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ORIGINAL RESEARCH ARTICLE

Maternal and Early-Life Exposure to Antibiotics and the Risk of Autism and Attention-Deficit Hyperactivity Disorder in Childhood: a Swedish Population-Based Cohort Study

Lembris L. Njotto[1,2] · Johanna Simin[3] · Romina Fornes[3] · Ingvild Odsbu[4] · Isabelle Mussche[5] · Steven Callens[6,7,8] · Lars Engstrand[3] · Robin Bruyndonckx[1,9] · Nele Brusselaers[3,10,11]

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Abstract

Introduction Antibiotics represent the most common type of medication used during pregnancy and infancy. Antibiotics have been proposed as a possible factor in changes in microbiota composition, which may play a role in the aetiology of autism and attention deficit/hyperactivity disorder (ADHD). Our aim was to investigate the association between maternal and early-life antibiotic use and autism and ADHD in childhood.

Methods This Swedish nation-wide population-based cohort study included all first live singleton births ($N = 483{,}459$) between January 2006 and December 2016. The association of dispensed antibiotics with autism and ADHD in children aged ≤ 11 years was estimated by applying multivariable logistic regression and generalised estimating equations models.

Results Of the mothers, 25.9% ($n = 125{,}106$) were dispensed ≥1 antibiotic during the exposure period (from 3 months pre-conception to delivery), and 41.6% ($n = 201{,}040$) of the children received ≥ 1 antibiotic in early life (aged ≤ 2 years). Penicillin was the most prescribed antibiotic class (17.9% of mothers, 38.2% of children). Maternal antibiotic use was associated with an increased risk of autism [odds ratio (OR) = 1.16, 95% confidence interval (CI) 1.09–1.23] and ADHD (OR = 1.29, 95% CI 1.21–1.36) in childhood. Early-life exposure to antibiotics showed an even stronger association [autism (OR = 1.46, 95% CI 1.38–1.55); ADHD (OR = 1.90, 95% CI 1.80–2.00)]. Both maternal and childhood-exposure sub-analyses suggested a dose-response relationship.

Conclusion Maternal and early-life antibiotic use was associated with an increased risk of autism and ADHD in childhood. However, differences were noted by exposure period and antibiotic classes.

Plain Language Summary

Antibiotics are commonly prescribed to pregnant women, infants, and toddlers. Antibiotic use during pregnancy may alter the maternal microbiota, which can influence the microbial colonisation of the gastrointestinal system of the foetus. It has been claimed that antibiotic use during pregnancy may have an effect on the gut-brain axis and, as a result, neurodevelopment. Neurodevelopmental disorder (NDD) is a category of illnesses characterised by functional impairments that manifest early in development. The most frequent NDDs are autism and attention-deficit/hyperactivity disorder (ADHD). In this large Swedish nation-wide study, we assessed whether antibiotic use during pregnancy and/or early in life affects the risk of developing autism and ADHD. The study found that both maternal antibiotic usage, as well as early childhood antibiotic use, were associated with an increased risk of autism and ADHD in children. These associations were altered by the quantity, type, and timing of antibiotic exposure.

Lembris L. Njotto and Johanna Simin shared first authorship.

Robin Bruyndonckx and Nele Brusselaers shared the last authorship.

✉ Nele Brusselaers
nele.brusselaers@ki.se

Extended author information available on the last page of the article

Key Points

Changes in microbiota caused by antibiotic usage have been postulated to play a role in the development of autism and attention deficit/hyperactivity disorder (ADHD).

Both maternal and early-life exposure to antibiotics were associated with increased risks of autism and ADHD.

1 Introduction

Neurodevelopmental disorders (NDDs) are chronic disabilities affecting the function of the nervous system and the brain, with onset during childhood [1]. The two most common NDDs among children are attention-deficit/hyperactivity disorder (ADHD) diagnosed in 5–10% and autism diagnosed among 1–3%, of children globally [2–4]. Several of the risk factors are known including (epi-)genetic, environmental, and psychosocial factors including pre- and perinatal factors such as preterm birth, pollutants, dietary factors and parental characteristics (obesity, age)—yet familial confounding makes it difficult to establish independent effects [2–5]. However, evidence indicates that exposure to systemic antibiotics during the developmental phase (and in early life) may pose a risk factor for these disorders through mechanisms grounded in the gut microbiome [6, 7]. The estimated antibiotic consumptions account for 80% of prescribed medications during pregnancy [8], and antibiotics are also commonly prescribed in early life and childhood in the Western world [9, 10]. In Sweden, approximately 10–12% of women receive antibiotics during each trimester of pregnancy separately [11]. Since the maternal microbiome influences the offspring's microbiome, exposure to antibiotics during the perinatal period and early life is believed to impact the child's microbiome biodiversity, directly, and indirectly through changes in the maternal microbiome [12, 13]. This could influence metabolism, bone growth, immunological functions, and it could potentially have effects on the developing gut-brain axis and behaviour of the child [14, 15].

Previous studies have linked maternal and early-life antibiotic use with a modestly increased risk of autism and ADHD among children, especially in different Western settings [1, 7, 16, 17]. However, several previous studies have been conducted on limited populations, and it remains unclear if the different exposure periods (i.e., pre-conception, different trimesters, and early life) could influence these outcomes. With the aim of filling these knowledge gaps, we performed this Swedish population-based cohort study to investigate the association between maternal and early-life exposure to commonly prescribed antibiotics and autism and ADHD during childhood.

2 Methods

This large population-based study was based on four Swedish nationwide registries, and included all first live singleton births ($N = 483{,}459$) between January 2006 and December 2016; and all live singleton births ($N = 1{,}095{,}645$) for the additional parity analyses. The unique Swedish personal identification number allowed for a valid data linkage across the Medical Birth Registry, Causes of Death Registry, Swedish Prescribed Drug Registry, and the National Patient Registry (inpatient and specialist outpatient care) [18]. This cohort has previously been described in more detail [19, 20], and was approved by the Regional Ethics Committee of Stockholm (2017/2423–31), which waived the need for informed consent because of the registry-nature of the data. The study accords to European General Data Protection Regulations, and the Declaration of Helsinki.

2.1 The Swedish National Health Registries Used in the Study

The Medical Birth Registry (established in 1973) contains data on > 99% of births including information from all antenatal visits, delivery, and early-life paediatric examinations [21, 22]. This registry was used to ascertain data on maternal characteristics, outcomes (maternal and offspring), lifestyle factors, and socioeconomic indicators. The Swedish Prescribed Drug Registry was used to ascertain data on all dispensed prescriptions in outpatient care since July 2005 [23]. The Swedish Causes of Death Registry, which was founded in 1952 [24], was used to acquire information on the date of death, and the main cause and underlying and contributing causes of all deaths among children. The National Patient Registry provides information on inpatient psychiatric diagnoses since 1973 and hospital-based outpatient visit data since 2001. This registry was used to ascertain data on all children diagnosed with autism and ADHD based on the Swedish version of the International Classification of Diseases 10th edition—ICD-10-SE codes (to note, only the code F90.0B is a Swedish-specific code). The in-patient care registry is nationwide complete from 1987 and onwards, with high coverage of the diagnoses [25], with 96% validity for the autism diagnosis according to a 2001–2007 validation study [26, 27]. The outpatient care registry was established in 2001. Since 1st July 2005, the Swedish Prescribed Drug

Registry has recorded individual-level data on all outpatient care drugs dispensed in Sweden with > 99% completeness [23]. Notably, the Registry contains data on both prescription and dispensing dates, but the prescription dates are only available for drugs that were actually dispensed. Details of drugs sold over the counter or administered in hospitals are not available; however, information on drugs dispensed at a hospital pharmacy is available.

2.2 Exposure

The exposure was based on filled prescriptions/dispensed drugs (referred to as drug use), ascertained from the Swedish Prescribed Drug Registry and defined by the Anatomical Therapeutic Chemical (ATC) codes for maternal antibiotic use and early-life exposure among children (different subclasses are described in Supplementary Table 1A). The ATC system codes are used for the classification of active ingredients of drugs according to the organ or system on which they act and their therapeutic, pharmacological, and chemical properties [28]. Maternal exposure was defined as having filled ≥ 1 antibiotic prescription at any time during the four exposure periods. The exposure periods were defined as follows: (1) pre-conception period as 90 days before the start of the pregnancy, (2) first trimester as 0–97 days of gestation, (3) second trimester from 98 to 202 days of gestation, and (4) third trimester from 203 days of gestation until delivery. The last menstrual period date was calculated by extracting the gestational age (as dated at ultrasound, from embryo transfer or last menstrual period) from the date of birth. As the date of birth was provided in year/month format due to privacy regulations, it was set as the 15th of each month in all the observations [29, 30]. Early-life exposure was defined as having received ≥ 1 dispensed antibiotic from birth until 2 years of age, ensuring that exposure occurred prior to the diagnosis of the outcome; and the first 2 years of life are regarded as being the most critical in the formation of a healthy microbiome [31].

The utilisation units for drugs were the number of filled prescriptions and the estimated duration of treatment, which were based on the number of defined daily doses (DDDs) per dispensed package. The DDD is "the assumed average maintenance dose per day for a drug used for its main indication in adults" as defined by the World Health Organization. The total dosage of each antibiotic class was calculated by adding all prescriptions or by estimating the number of days exposed throughout the study period [28].

We considered only systemic antibiotics in this study because these are more likely to have a systemic effect than local antibiotics.

2.3 Outcomes

The primary outcomes were autism and ADHD, the two most common NDDs among children, diagnosed before the age of 11 years [31, 32]. These outcomes were identified from the Patient Registry (Supplementary Table 1B) [25].

2.4 Covariates

Several potential confounders were a priori selected based on clinical knowledge. The maternal characteristics included country of birth (Nordic: Denmark, Norway, Sweden, Finland, Iceland, the Faroe Islands, Greenland, and Åland; or non-Nordic), delivery mode (vaginal, caesarean section), maternal age at gestation (< 25, 25–29, 30–34, or ≥ 35 years), maternal body mass index (BMI) at gestation in kg/m^2 (categorised as under-, normal-, over-weight, obese, missing) tobacco consumption (self-reported smoking and/or moist snuff use) during pregnancy (yes/no), parity (first born, second, third, and fourth or higher) and family situation (single, cohabiting or other). Child characteristics included sex, age, gestational age at birth (< 37 weeks, or ≥ 37 weeks), Apgar score at 5 min (< 7 or ≥ 7 out of 10), and size of gestational age, which was categorised into small for gestational age (SGA), appropriate for gestational age (AGA), and large for gestational age (LGA).

2.5 Statistical Analysis

Multivariable logistic regression models were used to assess the risk of autism and ADHD in children associated with maternal and early-life exposure to antibiotics, presented as adjusted odds ratios (ORs) with 95% confidence intervals (CIs), controlling for other potential covariates. The maternal and child exposure to antibiotics was assumed mutually exclusive, if not labelled otherwise. A purposeful selection strategy was employed to fit a parsimonious multivariable logistic regression model**.** A purposeful selection was applied to fit a parsimonious multivariable logistic regression model, as described by Hosmer and Lemeshow for the modelling process. Any variable with a significant univariable Wald test at the 25% significance level was chosen as a candidate for multivariable analysis. Iteratively, predictors that were not significant at the 5% level were removed one at a time in the multivariable model. Several models were compared using the likelihood ratio test. The impact of removing individual covariates on parameter estimates for another important variable was investigated. A change in a parameter estimate above a priori defined threshold (i.e., more than 20% adjustment in coefficient magnitude of another variable) was considered indicative of the omitted variable being a true confounder and the variable was retained in the model.

Furthermore, reasonable interactions with the variables of interest were examined using likelihood ratio tests from a biological or clinical standpoint. These included the interaction between maternal age and antibiotic usage, mode of delivery and maternal age, antibiotic usage and maternal country of birth, and antibiotic usage by mothers interacting with antibiotic usage by toddlers under the age of two.

Furthermore, an extra category was created for missing values because otherwise, a large proportion of the cohort would have to be excluded (6.8% missingness for BMI) and due to the cohort size, it was not computationally feasible to impute the missing values.

Supplementary analyses were conducted for all pregnancies. Due to correlated data Generalised Estimating Equations (GEE) models were fit using independent, exchangeable, and unstructured working correlation structures. The exchangeable correlation structure model gave the most comparable naïve and robust standard error estimates and had the lowest quasi-likelihood under the independence model criterion (QIC).

To examine whether the multivariable logistic regression model fit the data well, a formal test such as Hosmer and Lemeshow statistics was used [33]. The presence of multicollinearity was assessed using the variance inflation factors (VIF). For GEE, quasi-likelihood under the QIC was used to examine the most suitable working correlation structure. All analyses were conducted with R version 4.1.0 [34].

3 Results

The initial cohort included a total of 1,099,506 Swedish singleton births. After the exclusion of 3861 stillbirths, the final cohort consisted of 1,095,645 live births (Fig. 1). Altogether, the study included 483,459 unique mothers with their first live singleton delivery between January 1st, 2006, and December 31st, 2016.

Overall, 25.9% of mothers ($n = 125{,}106$) were exposed to ≥ 1 antibiotic(s) at any time during the exposure period (from 3 months preconception and onwards) (Table 1). In total, 201,040 children (41.6%) received out-patient antibiotics during the first 2 years of life (Supplementary Table 2). Most mothers were Nordic (78.8%), and cohabiting (87.4%) in both groups. More women were aged < 25 years among the antibiotic users than non-users (28.2% vs 21.3%). Vaginal delivery was marginally less common among antibiotic users (79.3%) than non-users (81.9%). Most mothers had a normal BMI in both groups. Most mothers reported no tobacco consumption during pregnancy, although the frequency of use was slightly higher among antibiotic users (9.1%) than non-users (5.9%). Among first singleton births, boys were slightly more represented (51.5%) than girls (48.5%). Most of the new-borns (98.1%) received an Apgar score of ≥ 7, the majority (94.2%) were delivered at ≥ 37 weeks, and 94.7% were AGA.

Of all children, 1.0% ($n = 4951$) were diagnosed with autism, with 78.4% ($n = 3882$) boys and 21.6% ($n = 1069$) girls. Overall, 1.2% ($n = 5611$) were diagnosed with ADHD, with 75.9% ($n = 4259$) boys and 24.1% ($n = 1352$) girls (Supplementary Table 3). Children diagnosed with ADHD had somewhat younger mothers (37.5% were aged < 25 years) compared to those diagnosed with autism (26% were aged < 25 years).

The different antibiotic classes prescribed to mothers during pre-conception and the different trimesters are shown in Fig. 2a and b. Of the various antibiotic classes, penicillin beta-lactam antibacterials were the most frequently prescribed antibiotic class across all exposure periods as well as during early life exposure, corresponding to 17.9% of mothers and 38.2% of children (Supplementary Table 4). The trend for penicillin beta-lactam antibacterials and other antibiotic classes increased in the first and second trimesters, yet decreased during the third. In contrast, the use of tetracyclines, nitroimidazole, sulphonamides/trimethoprim, and macrolides decreased during the exposure period. In contrast, the use of non-penicillin beta-lactams was slightly lower during the pre-conception period compared to all other classes, but increased throughout the pregnancy.

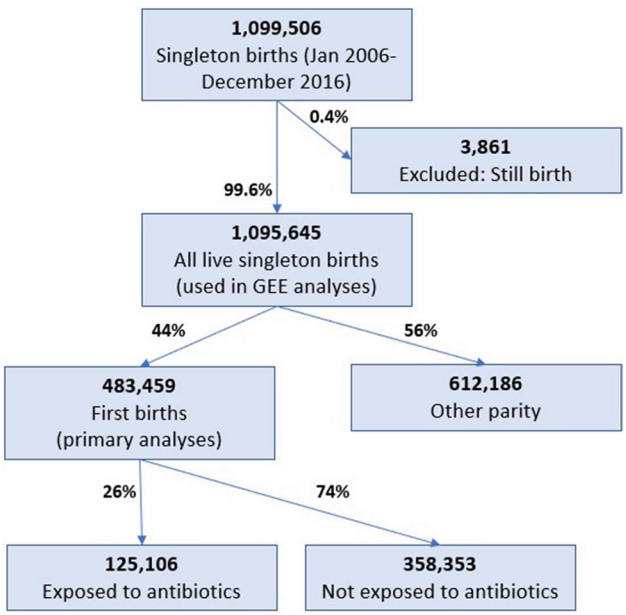

Fig. 1 Flowchart of the study cohort displaying the data filtration process and the final cohort selection, including sample size. *GEE* Generalised Estimating Equations

3.1 Association with Autism and ADHD

Compared to children with no maternal antibiotic exposure, autism (OR = 1.16, 95% CI 1.09–1.23) and ADHD (OR = 1.29, 95% CI 1.21–1.36) occurred more frequently among children whose mothers used systemic antibiotics at any time during the exposure period (Table 2). Compared to children without early-life exposure, early-life antibiotic use was more strongly associated with ADHD (OR = 1.90, 95% CI 1.80–2.00) than autism (OR = 1.46, 95% CI 1.38–1.55), independent of maternal antibiotic use (Table 2, Supplementary Table 5). During the model development process,

Table 1 Characteristics of mothers with their first live singleton delivery between January 1st, 2006, and December 31st, 2016 included in the cohort, by exposure to maternal antibiotic use (3 months pre-conception and/or during pregnancy)

	No antibiotics *n* (%)	Antibiotics *n* (%)	Total *n* (%)
	358,353 (74.1)	125,106 (25.9)	483,459 (100.0)
Maternal characteristics			
Mode of delivery			
Vaginal	293,562 (81.9)	99,238 (79.3)	392,800 (81.2)
Caesarean (acute or elective)	64,791 (18.1)	25,868 (20.7)	90,659 (18.8)
Maternal age at delivery, in years			
< 25	764,72 (21.3)	35,221 (28.2)	111,693 (23.1)
25–29	131,833 (36.8)	42,580 (34.0)	174,413 (36.1)
30–34	106,202 (29.6)	32,451 (25.9)	138,653 (28.7)
> 34	43,846 (12.2)	14,854 (11.9)	58,700 (12.1)
Smoking/snuff status			
No	337,273 (94.1)	113,748 (90.9)	451,021 (93.3)
Yes	21,080 (5.9)	11,358 (9.1)	32,438 (6.7)
Maternal country of birth			
Non-Nordic	75,712 (21.1)	26,670 (21.3)	102,382 (21.2)
Nordic	282,641 (78.9)	98,436 (78.7)	381,077 (78.8)
Family situation			
Cohabiting	316,201 (88.2)	106,402 (85.0)	422,603 (87.4)
Single	7269 (2.0)	3668 (2.9)	10,937 (2.3)
Other	19,125 (5.3)	9330 (7.5)	28,455 (5.9)
Missing	15,758 (4.4)	5706 (4.6)	21,464 (4.4)
Maternal body mass index at gestation			
Underweight	39,801 (11.1)	14,393 (11.5)	54,194 (11.2)
Normal weight	185,309 (51.7)	61,537 (49.2)	246,846 (51.1)
Overweight	76,040 (21.1)	26,735 (21.4)	102,775 (21.3)
Obese	33,004 (9.2)	13,664 (10.9)	46,668 (9.6)
Missing	24,199 (6.7)	8777 (7.0)	32,976 (6.8)
Child characteristics			
Sex			
Boy	184,366 (51.4)	64,709 (51.7)	249,075 (51.5)
Girl	173,987 (48.6)	60,397 (48.3)	234,384 (48.5)
Apgar scored at 5 min			
< 7	5424 (1.5)	2183 (1.7)	7607 (1.6)
≥ 7	351,889 (98.2)	122,536 (97.9)	474,425 (98.1)
Missing	1040 (0.3)	387 (0.3)	1427 (0.3)
Pre-term birth (< 37 weeks)			
No	338,238 (94.4)	117,389 (93.8)	455,627 (94.2)
Yes	20,115 (5.6)	7717 (6.2)	27,832 (5.8)
Size for gestational age			
Small	11,814 (3.3)	4207 (3.4)	16,021 (3.3)
Appropriate (normal)	339,794 (94.8)	118,013 (94.3)	457,807 (94.7)
Large	6272 (1.8)	2685 (2.1)	8957 (1.9)
Missing	473 (0.1)	201 (0.2)	674 (0.1)

no assessed potential interactions reached statistical significance—and therefore no interaction factors needed to be incorporated in the models.

3.2 Association with Other Risk Factors

Several maternal and child characteristics appeared to affect the outcomes (Table 2, Supplementary Table 3), including maternal age, region, mode of delivery, smoking, preterm birth, Apgar scores and SGA. Furthermore, autism (OR = 3.4, 95% CI 3.14, 3.60) and ADHD (OR = 2.9 95% CI 2.73, 3.08) were more common among boys than girls.

3.3 Antibiotic Classes

Compared to children with no maternal exposure to antibiotics, maternal penicillin and non-penicillin beta-lactam exposure were associated with higher odds of autism and ADHD (Table 3). Maternal exposure to all different antibiotic classes was associated with ADHD, apart from quinolones and nitroimidazole. Sulphonamides and trimethoprim showed the strongest association (OR = 1.88, 95% CI 1.54–2.29). In contrast, early-life exposure to all different antibiotic classes was associated with higher odds of autism and ADHD, apart from nitroimidazole.

3.4 Different Exposure Periods

Compared to children without pre-conceptional exposure, pre-conceptional exposure to antibiotics was associated with more frequently recorded ADHD (OR = 1.26, 95% CI 1.16–1.37) whilst the association with autism was marginal (OR = 1.10, 95% CI 1.00–1.21) (Table 4). Exposure to antibiotics during the first and second trimester was associated with higher risks of autism (OR = 1.22, 95% CI 1.11–1.34) and ADHD (OR = 1.29, 95% CI 1.19–1.40), compared to children with no maternal exposure to antibiotics during these periods.

3.5 Cumulative Exposure

The patterns of dose-response relationship were similar for maternal and early-life antibiotic exposure. For maternal exposure, the point estimates were highest for ≥ 3 filled prescriptions, which were associated with higher odds of autism (adjusted OR = 1.45, 95% CI 1.23–1.70) and ADHD (adjusted OR = 1.70, 95% CI 1.48–1.95) when compared to unexposed (Table 5). Similarly, among children with early-life exposure, the odds of autism and ADHD were the highest among those with a cumulative exposure of ≥ 3 weeks.

3.6 Supplementary Analysis

The results from the supplementary analyses utilising GEE models were consistent with those reported in the analyses including only first-born children (Supplementary Table 6). Overall, factors that were associated with an increased risk of child autism and ADHD in the main analysis showed the same associations in our GEE models. However, compared to the main analyses, higher parity (≥ 4) was associated with a higher risk of ADHD (OR = 1.26, 95% CI 1.16–1.37) among children.

4 Discussion

The findings of this population-based cohort study indicate that maternal and early-life antibiotic use was associated with an increased risk of autism and ADHD among children. However, we observed differences based on

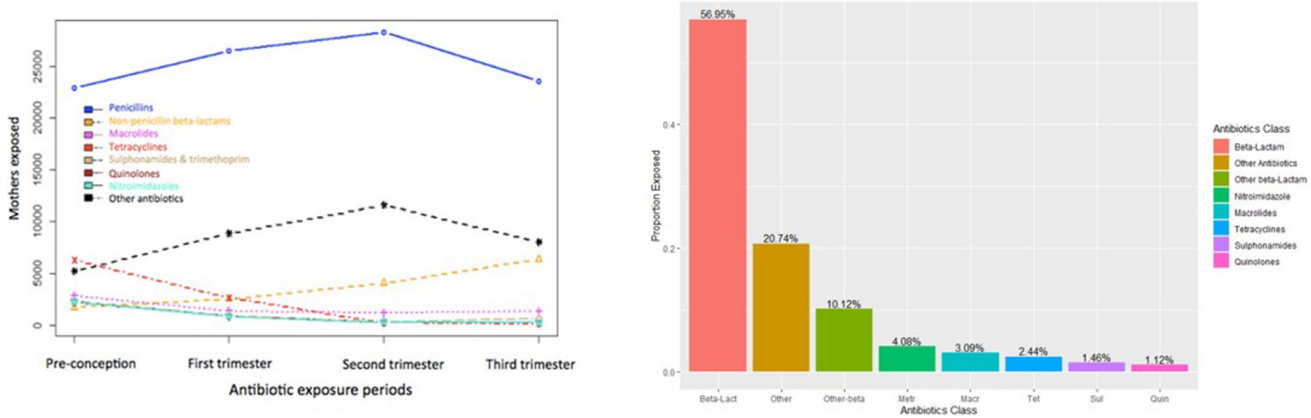

Fig. 2 Maternal antibiotic exposure, by the various exposure periods. The *y*-axis displays the maternal antibiotic consumption based on the number of women exposed. The *x*-axis displays the different time periods considered in the study. Pre-conception was defined as having received antibiotics 3 months before the conception.

Table 2 Odds ratios (ORs) with 95% confidence intervals (CIs) for child autism and attention-deficit/hyperactivity disorder (ADHD) in association with mother and child characteristics

	Child autism		Child ADHD	
	OR	95% CI	OR	95% CI
Antibiotics exposure				
Any antibiotics[a]				
Consumed by mothers	**1.16**	**(1.09–1.23)**	**1.29**	**(1.21-1.36)**
Consumed by child	**1.46**	**(1.38–1.55)**	**1.90**	**(1.80–2.00)**
Maternal characteristics				
Mode of delivery				
Caesarean (acute or elective)	**1.23**	**(1.15–1.32)**	**1.13**	**(1.05–1.21)**
Vaginal (ref)				
Maternal age (years)				
< 25	**0.91**	**(0.83–1.00)**	**1.80**	**(1.63–1.98)**
25–29	**0.81**	**(0.74—.88)**	**1.12**	**(1.01–1.23)**
30–34	**0.86**	**(0.78–0.94)**	1.00	(0.91–1.11)
> 34 (ref)				
Maternal country of birth				
Nordic	**0.62**	**(0.58–0.66)**	**1.66**	**(1.54–1.79)**
Non-Nordic (ref)				
Family situation				
Cohabits with father (ref)				
Single	**1.38**	**(1.19–1.61)**	**1.74**	**(1.53–1.98)**
Other	**1.29**	**(1.16–1.44)**	**1.50**	**(1.37–1.64)**
Missing	0.88	(0.74–1.04)	0.95	(0.81–1.10)
Smoking/snuff status				
Yes	**1.28**	**(1.15–1.41)**	**1.95**	**(1.81–2.10)**
No (Ref)				
Maternal BMI at gestation				
Underweight	0.98	(0.89–1.08)	**0.89**	**(0.81–0.98)**
Normal weight (ref)				
Overweight	**1.15**	**(1.07–1.24)**	**1.15**	**(1.07–1.23)**
Obese	**1.48**	**(1.35–1.61)**	**1.66**	**(1.54–1.80)**
Missing	**1.39**	**(1.22–1.59)**	**1.65**	**(1.46–1.85)**
Child characteristics				
Sex				
Boy	**3.36**	**(3.14–3.60)**	**2.90**	**(2.73–3.08)**
Girl (ref)				
Pre-term birth (< 37 weeks)				
Yes	**1.35**	**(1.22–1.50)**	**1.19**	**(1.08–1.32)**
No (ref)				
Apgar score at 5 min				
< 7	**1.52**	**(1.28–1.80)**	1.00	(0.82–1.22)
≥ 7 (Ref)				
Missing	1.01	(0.63–1.62)	**1.82**	**(1.30–2.55)**
Size for gestational age				
Small (< 2500 g)	**1.32**	**(1.16–1.51)**	**1.4**	**(1.24–1.59)**
Appropriate (normal) (ref)				
Large (macrosomia> 4500 g)	1.01	(0.83–1.24)	1.08	(0.90–1.29)
Missing	1.74	(1.00–3.02)	**1.80**	**(1.09–2.96)**

Bold values indicate statistically significant p values ($p < 0.05$)

BMI body mass index

[a]Unexposed was the reference

the timing of the exposure period and the various antibiotic classes. Furthermore, the risk of autism and ADHD increased with cumulative exposure to antibiotics indicating a dose-response relationship. Overall, maternal antibiotic use was more strongly associated with ADHD (OR = 1.29, 95% CI 1.21–1.36) than autism (OR = 1.16, 95% CI 1.09–1.23). The associated risks seemed even higher for early-life exposure to antibiotics, for both ADHD (OR = 1.90, 95% CI 1.80–2.00) and autism (OR = 1.46, 95% CI 1.38–1.55). Moreover, exposure to antibiotics during the pre-conception period was associated with the risk of ADHD, yet only marginally with autism. As expected, the use of contraindicated antibiotics such as tetracyclines and sulphonamides/trimethoprim decreased during the exposure period.

The largest strength of our study was the nationwide and population-based design, increasing statistical power and limiting the risk of selection bias, facilitating the generalisability of our results. In addition, the study was based on an a priori defined study protocol, taking advantage of the high-quality Swedish health care registries with valid data on the exposure, outcomes, and covariates. The exposure was ascertained from the virtually 100% complete Swedish Prescribed Drug Registry for outpatient care filled drugs [18].

Antibiotics are not sold over the counter in Sweden, minimising the risk of exposure misclassification. Although full compliance cannot be ascertained, the primary non-compliance can be ruled out given that all prescriptions were dispensed [35]. In addition, we restricted our analyses to early-life antibiotic use during the first 2 years to limit the effect of reverse causation (in which the exposure occurred after the first signs and symptoms of our outcomes). We opted for a statistical approach dividing exposure by trimester, and not as "time-varying" exposure, since this trimester-division is very relevant for clinical practice. In addition, we have no information on the prescribed daily dose, needed to define exposure periods. Whereas we could adjust for several potential confounding factors, some variables such as obesity are underreported in the registries. Here, maternal obesity and over-weight were accurately measured because we calculated the BMI, yet weight information was missing in 6.8% of women. Tobacco consumption could potentially be underreported, given that these variables were based on self-reported data. Generalisability to non-Nordic populations with different prevalence of autism and ADHD may be limited, particularly considering the strong hereditary nature of both disorders.

Furthermore, some underreporting of ADHD and autism is likely because these disorders may also be diagnosed at a later age (maximal follow-up to 11 years). Therefore, we expect to cover the most severe cases and we also acknowledge the likely underdiagnosis/underreporting

Table 3 Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for child autism and attention-deficit/hyperactivity disorder (ADHD) assessing the maternal and early-childhood exposure to antibiotics, by various antibiotic classes

	Child autism		Child ADHD	
	OR	95% CI	OR	95% CI
Antibiotic classes dispensed by mothers				
Ref (unexposed)				
Beta-lactam antibacterials, penicillin	**1.16**	**(1.09–1.25)**	**1.14**	**(1.07–1.22)**
Non-penicillin beta-lactam antibacterials	**1.29**	**(1.11–1.49)**	**1.48**	**(1.31–1.68)**
Macrolides	1.22	(0.99–1.51)	**1.30**	**(1.09–1.56)**
Tetracyclines	1.13	(0.93–1.37)	**1.33**	**(1.13–1.56)**
Sulphonamides and trimethoprim	1.18	(0.90–1.55)	**1.88**	**(1.54–2.29)**
Quinolones	1.07	(0.80–1.44)	1.17	(0.91–1.51)
Nitroimidazole	1.13	(0.93–1.37)	1.17	(0.99–1.39)
Other antibiotics	1.10	(0.99–1.23)	**1.20**	**(1.09–1.32)**
Antibiotic classes received by the children in early childhood				
Ref (unexposed)				
Beta–lactam antibacterials, penicillin	**1.42**	**(1.35–1.51)**	**1.88**	**(1.78–1.99)**
Non-penicillin beta-lactam antibacterials	**1.15**	**(1.01–1.30)**	**1.14**	**(1.01–1.28)**
Macrolides	**1.26**	**(1.10–1.44)**	**1.35**	**(1.20–1.52)**
Nitroimidazole	**2.08**	**(1.39–3.12)**	1.19	(0.73–1.94)
Other antibiotics	**1.34**	**(1.16–1.54)**	**1.37**	**(1.20–1.56)**

All analyses were adjusted for the mode of delivery, maternal age, maternal country of birth, maternal family situation, cigarette/snuff consumption, maternal body mass index, sex of the child, preterm birth (< 37 weeks), Apgar at 5 min (< 7), and size for gestational age

Bold values indicate statistically significant p values ($p < 0.05$)

Table 4 Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for the association between maternal antibiotic use and autism and attention-deficit/hyperactivity disorder (ADHD) in childhood stratified by the exposure periods

	Child autism		Child ADHD	
	OR	95% CI	OR	95% CI
Reference: unexposed in the various periods				
Pre-conception (3 months before pregnancy)	1.10	(1.00–1.21)	**1.26**	**(1.16–1.37)**
First trimester	**1.22**	**(1.11–1.34)**	**1.21**	**(1.11–1.32)**
Second trimester	**1.17**	**(1.06–1.28)**	**1.29**	**(1.19–1.40)**
Third trimester	**1.16**	**(1.05–1.28)**	**1.24**	**(1.14–1.36)**

All analyses were adjusted for the mode of delivery, maternal age, maternal country of birth, maternal family situation, cigarette/snuff consumption, maternal body mass index, sex of the child, preterm birth (< 37 weeks), Apgar at 5 min (< 7), and size for gestational age

of these outcomes, particularly in girls [36, 37]. Nevertheless, we would expect this potential misclassification due to underreporting to occur at random between the groups, leading to a dilution (underestimation) of the effect because of nondifferential misclassification [38]. Yet, the opposite could also be true, with a differential over-diagnosing particularly of ADHD in those families most frequently exposed to antibiotics [38]. Especially with the ADHD association, we cannot rule out some confounding by medicalisation: some parents and physicians might want to use antibiotics 'just in case' an infection might be bacterial (although it is probably viral), and the same preference for medications 'just in case' might cause greater use of ADHD medications for social behaviour problems, and thereby an overdiagnosis of ADHD. With autism, protective parenting might increase the chance of overuse of antibiotics. The relatively similar effects of antibiotic exposure during all time-periods, including the pre-conception period, might also support confounding by medicalisation.

Supplementary analyses were performed applying GEE models because the multivariable logistic regression models cannot account for the occurrence of correlation between pregnancies from the same mother. The obtained results were similar, indicating the robustness of our findings. Only among the fourth and subsequent childbirths, the effect of parity was associated with higher odds of ADHD (OR = 1.26, 95% CI 1.16–1.37), compared to the first-born child. Unfortunately, no data were available on potential neonatal exposure to antibiotics through breastfeeding [39].

Our findings are consistent with a previous meta-analysis of ten studies (published between 2016 and 2020), which found an association of antibiotic use in childhood and an increased risk of autism spectrum disorder (OR = 1.13, 95%

Table 5 Dose-response relationship between exposure to any antibiotics during pregnancy and the odds of autism and attention-deficit/hyperactivity disorder (ADHD), expressed as adjusted odds ratios (ORs) and 95% confidence intervals (CIs)

	Child autism		Child ADHD	
	OR	95% CI	OR	95% CI
Maternal dose-response				
Number of antibiotic courses (continuous)				
	1.11	**(1.08–1.15)**	**1.16**	**(1.13, 1.19)**
Categorised number of antibiotics courses				
Non-users (ref)				
One prescription	**1.14**	**(1.05–1.23)**	**1.25**	**(1.17–1.34)**
Two prescriptions	**1.38**	**(1.22–1.56)**	**1.49**	**(1.33–1.66)**
Three or more prescriptions	**1.45**	**(1.23–1.70)**	**1.70**	**(1.48–1.95)**
Cumulative antibiotic duration (days) (continuous)				
	1.01	(1.00–1.01)	1.01	(1.01–1.01)
Categorised cumulative antibiotic duration				
Non-users (ref)				
1 week	1.09	(0.99–1.21)	**1.27**	**(1.16–1.39)**
2 weeks	**1.26**	**(1.12–1.41)**	**1.36**	**(1.23–1.51)**
3 weeks	**1.35**	**(1.19–1.53)**	**1.29**	**(1.15–1.46)**
More than 3 weeks	**1.33**	**(1.15–1.55)**	**1.63**	**(1.43–1.85)**
Child dose-response				
Number of antibiotic courses				
	1.08	**(1.07–1.09)**	**1.10**	**(1.10–1.11)**
Categorised number of antibiotics courses				
Non-users (ref)				
One prescription	**1.29**	**(1.20–1.39)**	**1.54**	**(1.44–1.65)**
Two prescriptions	**1.45**	**(1.32–1.58)**	**1.94**	**(1.79–2.11)**
Three or more prescriptions	**1.84**	**(1.70–1.99)**	**2.66**	**(2.48–2.86)**
Cumulative antibiotic duration (days) (continuous)				
	1.02	**(1.02–1.02)**	**1.02**	**(1.02–1.02)**
Categorised cumulative antibiotic duration				
Non-users (ref)				
1 week	**1.32**	**(1.23–1.41)**	**1.63**	**(1.53–1.74)**
2 weeks	**1.50**	**(1.37–1.63)**	**2.10**	**(1.95–2.27)**
3 weeks	**1.84**	**(1.63–2.07)**	**2.67**	**(2.40–2.96)**
More than 3 weeks	**2.15**	**(1.89–2.45)**	**2.75**	**(2.45–3.09)**

All analyses were adjusted for the mode of delivery, maternal age, maternal country of birth, maternal family situation, cigarette/snuff consumption, maternal body mass index, sex of the child, preterm birth (< 37 weeks), Apgar in 5 min (< 7), and size for gestational age

Bold values indicate statistically significant p values ($p < 0.05$)

CI 1.07–1.21) and ADHD (OR = 1.18, 95% CI 1.10–1.27, $N = 6$), yet did not distinguish between different antibiotic classes or exposure periods, and maternal exposure [40]. Furthermore, a Finnish population-based study including 990,098 live births between 1996 and 2012 found a modestly increased association with in utero and early-life exposure to antibiotics and ADHD later in childhood [41]. Our estimates suggest that early-life exposure is associated with higher odds of ADHD and autism than exposure to maternal antibiotic use. Of note, pre-conceptional antibiotic exposure was associated with an increased risk of ADHD in children, whereas the association with autism was marginal.

Furthermore, we found that maternal use of penicillin and other non-penicillin beta-lactam antibacterials were associated with an increased risk of autism, whereas sulphonamides and trimethoprim suggested the highest risk of ADHD. In contrast, early-life exposure to all antibiotic classes showed an increased risk of autism and ADHD, apart from nitroimidazole, for which no association was found with ADHD.

Current evidence indicates that the child's microbiome is largely inherited from the mother [12]. These findings, however, appear to highlight that the two first years of life are critical in the formation of a healthy microbiome in the offspring. Furthermore, our findings also suggest that boys (exposed to antibiotics) have a higher risk of being diagnosed with autism and ADHD than girls. This gender disparity is consistent with prior research, which shows that men are diagnosed with ADHD and autism at a higher rate and at a younger age than women [36, 37].

Overall, antibiotic use during pregnancy has been suggested as a risk factor for NDDs among children, potentially through changes in the gut microbiome [31, 32]. Our rather consistent risk increase among all antibiotic classes may indeed be more supportive of an indirect effect through microbiome changes, than a direct effect on neurodevelopment from a specific antibiotic subtype. Despite varying aetiologies, early gastro-intestinal tract symptoms (including diarrhoea, constipation, bloating) seem to be prevalent among children with autism and ADHD [42–44]. This may support a potential (bi-directional) role of the gut microbiome through dysbiosis and the gut-brain axis [31]. Whereas the effect of different antibiotic classes remains unclear, varying patterns and especially the abundance of some *Clostridia* and *Bacteroidetes* species have been reported for autism further supporting the involvement of the gut microbiome [17, 42, 45]. Moreover, the caesarean section (and being born prematurely) is associated with microbiome alterations giving rise to short- and long-term health effects in the offspring [46]. Our identified higher risk after caesarean section also supports the potential involvement of the microbiome in early-life neurodevelopment. In addition, our data showed that prematurely born children (< 37 weeks) or SGA children had a higher risk of autism and ADHD, but factors other than the microbiome are likely to be involved [46]. In a recent French cohort, children of lower socioeconomic status (SES) families appeared to present more severe intellectual impairment and received an earlier diagnosis of autism compared to children from higher SES families [47]. In Sweden the access to health care is equal to everyone, yet it is possible that SES and other sociocultural factors could

influence the utilisation of health care services. The use of antibiotics might vary across these parameters, even if prescriptions are filled [48].

To note, almost all preterm babies are exposed to antibiotics in hospital settings and during early life. In our study, we did not assess in-hospital antibiotic use, as it is not recorded in the Drug Registry. This could result in misclassification of new-borns that received only in-hospital antibiotics, potentially underestimating/diluting the effect. However, 28% of the preterm babies in our cohort received outpatient care antibiotics. In total, 5.7% of the children were born pre-term, and thus the antibiotic effect through confounding by indication (prematurity itself and early-life infections) cannot be ruled out.

Confounding by indication, genetics, and other confounding factors cannot be entirely ruled out with the present design and data sources, as the Swedish prescribed drug registry does not record indications. It has been hypothesised that major infections, particularly during the third trimester, could influence foetal brain development [49]. Yet, to date, the Swedish prescribed drug registry does not contain in-patient antibiotic use. To what extent the potential effect of antibiotic exposure during pregnancy and early life differs depending on (epi-)genetic predisposition to autism and ADHD, remains to be explored [40, 50]. For both disorders, heritability estimates up to 80% have been reported, with at least 200 different genes being linked to the risk of autism spectrum disorders, and ADHD also being described as polygenic (many genes have been associated but all with small individual effects) [2, 4, 50, 51].

Antibiotic exposure was high in this cohort, with 26% of mothers and 42% of children using out-patient antibiotics during pregnancy (including 3 months pre-conception) and first 2 years of life, respectively. However, these figures are comparable to antibiotic consumption in other Nordic countries [52–55]. Whereas this study cannot determine the underlying pathophysiological mechanisms or establish causality, these results underscore that potential short- and long-term consequences for the mother and child should be considered when prescribing antibiotics during pregnancy and early life.

To conclude, our results suggest that maternal and early-life antibiotic use is associated with a higher risk of autism and ADHD. However, the risks seem to differ by the timing of exposure and antibiotic class consumed, and we found evidence for a dose-response relationship.

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Declarations

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Ethics approval This study was approved by the Regional Ethics Committee of Stockholm (2017/2423–31), which waived the need for informed consent because of the registry-nature of the data. The study accords to European General Data Protection Regulations, and the Declaration of Helsinki.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material (data transparency) De-identified data are available upon reasonable requested (contact corresponding author NB, principal investigator of the study) under the condition that all ethical and data-sharing requirements are fulfilled. Due to the registry-based nature of the data, containing clinical and personal information, it is not allowed by the National Board of Health and Welfare to deposit these data publicly.

Code availability Available by contacting the corresponding author.

Authors' contributions LLN, JS, RB and NB designed the study. Data management was conducted by LLN, RF and JS. LLN, junior statistician, conducted the statistical analyses under supervision of JS, NB as clinical epidemiologists, and senior statistician RB. All authors interpreted the results, with IM (child psychiatrist) and SC (infectious diseases/internal medicine expert) as clinical experts, and IO as expert on neurodevelopmental disorders, and LE as expert in clinical microbiology/microbiome. LLN, JS and NB drafted the manuscript, which was critically revised and approved for submission by all authors (RF, IM, SC, IO, LE, RB). All authors (LLN, JS, RB, IO, IM, SC, LE, RB, NB) agree to be personally accountable for their own contributions, and ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved and the resolution documented in the literature. All authors read and approved the final version.

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Authors and Affiliations

Lembris L. Njotto[1,2] · Johanna Simin[3] · Romina Fornes[3] · Ingvild Odsbu[4] · Isabelle Mussche[5] · Steven Callens[6,7,8] · Lars Engstrand[3] · Robin Bruyndonckx[1,9] · Nele Brusselaers[3,10,11]

1 Interuniversity Institute for Biostatistics and statistical Bioinformatics (I-BIOSTAT), Data Science Institute, Hasselt University, Diepenbeek, Belgium

2 Department of Mathematics and ICT, College of Business Education (CBE), Dar es Salaam, Tanzania

3 Department of Microbiology, Tumour and Cell Biology, Centre for Translational Microbiome Research (CTMR), Karolinska Institutet, Solnavägen 9, 171 77 Stockholm, Sweden

4 Division of Mental and Physical Health, Department of Mental Disorders, The Norwegian Institute of Public Health, Oslo, Norway

5 Child and Youth Psychiatry, Centre for Ambulatory Revalidation (CAR) Ascendre, Eeklo/Wetteren, Belgium

6 Department of General Internal Medicine, Ghent University Hospital, Ghent, Belgium

7 Global Health Institutet, Antwerp University, Antwerp, Belgium

8 Department of Head and Skin, Ghent University, Ghent, Belgium

9 Laboratory of Medical Microbiology, Vaccine and Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium

10 Global Health Institute, Antwerp University, Antwerp, Belgium

11 Department of Head and Skin, Ghent University, Ghent, Belgium