Association of Antibiotics Exposure Within the First 2 Years After Birth with Subsequent Childhood Type 1 Diabetes

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Research Article

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Abstract

Purpose: Antibiotics prescription in early life can cause dysbiosis, an imbalance of gut microbiota. We aimed to reveal the relationship between antibiotics exposure during the first 2 years after birth and type 1 diabetes risk in children under 8 years of age using a nationally representative data from South Korea.

Methods: The final study population consisted of 63,434 children from the National Health Insurance Service (NHIS) database from 2008 to 2015. The primary exposure of interest was antibiotics prescription in first 2 years after birth. The analysis was conducted with cumulative defined daily dose (cDDD; 0-29, 30-59, ≥ 60 cDDD), the number of antibiotics classes (0-3, 4, ≥ 5 classes), and age at first antibiotics prescription (0-119, 120-239, ≥ 240 days). Age, sex, household income, and overweight were considered as potential confounding covariates.

Results: Compared to those within the less than 30 cDDD, other groups that were prescribed more antibiotics did not have a significant difference in diabetes risk (aHR 0.86, 95% CI 0.37-2.02 in ≥ 60 cDDD). The number of antibiotics classes and age at first antibiotics prescriptions were also not associated with the risk of type 1 diabetes. The development of diabetes was not related to the cDDD, the number of antibiotics classes, and age at first antibiotics prescription according to subgroup analysis which was stratified by overweight.

Conclusions: Antibiotics exposure within the first 2 years of life was not associated with subsequent diabetes risk. Future studies using a larger number of long-term follow-up data are needed.

Declarations

Funding

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Conflicts of Interest Disclosures

None of the authors reported disclosures.

Availability of data and material

This study used the NHIS database (NHIS-2020-2-072).

Code availability

SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC, USA) was used to conduct all statistical analyzes.

Author Contributions

SM Park had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: DH Lee, SG Choi, SM Park

Acquisition of data: DH Lee, JY Chang, SM Park

Analysis and interpretation of data: DH Lee, SG Choi, JY Chang, YJ Park, SM Park

Drafting of the manuscript: DH Lee, SG Choi, SM Park

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Statistical analysis: DH Lee, SG Choi, JY Chang, YJ Park

Administrative, technical, or material support: JY Chang, SG Choi, SM Park

Ethics approval

This study was approved by the Seoul National University Hospital Institutional Review Board (IRB number: E-1904-003-1021).

Consent to participate

The requirement for informed consent was waived as the NHIS data is anonymized according to strict confidentiality guidelines prior to distribution.

Consent for publication

The Ministry of Health and Welfare and National Research Foundation of Korea had no role in the design and conduct of the study, the collection, management, analysis, and interpretation of the data, or the preparation, review, or approval of the manuscript, and decision to submit for publication.

Introduction

The incidence of type 1 diabetes in children and adolescents is increasing worldwide [1,2]. Type 1 diabetes incidence is rapidly increasing, especially at a young age of 10 years or younger than adolescents [3]. The incidence of type 1 diabetes varies remarkably by ethnicity [3,4]. In Asia including South Korea, incidence of type 1 diabetes is lower than that of Western countries [5,6]. The incidence between Asia and Europe in childhood type 1 diabetes is reported to be more than 350 folds different [7]. Diabetes is a disease that has a high socioeconomic burden due to morbidity and mortality via complications related to diabetes [8,9]. The importance of predicting the occurrence of childhood diabetes and preventing risk factors before developing diabetes is of clinical importance.

Type 1 diabetes is caused by pancreatic islet beta cell destruction by autoreactive T lymphocyte [10]. Factors that trigger pancreatic beta cell destruction include genetic defect, infection, drug, and chemicals [6,11,12]. Recently, gut microbiota is also known to be associated with the development of type 1 diabetes [13]. Antibiotics prescription in early life can cause dysbiosis, which is an imbalance of gut microbiota [14]. Recently, research on how dysbiosis caused by antibiotics for early infancy affects health in the future is being actively conducted. Antibiotics exposure in early life is known to increase obesity [15,16]. Due to the fact that obesity is associated with diabetes risk [17,18], antibiotics exposure may also be associated with the subsequent development of diabetes.

Whether antibiotics exposure affects the development of childhood diabetes is yet unclear. Few studies have investigated the association of antibiotics exposure with diabetes risk, but the link between antibiotics exposure and type 1 diabetes prevalence is not clear [14,19,20]. All of these studies were conducted on Caucasians, therefore limiting the generalizability of the results to populations outside of the Western population. Previous studies on the association of antibiotics and diabetes failed to take obesity into consideration due to a lack of information. This may be particularly relevant as obesity may be an important confounder that is associated with both antibiotics and diabetes. Therefore, study that takes into account obesity on the association of antibiotics with diabetes is needed.

In this study, we aimed to reveal the relationship between antibiotics exposure during the first 2 years and the development of diabetes in children under 8 years of age using a nationally representative data from South Korea. Additionally, overweight was adjusted for and stratified in the relationship between antibiotics exposure and diabetes risk.

Methods

Study population

In South Korea, the National Health Insurance Service (NHIS) provided the National Health Information Database (NHID) established by integrating regular health examination data, claim data for hospital use, socio-demographic profile, and mortality [21]. National infant and child medical check-ups are provided to all Korean children from 4 months-old to 6 years-old on a total of 7 occasions. Each examination includes physical examinations, measuring anthropometric data such as height, weight, head circumference, and developmental assessment. The NHIS provided the 5% sampled data which was extracted randomly from the total examination data of children received at least the first or second check-up once in 2008-2012. This is provided through the establishment of an infant medical check-up cohort database as follow up data during 2008-2015.

Among 84,005 participants included in the database, children who met the following exclusion criteria were excluded: missing values on covariates including age, sex, and household income (n=39), missing values on height or weight in the third medical check-up (n=20,458), outliers of height or weight which has lesser than 1 % or more than 99 % according to sex and age (n=39), and those who died or were

diagnosed with diabetes before the age of 2 (n=35). The final study population consisted of 63,434 children.

Key variables

The diagnosis of diabetes as an outcome variable was identified by using the ICD-10 codes, E10, E11, and E14. E12, malnutrition related diabetes, and E13, other specified diabetes mellitus was excluded from the analysis because they were considered as temporary diabetes due to other causes such as malnutrition, drug, etc. The incidence of type 2 diabetes among children aged <10 years in South Korea is very low, as can be seen in 0 aged 0-4 regardless of sex, and 0.14 in boys and 0.21 in girls aged 5-9 [22]. The diagnosis of diabetes in this study of children under 8 years of age was considered as type 1 diabetes considering the incidence of type 2 diabetes in South Korea. Even if type 2 diabetes was diagnosed, we thought it would be a misdiagnosis and considered it type 1 diabetes since the incidence of type 2 diabetes is too low at this age. Moreover, outliers for weight were excluded.

The primary exposure of interest was antibiotics exposure within the first 2 years after birth since healthy infants reach adult gut microbiome after undergoing compositional change during the first 2 years [23]. In South Korea, antibiotics can be prescribed only through a doctor's prescription. Through the database provided by NHIS, information on the prescription date, the class of antibiotics prescribed, and the prescribed dose was collected. Antibiotics prescriptions were confirmed using the Anatomic Therapeutic Chemical (ATC) code since all drugs are classified according to ATC classification system [24].

The analysis was conducted with three ways of cumulative defined daily dose (cDDD), the number of antibiotics classes, and age at first antibiotics prescription. The defined daily dose (DDD) is defined by the World Health Organization as "The assumed average maintenance dose per day for a drug used for its main indication in adults." And is the most commonly used method for quantifying antibiotics [24]. We used cDDD in the analysis because there was no established method in children. cDDD was calculated by adding up the DDD of each antibiotic multiplied by the number of prescription days.

Each exposure, cDDD, classes of antibiotics, and the age at first antibiotics prescription, was divided into 3 groups (0-29 cDDD, 30-59 cDDD, and 60 cDDD or more; 0-3 classes, 4 classes, and 5 classes or more; 0-119 days, 120-239 days, and 240 days or more). In the cDDD analysis, additional analysis was conducted on each antibiotics class with more than 2 events of type 1 diabetes in each cDDD group. The classification of the antibiotics class follows the ATC classification system [24].

Age (continuous, years), sex (categorical, boys or girls), household income (categorical, first, second, third, or fourth quartiles), and overweight (categorical, yes or no) was considered as potential confounding covariates. Household income was acquired from the insurance premium. The weight percentile based on length, weight for length (WFL), referring to growth chart was used as an indicator of overweight under 24 months of age [25,26]. Overweight was defined as WFL of 85th percentile or higher referred to the Korean National Growth Charts for children and adolescents [26]. As the number of events was too small, 85th percentile, which was alleviated than the 95th percentile suggested by WHO, was

used as a criterion for overweight. WFL was used as an index of overweight instead of body mass index because weight and height values of the third examination, which was performed around 18 to 24 months after birth, were used closest to the index date when the patient was 24 months of age.

Statistical analysis

Follow up duration was defined as the interval between the age of 2 years and the earliest date among the diagnosis of diabetes, death, or December 31st, 2015. The hazard ratios (HRs) and 95% confidence interval (CIs) of diabetes development of antibiotics prescribed before 2 years old was calculated by multivariate Cox proportional hazard regression analysis. The group with the smallest amount of antibiotics exposed (0-30 cDDD; 0-3 classes; 0-120 days) were considered as the reference group in each analysis.

Stratified analysis of antibiotics prescription on the risk of diabetes was conducted according to subgroups of WFL. Sensitivity analysis was performed according to preterm birth, neonatal intensive care unit (NICU) admission, and breastfeeding through questionnaire surveyed at the first medical check-up.

Statistical significance was defined when the *p*-value was less than 0.05. SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC, USA) was used to conduct all statistical analyzes.

Results

Table 1 showed the clinical characteristics in the study population according to cDDD of prescribed antibiotics before 2 years old. Among 63,434 study participants, 28,706 (45.3 %), 18,188 (28.7 %), and 16,540 (26.1 %) were allocated for the 0-30 cDDD, 30-60 cDDD, and more than 60 cDDD group. The age was the same as all 2 years since the index date was set based on the participant's age of 2. The proportion of boys was 41.3 %, 52.9 %, and 58.3 %, respectively. Participants who were boys and had lower household income prescribed more antibiotics (*p* for trend <0.001). The proportion of overweight was 18.3 %, 22.0 %, and 27.6 %, respectively. The overweight ratio was higher in the group that received more antibiotics prescribed (*p* for trend <0.001).

The association between cDDD of antibiotics and diabetes was shown in Table 2. Compared to those within the less than 30 cDDD, other groups which were prescribed more antibiotics did not have a significant difference in diabetes risk (aHR 0.86, 95% CI 0.45-1.65 in 30-60 cDDD; aHR 0.81, 95% CI 0.41-1.62 in >60 cDDD). The same tendency was observed when analyzed for each antibiotics class, penicillin, cephalosporine, macrolide, and fluoroquinolone. The risk of diabetes did not increase upon greater cDDD (*p* for trend 0.556 for penicillin; 0.441 for cephalosporin; 0.563 for macrolide; 0.411 for fluoroquinolone).

The number of antibiotics classes and age at first antibiotics prescriptions were also not associated with the risk of type 1 diabetes (Table 3). Compared to 0-3 antibiotics classes, more than 5 classes of antibiotics classes did not show a significant increase in diabetes (aHR 1.20, 95% CI 0.54-2.70; *p* for trend 0.862). Age at first antibiotics prescription was also not significantly related to the development of

diabetes. More than 240 days at first prescription did not increase the risk of diabetes compared to less than 120 days (aHR 1.25, 95% CI 0.65-2.39).

Table 4 depicts the stratified analysis of the antibiotics on diabetes according to subgroups of overweight. The development of diabetes was not related to the cDDD, the number of antibiotics classes, and age at first antibiotics prescription among overweight or not.

The sensitivity analysis of the association antibiotics prescribed before the age of 2 years and diabetes after additional adjustments for preterm birth, NICU admission, and breastfeeding are shown in Table 5. As the previous results, an increase in the cDDD, the number of antibiotics classes, and age at first antibiotics prescription was not associated with diabetes risk after additional adjustments for preterm birth, NICU admission, and breastfeeding.

Discussion

This study is significant as it is the first paper to study antibiotics exposure in early life and diabetes in Asian countries. Antibiotics exposure in the first 2 years did not affect the development of type 1 diabetes in children under 8 years of age. We conducted the analysis using nationally representative data sampled from the longitudinal national cohort in the NHIS database from South Korea. The incidence of type 1 diabetes was not related to cDDD, the number of antibiotics classes, and age at first antibiotics prescription for the first 2 years.

One of the most commonly prescribed medications in children is antibiotics [27]. Since the gut microbiome is not stable in early life, an imbalance of gut microbiome caused by antibiotics prescribed during the neonatal period causes various health problems [28,29]. Studies have reported that obesity was associated with dysbiosis caused by antibiotics [15,30]. Moving further from obesity, diabetes has also recently been increasingly noted for its relevance to the intestinal microbiome [31]. Animal studies have reported that the prevalence of type 1 diabetes increases with partial ablation of gut microbiota due to antibiotics exposure in early life [13]. It was previously speculated that dysbiosis could lead to type 1 diabetes by autoimmunity damage [32,33]. We conducted a study assuming that diabetes may be related to the exposure of antibiotics in early life. In this study of more than 60,000 children, antibiotics use was not associated with childhood diabetes.

Few papers have studied the relationship between antibiotics exposure and the development of childhood diabetes. Whether there is a relationship between antibiotics exposure and diabetes is still controversial. The use of antimicrobials during pregnancy was not associated with the occurrence of type 1 diabetes, but it has been reported that using the penicillin and macrolide series may increase the risk [19]. Although the exposure group is different due to the use of antibiotics before pregnancy, this also reminds us of the link between antibiotics in early life and childhood diabetes. A Danish cohort study has been reported that the use of broad-spectrum antibiotics increases type 1 diabetes in the first 2 years of life among children born in the cesarean section, but children who were delivered by vaginal delivery was not relevant [20]. In studies using national data in Norway, the history of antibiotics in the first 2 years and infection were not

associated with the development of type 1 diabetes [14]. All of the above studies were conducted on Caucasians.

Diabetes is also a disease that is highly affected by ethnicity regardless of type [4,34]. The incidence of diabetes is increasing in South Korea, and the incidence of children under 15 years of age was reported to be 1.19 per 100 000 population in 1995-2000 and 3.19 in 2012-2014, where the annual increase was 5.6 % [35]. The incidence of South Korea is lower than that of the United States, 21.7 cases per 100 000 youth in 2011-2012 [36]. This is the first study in Asian childhood, and this study can provide insight into the relationship between antibiotics exposure and diabetes in Asian children by using nationally representative data. Furthermore, weight and height that were not considered in the previous studies due to limitations of the retrospective study were in this paper for a more accurate correlation between antibiotics and diabetes. The overweight was adjusted as a covariate variable using WFL percentage of each participant. This study attempted to perform a more accurate analysis through the relationship with an accumulated dose of antibiotics by cDDD, unlike previous studies that only analyzed antibiotics repeated courses.

This study confirmed whether diabetes is related according to the accumulated doses of antibiotics prescribed for 2 years of age and prescribed antibiotics classes. We performed statistical analysis by adjusting age, sex, income, and overweight. The overweight tended to increase as the group receiving more antibiotics was prescribed. Given the reports that obesity can increase the risk of infection [37,38], it suggests that overweight may increase the risk of infection and may be related to antibiotics prescriptions. The first 2 years of antibiotics exposure has null-association with the occurrence of type 1 diabetes, whether according to cDDD or the number of antibiotics class. Even when the stratified analysis was performed by dividing the overweight or not, the relationship between antibiotics exposure and type 1 diabetes was not clear. In an additional analysis, preterm birth, history of NICU care, and breastfeeding were additionally adjusted. Antibiotics exposure was not also statistically significantly related to the development of type 1 diabetes in the sensitivity analysis.

The limitations of this study are as follows. First, there were difficulties in statistical analysis that there were not many numbers of diabetes in children due to the limitation of sampled data. Only 53 children were diagnosed with diabetes among total children. The possibility that the number of diabetes of each group was too small to derive statistical significance cannot be ignored. Further studies using the entire data rather than sample data are needed to confirm more accurate relevance between antibiotics exposure in early life and diabetes. Second, the average follow-up duration is as short as 3 years. This study assumed that children were diagnosed as type 1 diabetes by referring to the prevalence of Korea under 8 years of age [22]. There may be overdiagnosis and misclassification because diabetes is defined only by ICD-10 codes. There may be type 2 diabetes and maturity-onset diabetes of the young, but we considered the impact would be minimal because the number is too small to change the result. The incidence of type 2 diabetes increases toward adolescence rather than early age, so further studies with a longer follow up period are needed for type 2 diabetes studies. Third, there is no guarantee that antibiotics are actually taken through register-based data. Patients can be prescribed medicine in several

hospitals given that Korea is an environment with high access to primary care physicians. Finally, it is difficult to confirm the time-temporal relationship between exposure and outcome due to the retrospective study design.

In conclusion, antibiotic exposure within the first 2 years of life was not associated with subsequent type 1 diabetes risk. Even when considering overweight, the relationship between antibiotics exposure and type 1 diabetes did not change. This study is meaningful in that this is the first study conducted in an Asian group and also it investigated the effect of overweight on the relationship between antibiotics exposure in early life and type 1 diabetes. Physicians should interpret the results with care as the impact of antibiotics on other health problems such as obesity appear to be prevalent. Further studies using a larger number of long-term follow-up data are needed.

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Tables

Table 1. Clinical characteristics in the study population according to the cumulative defined daily dose of antibiotics.

	The cumulative defined daily dose of antibiotics			
	0-29	**30-59**	**≥ 60**	***p* for trend**
N (%)	28,706 (45.3)	18,188 (28.7)	16,540 (26.1)	
Age, years, mean (SD)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	-
Sex, N (%)				<0.001
Boys	13,531 (41.3)	9,614 (52.9)	9,634 (58.3)	
Girls	15,175 (52.9)	8,574 (47.1)	6,906 (41.8)	
Income, quartiles, N (%)				<0.001
1st (highest)	7,553 (26.3)	4,194 (23.1)	3,640 (22.0)	
2nd	13,480 (47.0)	8,705 (47.9)	7,873 (47.6)	
3rd	4,746 (16.5)	3,171 (17.4)	2,994 (18.1)	
4th (lowest)	2,927 (10.2)	2,118 (11.7)	2,033 (12.3)	
Overweight†, N (%)				<0.001
No	23,464 (81.7)	14193 (78.0)	11,972 (72.4)	
Yes	5,242 (18.3)	3,995 (22.0)	4,568 (27.6)	

p-value was calculated by the Chi-squared test for categorical variables, and analysis of variance for continuous variables.

† Overweight was based on weight for length of 85 percentile or more.

Acronyms: SD, standard deviation.

Table 2. The association between the cumulative defined daily dose of antibiotics and type 1 diabetes

	The cumulative defined daily dose of antibiotics			
	0-29	30-59	≥ 60	*p* for trend
Total antibiotics				
N	28706	18188	16540	
Event (%)	27 (0.09)	14 (0.08)	12 (0.07)	
aHR (95% CI)	1.00 (ref)	0.86 (0.45-1.65)	0.81 (0.41-1.62)	0.532
Penicillin				
N	28885	18060	16489	
Event (%)	27 (0.09)	14 (0.08)	12 (0.07)	
aHR (95% CI)	1.00 (ref)	0.88 (0.46-1.68)	0.82 (0.41-1.64)	0.556
Cephalosporin				
N	29857	17352	16225	
Event (%)	28 (0.09)	14 (0.08)	11 (0.07)	
aHR (95% CI)	1.00 (ref)	0.90 (0.47-1.72)	0.76 (0.37-1.53)	0.441
Macrolide				
N	30218	16924	16292	
Event (%)	28 (0.09)	13 (0.08)	12 (0.07)	
aHR (95% CI)	1.00 (ref)	0.86 (0.45-1.67)	0.83 (0.42-1.65)	0.563
Fluoroquinolone				
N	32755	14941	15738	
Event (%)	32 (0.10)	9 (0.06)	12 (0.08)	
aHR (95% CI)	1.00 (ref)	0.64 (0.30-1.34)	0.81 (0.42-1.59)	0.411

Adjusted hazard ratio was calculated by Cox proportional hazard regression after adjustments for age, sex, house income, and overweight. The least prescribed antibiotics group (0-30) was considered as the reference group.

Acronyms: aHR, adjusted hazard ratio; CI, confidence interval.

Table 3. The association between the number of antibiotics classes and type 1 diabetes

	Type 1 Diabetes		
	N	**Event (%)**	**aHR (95% CI)**
The number of antibiotics classes			
0-3	23849	23 (0.10)	1.00 (ref)
4	33050	22 (0.07)	0.70 (0.39-1.26)
≥ 5	6482	8 (0.12)	1.20 (0.54-2.70)
p for trend			0.862
Age at first antibiotics prescription (days)			
0-119	25997	22 (0.08)	1.00 (ref)
120-239	21454	15 (0.07)	0.85 (0.44-1.65)
≥ 240	15983	16 (0.10)	1.25 (0.65-2.39)
p for trend			0.573

Adjusted hazard ratio was calculated by Cox proportional hazard regression after adjustments for age, sex, house income, and overweight. The smallest number of antibiotics classes (0-3) was considered as the reference group.

Acronyms: aHR, adjusted hazard ratio; CI, confidence interval.

Table 4. Stratified analysis on the association of antibiotics and type 1 diabetes according to groups of weight for length

	Overweight†					
	No			Yes		
	N	event (%)	aHR (95% CI)	N	event (%)	aHR (95% CI)
cDDD						
0-29	23464	22 (0.09)	1.00 (ref)	5242	5 (0.10)	1.00 (ref)
30-59	14193	9 (0.06)	0.72 (0.33-1.56)	3995	5 (0.13)	1.42 (0.41-4.93)
≥ 60	11972	8 (0.07)	0.76 (0.34-1.73)	4568	4 (0.09)	1.01 (0.27-3.78)
p for trend			0.433			0.965
The number of antibiotics class						
0-3	19173	16 (0.08)	1.00 (ref)	4699	7 (0.15)	1.00 (ref)
4	25474	18 (0.07)	0.87 (0.44-1.70)	7598	4 (0.05)	0.36 (0.11-1.23)
≥ 5	4982	5 (0.10)	1.15 (0.42-3.14)	1508	3 (0.20)	1.25 (0.32-4.84)
p for trend			0.991			0.720
Age at first antibiotics prescription (days)						
0-119	19926	16 (0.08)	1.00 (ref)	6071	6 (0.10)	1.00 (ref)
120-239	16729	10 (0.06)	0.77 (0.35-1.70)	4725	5 (0.11)	1.09 (0.33-3.59)
≥ 240	12974	13 (0.10)	1.29 (0.62-2.68)	3	5 (0.10)	1.11 (0.28-4.44)
p for trend			0.569			0.873

The number of events was expressed as the total number of diabetes. Adjusted hazard ratio was calculated by Cox proportional hazard regression after adjustments for age, sex, and house income. The least prescribed antibiotics group (0-30 cDDD), the smallest numbers of antibiotics classes (0-3 classes), the earliest days of age at first antibiotics prescription (0-120 days) was considered as the reference group.

† Overweight was based on weight for length of 85 percentile or more.

Acronyms: Abx, antibiotics; aHR, adjusted hazard ratio; cDDD, cumulative defined daily dose; CI, confidence interval.

Table 5. Sensitivity analysis on the association between antibiotics and type 1 diabetes after additional adjustments for preterm birth, NICU admission, and breastfeeding

	N	Events (%)	aHR (95% CI)
cDDD			
0-29	23907	21 (0.09)	1.00 (ref)
30-59	14994	11 (0.07)	0.86 (0.42-1.80)
≥ 60	13346	8 (0.06)	0.69 (0.30-1.58)
p for trend			0.354
The number of antibiotics class			
0-3	19833	16 (0.08)	1.00 (ref)
4	27113	19 (0.07)	0.86 (0.44-1.68)
≥ 5	5291	5 (0.09)	1.08 (0.39-2.95)
p for trend			0.933
Age at first antibiotics prescription (days)			
0-119	21245	17 (0.08)	1.00 (ref)
120-239	17590	11 (0.06)	0.87 (0.40-1.89)
≥ 240	13402	12 (0.09)	1.27 (0.60-2.71)
p for trend			0.588

The number of events was expressed as the total number of diabetes. Adjusted hazard ratio was calculated by Cox proportional hazard regression after adjustments for age, sex, house income, overweight, preterm birth, NICU admission, and breastfeeding. The least prescribed antibiotics group (0-30 cDDD) , the smallest numbers of antibiotics classes (0-3 classes), the earliest days of age at first antibiotics prescription (0-120 days) was considered as the reference group.

Acronyms: aHR, adjusted hazard ratio; cDDD, cumulative defined daily dose; CI, confidence interval; NICU, neonatal intensive care unit.