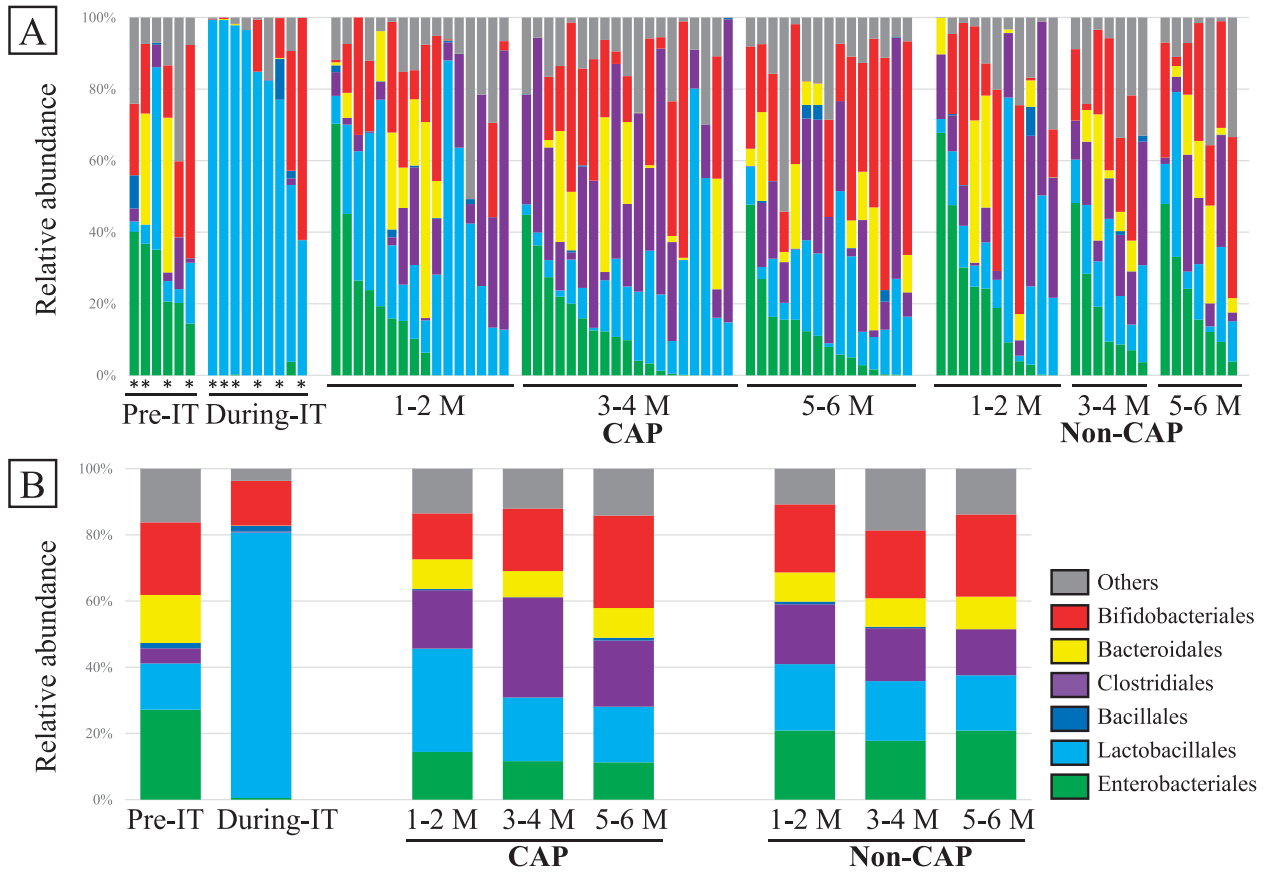

Figure 2. Relative abundance of taxa at order level in each sample (*A*) and average of each group (*B*). Height color bars indicate percentage of each taxon. Asterisk represents patients in CAP group. *IT*, initial antibiotic treatment. *M*, months.

our results. Dethlefsen et al reported decreased gut microbial diversity after oral ciprofloxacin treatment in 3 adults.[21] Panda et al also reported decreased gut microbial diversity in 21 adults treated with fluoroquinolones and β-lactams.[22] Yassour et al compared 20 pediatric patients who received antibiotic treatment to 19 controls, and found decreased microbial diversity in the treated children.[23] Focusing on recovery after antibiotic treatment, Dethlefsen et al,[21] and Dethlefsen and Relman[24] reported that gut microbial diversity began to be restored 1 week after the end of treatment and then resembled the pretreatment state by the fourth week.

Our finding that antibiotic treatment in the acute phase of fUTI affected gut microbiota is similar to that of previous studies. However, there is little to no evidence regarding the effect of low dose antibiotic treatment on pediatric gut microbiome. Our results suggest that long-term low dose antibiotic treatment with TMP-SMX exerted only a small effect on pediatric gut microbiota.

Regarding microbial composition, bacteria of the order Lactobacillales were the main microorganisms remaining after the initial antibiotic treatment for fUTI. This finding can be explained by the microbial target of ceftriaxone and cefditoren pivoxil. In our study Enterococcus faecium was one of the main bacteria of the order Lactobacillales remaining, which is known to be resistant to β-lactam antibiotics including ceftriaxone and cefditoren pivoxil.[25] CAP using TMP-SMX did not decrease microbial diversity and, interestingly, Enterobacteriales bacteria were less abundant in the CAP group than in the nonCAP group.

Although the effect of treatment dose of TMP-SMX on gut microbiota is unclear, one possible reason for the small effect on diversity in our study is the low dosage. Sekirov et al reported that the effects of streptomycin and vancomycin in mouse gut microbiota are dose dependent.[26] Moreover, the lower composition of Enterobacteriales bacteria in the CAP group could be caused by differences in susceptibility to TMP-SMX among gut microbiota. Although TMP-SMX has a wide antibacterial spectrum including urinary tract pathogens (mainly belonging to the order Enterobacteriales), Lactobacillales bacteria and several anaerobic bacteria including Bacteroides fragilis (order Bacteroidales) and Clostridium species (order Clostridiales) are less susceptible to TMP because dihydrofolate reductase enzymes have low affinity to the drug.[27] Moreover, Magruder et al observed that increased abundance of

Escherichia (order Enterobacteriales) was an independent risk factor for Escherichia bacteriuria and UTI among patients receiving a kidney transplant, suggesting an association between gut microbial composition and UTI.[28] These facts led to our assumption that continuous antibiotic prophylaxis using TMP-SMX is an efficient choice of treatment to avoid fUTI recurrence by selectively inhibiting the growth of bacteria causing fUTI.

Our study has several limitations. We could not evaluate the independent effect of TMP-SMX because it was the only antibiotic treatment used as CAP and because changes after completing CAP were not yet studied. However, TMP-SMX is a widely accepted choice for CAP based on its effectiveness as demonstrated in the RIVUR trial. Therefore, we will further investigate its long-term effect. In addition, because we performed 16S rRNA gene sequencing, bacterial functional profile, including antibiotic resistant gene classification, was not analyzed. Although the RIVUR trial revealed that E. coli isolated from children receiving CAP had higher resistance to TMP-SMX than from those receiving placebo,[19] further investigation of antibiotic resistance remains essential.

Patient age (in months) also varied in this study. Following birth bacteria belonging to orders Bifidobacteriales, Bacteroidales and Clostridiales begin to colonize the intestine at low diversity. Toward weaning, the order Bifidobacteriales becomes dominant, followed by an increase in Bacteroidales and Clostridiales, thus producing a more diverse environment and resulting in an adult-like bacterial composition by age 3 years.[1] As gut microbiota changes dramatically during infancy owing to several factors, including nutrition, it would be ideal to conduct a study with more subjects limited to a certain age. In addition, the functional consequences of CAP on gut microbiota are yet to be determined. Several cohort studies suggest that antibiotic treatment during childhood leads to increased risk of obesity and allergic diseases.[16,17,29,30] Finally, our sample size was rather small, especially for the nonCAP group. However, to our knowledge there is no previous study assessing the effect of low dose TMP-SMX on gut microbiota, and thus we believe our findings are novel and provide new insights into clinical practice.

CONCLUSIONS

We report the first known study on the effect of long-term low dose antibiotic treatment using TMP-SMX on pediatric gut microbiota. Our results show that the gut microbiota diversity of TMP-SMX CAP treated patients did not decrease, indicating that the effect of TMP-SMX CAP on gut microbiota was insignificant. Furthermore, TMP-SMX CAP specifically suppressed the growth of bacteria belonging to the order Enterobacteriales, such as E. coli and Klebsiella species, which are the main causative bacteria of fUTI. This finding suggests that TMP-SMX continuous antibiotic prophylaxis is a favorable treatment option not only for avoiding fUTI recurrence, but also for preserving pediatric gut microbiota.

ACKNOWLEDGMENT

Editage (www.editage.com) performed English language editing.

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