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Antibiotic treatment during early childhood and risk of type 1 diabetes in children: A national birth cohort study

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Abstract

Objective/Background: Antibiotics are widely used during childhood infections and influence the composition of the microbiota, which is established during the first years of life. Evidence from animal models of type 1 diabetes shows that antibiotics might accelerate disease progression, and altered intestinal microbiota has been reported in association with type 1 diabetes in humans. We aimed to test the hypothesis that early exposure to antibiotics (0-24 months of age) was associated with an increased risk of childhood type 1 diabetes development.

Methods: We studied 75 615 mother-child dyads from the Danish National Birth Cohort. Information on the use of antibiotics during early childhood and type 1 diabetes development in childhood was available for all children via linkage to the Danish National Prescription Registry and the Danish National Patient Register, respectively. The mean follow-up time was 14.3 years (range 11.5 to 18.4 years, SD 1.4).

Results: After adjustment for confounders, we found no association between antibiotic exposure and risk of type 1 diabetes (HR 1.26, 95% CI 0.89-1.79). The number of antibiotic courses during early childhood was not associated with type 1 diabetes development when analyzing for one (HR 1.31, 95% CI 0.87-1.99), two (HR 0.99, 95% CI 0.61-1.63), or 3 or more (HR 1.42, 95% CI 0.95-2.11) courses. Furthermore, no specific types of antibiotics (penicillins/beta-lactam antibacterials, sulfonamide/trimethroprim, or macrolides/lincosamides/streptogramins) were associated with increased risk of type 1 diabetes.

Conclusion: Our nationwide cohort study suggests that postnatal exposure to antibiotics does not influence the development of childhood type 1 diabetes.

KEYWORDS

antibiotics, children, early childhood, postnatal exposure, type 1 diabetes

1 | INTRODUCTION

Type 1 diabetes is a multifactorial disease, where genes predispose to the disease, but environmental factors are assumed to trigger the development. The incidence of the disease has increased during the last decades more rapidly than can be accounted for by genetic change, thus supporting the role of other factors including diet, birth mode, infections, and changes in the composition of the intestinal microbiota.[1]

The development of the microbiome in early life is dependent on many different factors, and crosstalk between the microbiome and the immune system is thought to be involved in the pathogenesis of islet autoimmunity and later overt type 1 diabetes development.[2] Antibiotics are widely used during childhood infections and influence the composition of the microbiota, which is established during the first years of life.[3] Thus, exposure to antibiotics could result in persistent changes in the microbiota, which could affect the risk of type 1 diabetes development.

Earlier studies have investigated the use of pre-[4,5] and postnatal antibiotics[4,6-13] on the risk of type 1 diabetes development, and the results show that the effect of antibiotics is influenced by time of administration, the type of antibiotics, and potential effect modification by for example, mode of delivery.[9] However, more studies are warranted as the results are not unequivocal.

Previous Danish studies have been based on national registers.[9,11,13] In this study, we used the Danish National Birth Cohort (DNBC) that is particularly well described to conduct a nationwide, observational study to investigate if the use of antibiotics from 0 to 24 months of age influences the risk of type 1 diabetes development in childhood. DNBC allowed for a more detailed analysis as it included multiple possible confounders, compared with the two previous Danish cohort studies.[9,13]

2 | METHODS

2.1 | Study population

This study was carried out within the Danish National Birth Cohort (DNBC), a nationwide, ongoing study of pregnant women and their offspring. During years from 1996 to 2002, women were invited to the cohort at their first antenatal visit at the general practitioner. It is estimated that about 30% of all Danish pregnant women in the years between 1996 and 2002 were recruited to the cohort. The women were included if they intended to carry the pregnancy to term, had a permanent address in Denmark and spoke Danish well enough to participate in telephone interviews. They provided information on exposures during and after pregnancy by means of four computer-assisted telephone interviews, scheduled to take place in gestational weeks 12 and 30 and post-partum when the child was 6 and 18 months old. The cohort is described in detail elsewhere.[14,15] A total of 97.4% of the women were of Danish origin, 2% were from Scandinavia or other OECD countries, and 0.5% from the rest of the world (0.1% unknown origin).

Information on the use of antibiotics during early childhood and type 1 diabetes development in childhood was available for all children in the DNBC via linkage to the Danish National Patient Register and the Danish National Prescription Registry. The children were followed via the registers until 31 December 2014. The mean follow-up time was 14.3 years (range 11.5-18.4 years, SD 1.4). During the recruitment period, 100 418 pregnancies were enrolled in the cohort. For this study, we initially included all children born between 1997 and 2003 (n = 96 840). Subsequently, we excluded twins and triplets (n = 4165), children born before 25 completed gestational weeks (n = 151), children with a birth weight < 500 g (n = 453), stillbirths (n = 59), and children who developed diabetes before 24 months of age (n = 14). Moreover, some had missing information on socioeconomic status (n = 10 310), parity (n = 52), maternal diabetes (n = 5833), smoking during pregnancy (n = 187), or cesarean section (n = 1). Additionally, some individuals had missing information on breastfeeding (n = 24 695). Thus, 91 998 were eligible for crude analyses on the use of antibiotics and the risk of type 1 diabetes, 75 615 were eligible for adjusted analyses, and 50 924 were eligible for analyses adjusted for breastfeeding (Supplementary Figure 1).

2.2 | Measurement of outcome

The outcome measure of interest was onset of type 1 diabetes. Information on age of type 1 diabetes diagnosis was obtained by linking the children in the DNBC to the Danish National Patient Registry via the unique Danish identification number (CPR-number). We included the ICD-10 diagnoses DE10 and DE14 with onset after 24 months of age.

2.3 | Measurement of exposure

Information on use of all systemic antibiotics with Anatomical Therapeutic chemical Code (ATC) J01 was obtained by linking the children in the DNBC to the Danish National Prescription Registry by using the CPR-numbers of the children.

Use of antibiotics was recorded for the period 0 to 24 months of age and quantified as courses. A new course was defined by a new prescription, 14 days after the previous prescription of antibiotics.

For analyses of the type of antibiotics, we categorized the children into "ever users", meaning that a child had also received other types of antibiotics or "only users" in which the child only received that particular antibiotic. Both groups of children were compared with a group of "never users" of any antibiotic during 0 to 24 months of age. The antibiotics were categorized according to type (a) penicillins/beta-lactam antibacterials (J01C), (b) sulfonamide/trimethoprim (J01E) or (c) macrolides/lincosamides/streptogramins (J01F). These three groups were compared with a group of children who had not been using antibiotics during early childhood (0-24 months of age).

The use of antibiotics during early childhood was analyzed as (a) yes/no (0-24 months of age) (b) number of courses (0-24 months of age, categorized as(0, 1, 2, ≥ 3 courses), (c) type of antibiotic used (0-24 months of age); use among ever users of penicillins/beta-lactam antibacterials (J01C), sulfonamide/trimethoprim (J01E), or macrolides/lincosamides/streptogramins (J01F) (d) type of antibiotic used (0-24 months of age); use among only users of penicillins/beta-lactam antibacterials (J01C), sulfonamide/trimethoprim (J01E), or macrolides/lincosamides/streptogramins (J01F).

2.4 | Potential confounders and characteristics of the study population

A large variety of information on mothers and children was available from the DNBC. Information on maternal pre-pregnancy BMI was obtained in gestational week 12, and information on paternal BMI was obtained at the 18 months' interview. Information on maternal age at conception, socio-economic status, parity, and smoking during pregnancy was obtained from the first gestational interview. Socio-economic status was based on the highest level of the maternal or paternal level of education and occupation and classified as low, medium, or high. The measure was based on the current or most recent job within the last 6 months or, if the father/mother was studying, on the type of education he/she headed for.[16] Parity was categorized as 0 or ≥ 1 previous births, and smoking during pregnancy was categorized as never smoked, 1 to 10 cigarettes per day and above 10 cigarettes per day. Information on gestational weight gain was obtained from the interview 6 months post-partum and was converted into kg per week of the actual gestational length and included as a continuous measure (100 g increment intervals). A complete analysis diagram can be found in Supplementary Figure 2.

2.5 | Potentially mediating individual risk factors

Information on cesarean section was obtained from the Danish National Patient Registry. Information of breastfeeding and duration of breastfeeding was gained from the interview 6 and 18 months post-partum. In the interview 6 months post-partum, the women were asked about the duration of any and exclusive breastfeeding; 18 months post-partum the women were only asked about the duration of any breastfeeding. We could therefore not include exclusive breastfeeding beyond the time of the 6 months interview. The duration of any breastfeeding was categorized into quartiles (<20, 20-31.9, 32-39.9, ≥40 weeks).

2.6 | Statistical analyses

The hazard ratios (HR) of development of type 1 diabetes in childhood according to use of antibiotics during early childhood were estimated using Cox regression. Child age in days was used as the underlying time. Children were considered to be at risk for type 1 diabetes development from the date of their 2-year birthday. The follow-up ended at the onset of type 1 diabetes, emigration, death or by the end of follow-up at 31 December 2014, whichever came first. The children in the DNBC contributed a total of 1 115 847 person years at risk. We conducted three sets of analyses estimating the relations between the use of antibiotics (any antibiotics, dose, and type of antibiotics) during early childhood and the risk of type 1 diabetes in children and adolescents. An interaction term was included to test whether the association between antibiotics during early childhood and the risk of type 1 diabetes differed for children born by cesarean section. The proportional hazards assumption was tested by including a time-varying effect of any antibiotics, dose, and type of antibiotics during early childhood in the model. A *P*-value <.05 was considered statistically significant. All statistical analyses were performed using the SAS software package version 9.4.

3 | RESULTS

From the DNBC, a total of 91 998 children were available for unadjusted analysis. Of these, 322 children developed type 1 diabetes. For adjusted analysis, 75 615 mother-child dyads were identified.

In total, 73.0% of the children, who developed type 1 diabetes, had received antibiotic treatment from 0 to 24 months of age, vs 70.4% of children not developing type 1 diabetes (*P* = .3). Of these, 33.5% were exposed to ≥3 antibiotic courses, compared with 28.7% of children that did not develop type 1 diabetes (*P* = .2). Among children ever receiving antibiotics, 72.3% of the children that developed type 1 diabetes were treated with penicillins/beta-lactam antibacterials vs 69.4% in the group of children not developing type 1 diabetes (*P* = .3). The corresponding numbers for sulfonamide/trimethroprim and macrolides/lincosamides/ streptogramins were 0.0% vs 1.5% (*P* = .3) and 73.1 vs 75.1 (*P* = .6). Among children that only received one type of antibiotics, 70.0% had been treated with penicillins/beta-lactam antibacterials vs 67.1% (*P* = .3) of children that did not develop type 1 diabetes. For sulfonamide/trimethroprim or macrolides/lincosamides/streptogramins, the numbers were 0.0% vs 0.4% (*P* = .5) and 8.4% vs 89.8% (*P* = .6), respectively (Supplementary Table 1). A similar result was obtained when the analysis was carried out from 0 to 12 months of age (Supplementary Figure 3 and Supplementary Table 2-5).

Table 1 shows the characteristics of the study population according to the use of antibiotics. Children who had been treated with antibiotics during early childhood, were associated with parents with slightly higher BMI than children who had not been treated. In addition, use of antibiotics was associated with lower educational level of the family, a mother that was more likely to smoke, and a duration of breastfeeding that tended to be lower. Likewise, parity, short gestational age, caesarian section, and birth weight were associated with the use of antibiotics during early childhood. Table 2 shows the characteristics for the children developing type 1 diabetes: type 1 diabetes was associated with higher maternal pre-pregnancy BMI, higher paternal BMI and a type 1 diabetes diagnosis in the mother and was inversely associated with weekly gestational weight gain. We did not see any association between caesarian section or duration of breastfeeding and the risk of development of type 1 diabetes in childhood.

3.1 | Antibiotics and childhood type 1 diabetes

The result of the unadjusted analyses showed that exposure to antibiotics from 0 to 24 months of age was not statistically significantly

TABLE 1 Characteristics of the study population according to use of antibiotics during infancy (0-24 months of age)

	n[a]	Antibiotics during infancy		P-value[b]
		Yes Mean ± SD or %	No Mean ± SD or %	
Maternal pre-pregnancy BMI (kg/m^2)	84 748	23.7 ± 4.4	23.3 ± 4.0	<.0001
Paternal BMI (kg/m^2)	62 275	25.2 ± 3.2	25.0 ± 3.1	<.0001
Maternal age at birth (years)	91 998	30.3 ± 4.3	30.6 ± 4.3	.08
Maternal diabetes	83 444			
Type 1 diabetes		0.3	0.2	.3
Type 2 diabetes		0.1	0.0	
Gestational diabetes		0.7	0.7	
No maternal diabetes		98.9	99.1	
Family education/occupational class (%)	81 688			
Highest level		66.4	71.4	<.0001
Middle level		30.2	25.7	
Lowest level		3.5	3.0	
Parity	86 108			
0 prior births (%)		46.6	47.8	.001
≥ 1 (%)		53.4	52.2	
Weekly gestational weight gain (kg)	69 247	0.38 ± 0.2	0.37 ± 0.1	<.0001
Smoking in pregnancy	85 935			<.0001
No		72.9	77.2	
1–10 cigarettes per day		20.5	17.6	
>10 cigarettes per day		6.6	5.3	
Cesarean section	91 860			<.001
Yes (%)		15.8	13.9	
No (%)		84.2	86.1	
Gestational age at birth, (days)	91 998	280.0 ± 12.7	280.7 ± 12.2	<.0001
Birth weight (kg)	91 998	3.6 ± 0.6	3.6 ± 0.6	<.0001
Breastfeeding (any breastfeeding in weeks, %)	59 172			
0.0-19.9		31.7	24.8	<.0001
20.0-31.9		18.1	17.4	
32.0-39.9		21.1	22.9	
40.0-95.0		29.1	34.8	

[a]n is the total number of individuals with available information on the variables in the left column and use of antibiotics during infancy.
[b]*P*-values from χ^2 or *t* test.

associated with the incidence of childhood type 1 diabetes (HR 1.12, 95% CI 0.87-1.43) (Table 3). Multivariate adjustment did not alter the result markedly (HR 1.26, 95% CI 0.89-1.79). We did not find an association between the number of antibiotic courses and type 1 diabetes development, when analyzing for one (HR 1.31, 95% CI 0.87-1.99), two (HR 0.99, 95% CI 0.61-1.63) or 3 or more (HR 1.42, 95% CI 0.95-2.11) treatments. Also, the result was not influenced by the type of antibiotics administered. Regarding ever users vs never users, the adjusted HRs were penicillin/beta-lactam antibiotics 1.30 (95% CI 0.92-1.85) and macrolides/lincosamides/streptogramins 1.29 (95% CI, 0.75-2.22) while there were not enough cases for analysis among children that received sulfonamides/trimethroprim. The corresponding numbers for only users were 1.27 (95% CI 0.89-1.81) and 0.61 (0.19-1.97), respectively.

The test for proportional hazard showed no significant time varying difference in the associations between the different measures of antibiotics during early childhood and the risk of development of type 1 diabetes (data not shown). Furthermore, we found no interaction between the use of antibiotics in early childhood and delivery by caesarian section for the risk of developing type 1 diabetes in childhood (data not shown).

Throughout the results, there is a tendency toward higher HR values with the use of antibiotics, including a dose effect and type of antibiotic. It is therefore possible that a higher statistical power of the study could reveal an effect of antibiotics on type 1 diabetes development.

TABLE 2 Characteristics of the study population according to type 1 diabetes in childhood

	n[a]	Type 1 diabetes in childhood or adolescence		P-value[b]
		Yes Mean ± SD or %	No Mean ± SD or %	
Maternal pre-pregnancy BMI (kg/m)	84 748	24.0 ± 4.3	23.6 ± 4.3	<0.0001
Paternal BMI (kg/m)	62 275	25.3 ± 3.5	25.2 ± 3.2	0.04
Maternal age at birth (years)	91 998	29.8 ± 4.4	30.4 ± 4.2	0.6
Maternal diabetes	83 458	100%	100%	
Type 1 diabetes		2.3	0.2	<0.0001
Type 2 diabetes		0.3	0.1	
Gestational diabetes		0.0	0.7	
No maternal diabetes		97.4	99.0	
Family education/occupational class (%)	81 688			
Highest level		62.3	67.9	0.08
Middle level		32.7	28.8	
Lowest level		5.0	3.3	
Parity	86 108			0.6
0 prior births		48.3	46.9	
(≥1) (%)		51.7	53.1	
Weekly gestational weight gain (kg)	69 247	0.37 ± 0.17	0.38 ± 0.14	0.004
Smoking in pregnancy, yes (%)	85 949			
No		76.5	74.2	0.2
1–10 cigarettes per day		16.1	19.7	
>10 cigarettes per day		7.4	6.2	
Cesarean section	91 860			0.8
Yes (%)		14.6	15.2	
No (%)		85.4	84.8	
Gestational age at birth, (days)	91 998	279.7 ± 12.2	280.2 ± 12.6	0.5
Birth weight (kg)	91 998	3.6 ± 0.6	3.6 ± 0.6	0.5
Breastfeeding (any breastfeeding in weeks, %)	59 172			
0.0–19.9		34.3	29.7	0.5
20.0-31.9		15.2	17.9	
32.0–39.9		20.1	21.6	
40.0–95.0		30.4	30.8	

[a]n is the total number of individuals with available information on the variables in the left column and use of antibiotics during infancy.
[b]*P*-values from χ^2 or *t* test.

4 | DISCUSSION

This nationwide cohort study, based on information from 75 615 mother-child dyads, found no statistically significant association of the use of antibiotics in infants from 0 to 24 months of age and subsequent development of childhood type 1 diabetes. Furthermore, we did not find any association between the number of antibiotic courses, the type of antibiotics used, or interactions between the use of antibiotics during early childhood and cesarean section on the risk for development of childhood type 1 diabetes.

There are substantial differences in pediatric antibiotic use across industrialized countries,[17] but in general, antibiotics are widely prescribed in early childhood, and an incidence rate of antibiotic treatment of 82.7% was observed in a Danish study among children younger than 24 months of age in 2012.[18] Although a more restrictive use of antibiotics has since been applied, we still found that 64.8% used antibiotics during the first 2 years of life, which is much higher than in for instance the Norwegian Mother and Child Cohort Study where 31.8% used antibiotics from 0 to 18 month of age. Considering this intense use of antibiotics in childhood, we found it important to investigate any possible association with type 1 diabetes.

Microbiota colonization of the child's intestine already starts prenatally[19] and occurs particularly during childbirth and continues to develop during the first 4 years of life, influenced by the diet and

TABLE 3 HR of the association between the use of antibiotics during infancy (0-24 months of age) and type 1 diabetes in childhood

Exposure	HR (95% CI) for type 1 diabetes unadjusted	HR (95% CI) for type 1 diabetes, adjusted	HR (95% CI) for type 1 diabetes, adjusted for breastfeeding
N	91 998	75 615	50 924
Antibiotics during infancy			
Yes	1.12 (0.87; 1.43)	1.03 (0.79; 1.35)	1.26 (0.89; 1.79)
No	1.00	1.00	1.00
Antibiotic courses during infancy			
0	1.00	1.00	1.00
1	1.08 (0.80; 1.46)	1.07 (0.77; 1.49)	1.31 (0.87; 1.99)
2	0.93 (0.66; 1.33)	0.86 (0.58; 1.27)	0.99 (0.61; 1.63)
≥3	1.26 (0.95; 1.67)	1.10 (0.80; 1.51)	1.42 (0.95; 2.11)
Antibiotics during infancy by type, ever user			
Penicillins/beta-lactam antibacterials (J01C)	1.13 (0.89; 1.45)	1.05 (0.80; 1.38)	1.30 (0.92; 1.85)
No antibiotics during infancy	1.00	1.00	1.00
Sulfonamides/trimethoprim (J01E)	–	–	–
No antibiotics during infancy	–	–	–
Macrolides/lincosamides/ streptogramins (J01F)	1.09 (0.73; 1.64)	0.99 (0.63; 1.55)	1.29 (0.75; 2.22)
No antibiotics during infancy	1.00	1.00	1.00
Antibiotics during infancy by type, only user			
Penicillins/beta-lactam antibacterials (J01C)	1.13 (0.88; 1.45)	1.04 (0.78; 1.37)	1.27 (0.89; 1.81)
No antibiotics during infancy	1.00	1.00	1.00
Sulfonamides/trimethoprim (J01E)	–	–	–
No antibiotics during infancy	–	–	–
Macrolides/lincosamides/ streptogramins (J01F)	0.80 (0.39; 1.65)	0.58 (0.23; 1.43)	0.61 (0.19; 1.97)
No antibiotics during infancy	1.00	1.00	1.00
1 No children exposed to J0E develops type 1 diabetes			

other environmental factors, including antibiotics. Postnatal antibiotic treatment could disturb the establishment of the microbiota in the child, and has been shown to reduce gut microbiota diversity, which in some cases is associated with later adverse health outcome.[20]

Our study found no significant association between the use of antibiotics in the first 2 years of life and risk of childhood type 1 diabetes. This is in accordance with the Norwegian Mother and Child Cohort Study investigating the use of antibiotics from 0 to 18 month of age,[4] and with the study of Hviid et al.[13] However, there could be an effect of antibiotics on development of type 1 diabetes in specific settings: *certain antibiotic groups* used by the mother before pregnancy and later by the child and *more than seven courses* of antibiotic treatment was related to greater risk of type 1 diabetes (OR = 1.66, 95% CI 1.24-2.24, P = .001).[6] *Five or more antibiotic prescriptions* in the first 2 years of life was likewise associated with a higher odds ratio of type 1 diabetes at 1.35 (95% CI 1.10-1.64) in a previous Danish study.[11] Moreover, *broad-spectrum antibiotics* during the first 2 years of life has been shown to be associated with an increased risk of childhood type 1 diabetes in children delivered by *cesarean section*[9](Figure 1). Three previous Danish studies[9,11,13] included some of the same individuals as the present study. However, we were not able to confirm the associations identified. In this study, many more confounders were available for the analysis, which reduced the risk of unmeasured confounding. On the other hand, our cohort is smaller, and might not have sufficient power for demonstrating all possible associations.

The strength of our study is that the comprehensive data collected from interviews during and after pregnancy made it possible to adjust for many potential confounders, and that the prospective design eliminated recall bias. The children's type 1 diabetes diagnosis was obtained through the Danish National Patient Registry[21] and the use of antibiotics from the Danish National Prescription Registry,[22] all

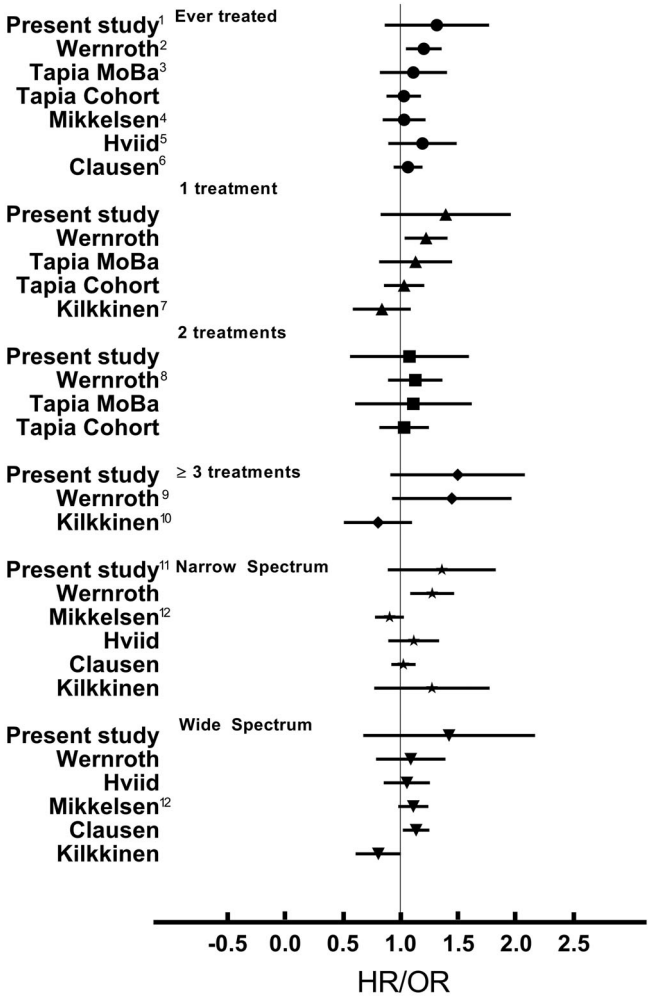

FIGURE 1 Forest plot of relative risk of developing type 1 diabetes following antibiotic treatment early in life. The results from the present study are marked "present study" and where comparable results from the literature exist, they are shown. Small differences between the studies exist, which exclude a direct comparison (some studies express results as hazard ratio while other use odds ratio, for instance). Antibiotic exposure was recorded [1]0 to 24 months, [2]0 to 12 months, [3]0 to 18 months, [4]0 to 16 years, [5]0 to 9 years, [6]0 to 2 years, [7]0 to 6 years. [8]2 to 3 treatments, [9] ≥ 4 treatments, [10]4 to 7 treatments, [11]"ever" treated, [12]1 to 4 treatments

linked by the CPR register. Further, our endpoint was type 1 diabetes and not development of autoantibodies, which is commonly used as marker of disease; however, not all patients positive for islet autoantibodies progress to overt type 1 diabetes. Due to the strict diagnostic criteria of type 1 diabetes, the risk of misclassification of type 1 diabetes is insignificant.

Limitations of our study are that we do not cover the prescribed antibiotics given to children during hospitalization, which has been estimated to account for 15% of all antibiotics prescribed.[23] This could affect our results, especially since infections have been linked to increased risk for type 1 diabetes development.[4,24-27] Furthermore, our study does not cover maternal use of antibiotics prenatally or during obstetric procedures (sectio, cervical cerclage, sphincter rupture, or manual placenta removal) in which national guidelines[28] requires prescription of antibiotics. Likewise, maternal antibiotic use was not taken into account. This could be relevant because the majority of antibiotics are found in breast milk, which could therefore affect the developing microbiota in the child. Finally, we only identified 322 cases of type 1 diabetes from our mother-child dyads, which only gives us a moderate statistical power and confounders, which are still unidentified or unmeasured, cannot be fully excluded in observational studies. The use of antibiotics reflects the occurrence of underlying infection. Thus, the possible effect of antibiotics on type 1 diabetes can also be due to infection alone, which we are not able to account for. Several examples of infections associated with type 1 diabetes are described in the literature.[29] In the TEDDY study, respiratory infections[30,31] are identified as a pathogenic factor and enteroviral infections have also been linked to disease.[32-34] Other studies have not found such an association.[4,35]

To conclude, our study suggests that the use of antibiotics in the first 2 years of life is not associated with later development of childhood type 1 diabetes.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

J.C.A and K.H.A designed the study. C.S.M performed the statistical analysis. J.C.A, C.S.M, and K.H.A analyzed the data. J.C.A drafted the manuscript. All authors contributed to the interpretation and the critical revision and approved the final version of the manuscript. C.S.M, T.J and K.H.A had full access to all the data in the study and take responsibility of the data and the accuracy of the data analysis.

ETHICS APPROVAL STATEMENT

The pregnant women participating in the study gave written, informed consent at enrolment and later follow-up surveys. The establishment of the cohort was approved under ref. no (KF) 01-471/94 by the Committee on Biomedical Research Ethics. The Danish Data Protection Agency approved the data collection of the cohort.

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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