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Article

Prenatal Environmental Antibiotic Exposure and Autism Spectrum Disorder Symptoms in Children at 3 Years of Age: Findings from the Ma'anshan Birth Cohort Study

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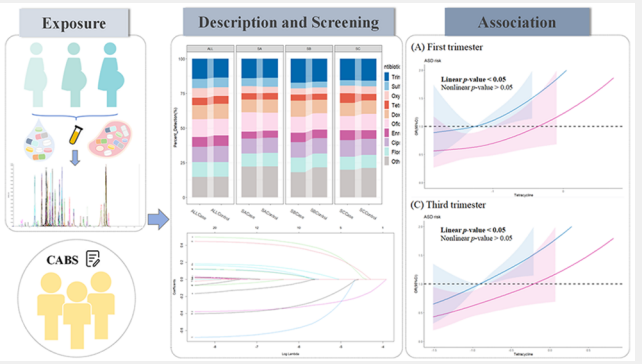
ABSTRACT: Antibiotic exposure during pregnancy may affect the neurodevelopment of children, but biomonitoring-based population studies on this class of new pollutants are lacking. We conducted a prospective birth cohort study of 2860 mother−child pairs, measured the urinary concentrations of 41 antibiotics and their two metabolites over three trimesters, and assessed children's autism spectrum disorder (ASD) symptoms at 3 years of age. We examined the associations between prenatal antibiotic exposure and children's ASD symptoms. The least absolute shrinkage and selection operator regression screened for Tetracycline and Ofloxacin as important predictors of ASD symptoms. Modified Poisson regression models revealed that maternal Tetracycline exposure throughout pregnancy increased the risk of ASD symptoms (RR: 1.66, 95% CI: 1.14, 2.40). Maternal Tetracycline exposure during the first (RR: 1.74, 95% CI: 1.13, 2.68) and third trimesters (RR: 1.86, 95% CI: 1.16, 3.00) increased the risk of ASD symptoms in boys, and Ofloxacin exposure during the first trimester (RR: 1.47, 95% CI: 1.07, 2.02) increased the risk of ASD symptoms in girls. No dose-dependent relationships between prenatal antibiotic exposure and ASD symptoms were validated by restricted cubic splines. Prenatal exposure to Tetracycline and Ofloxacin may increase the risk of ASD symptoms in children, and the first and third trimesters might be the key windows.



KEYWORDS: *Antibiotics, Biomonitoring, Urine biomarkers, Prenatal exposure, Autism, Birth cohort*

1. INTRODUCTION

Over the past century, the countless lives saved by antibiotics marked the beginning of a new epoch in human medical history. Moreover, antibiotics are also used to treat and prevent bacterial diseases in animals to improve agricultural and breeding productivity.[1] Between 2000 and 2018, the total consumption of antibiotics in 204 countries and cites increased by 46%.[2] In 2013, the total amount of antibiotics used in China was 162 000 tons, of which 48% were used for humans and 52% for livestock husbandry.[3] Due to the lack of effective control and disposal measures, a variety of antibiotics have been detected in food, drinking water, aquatic environments and soil,[4] and these antibiotics can be exposed to the human body through food and water.[5] In fact, previous biomonitoring-based studies have shown that pregnant women are commonly exposed to multiple antibiotics, including human antibiotics (HAs) and veterinary antibiotics (VAs).[6−8] Under long-term maternal antibiotic exposure, the fetal microbiome and metabolome may be altered, with adverse effects on early metabolism, physical growth, and neurodevelopment.[9,10] Previous studies have shown that the prescription of antibiotics during pregnancy is associated with an increased risk of nervous system diseases in children.[11,12] Therefore, it is particularly important to objectively elucidate the effects of multiple antibiotic exposures during pregnancy on offspring health outcomes.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that occurs mostly in children. It is thought to originate early in life and is influenced by a combination of genetic and environmental factors.[13,14] The age-standardized prevalence rate (ASPR) of ASD is increasing in the worldwide.[15] The estimated prevalence of ASD in the USA was 1 in 10 000 in the 1970s, 1 in 150 in 2000 and 1 in 54 in 2016.[16] In China, the ASPR for ASD was 357.68 per 100 000 in 1990 and 372.30 per 100 000 in 2019.[17] However, the

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pathogenesis of ASD is partly related to genetic factors, and dramatic changes in environmental factors in early life may have contributed to the rapid increase in ASD incidence.[18] The extensive use of antibiotics during pregnancy and the increased incidence of ASD in children have aroused the attention and exploration of association. Exposure to some antibiotics during pregnancy is associated with neurobehavioral function among children, including those with ASD.[19,20]

Early research on the association between early life antibiotic exposure and the development of ASD was mostly based on paper questionnaires and clinical data, and the results are inconsistent.[21,22] However, the possible adverse health effects of antibiotic exposure in the environment have been ignored. To fill this gap, we used repeated biological monitoring data to examine the available evidence on the association between maternal antibiotic concentrations during pregnancy and ASD symptoms among 3-year-old children in a large prospective birth cohort study.

2. MATERIALS AND METHODS

2.1. Study Participants

We used data from a longitudinal birth cohort, the Ma'anshan Birth Cohort (MABC) Study, in which 3474 pregnant women were recruited from the Ma'anshan Maternal and Child Health Care Hospital between May 2013 and September 2014. The pregnant women were followed from the first trimester to delivery and collected samples. In early life, we followed-up with mothers and their children to identify early environmental and genetic factors that influence child health and development. A total of 201 twins, 86 pregnant women without urine samples and 327 children with missing Clancy Autism Behavior Scale (CABS) data were excluded (Figure S1). Ultimately, a total of 2860 mother−child pairs were enrolled in our study. In total, the details of the cohort and inclusion criteria were published in our previous publications.[7,23] The study protocol was approved by the Ethics and Research Committees of Anhui Medical University (Number: 20131195). All participants provided written informed consent.

2.2. Antibiotic Exposure Assessment

After the urine samples were collected, they were placed in a polypropylene urine cup (10−40 mL), divided into 10 mL polypropylene freezer tubes and stored in a−80 °C refrigerator until detection. Based on the analytic chemistry method developed in our laboratory,[24] we measured the urinary concentrations of 8 antibiotics by category and divided these into 6 HAs, 16 VAs, 4 preferred HAs (PHAs), and 15 preferred VAs (PVAs). We also measured the urinary concentrations of two metabolites from the above 41 antibiotics. Before the experiment, urine was transferred from a−80 °C refrigerator to 4 °C and dissolved for 24 h. After centrifugation, 1 mL of urine supernatant was removed and spiked into 200 μL of Na2EDTA-Mcllvaine buffer (pH = 4.0), 20 μL of internal standard mixture and 15 μL of β-glucuronidase aqueous solution. Then, the mixture was incubated at 37 °C overnight for enzymatic hydrolysis. The urine was purified by solid phase extraction, and antibiotics in the urine were detected by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) using multiple stable isotope internal standard dilution techniques. Two blank urine samples were used for precision and accuracy monitoring. The range of limits of detection (LODs) for urinary antibiotics was 0.016 to 1.481 ng/mL. In the statistical analyses, we replaced antibiotic concentrations below the LOD with the LOD/2.[25] We measured urinary creatinine concentrations to explain the effect of urine dilution and adjust urinary antibiotic concentrations.[26] The creatinine-adjusted concentrations of log10-transformed values were used to standardize the distribution.

2.3. Assessment of ASD Symptoms

The CABS is a global early screening and diagnostic tool for autism that is suitable for children aged 2−4 years. The CABS is composed of 14 items completed by parents or guardians.[27] In 1983, Scholars in Taiwan, China, revised it and adopted a three-point scoring method, changing the options from "yes" and "no" to "never", "occasionally" and "often", and assigning the scores of each option to "0", "1" and "2", respectively. At present, the CABS of the revised version has been widely used in China.[28] All items were summed, and a total score ≥14 was considered indicative of suspected autism.[28] The score of this scale did not reach the threshold for an autism diagnosis, so it is more appropriate to identify these children as having ASD symptoms in this study.

2.4. Covariates

For pregnant women in the first trimester, demographic information, including maternal and paternal age, prepregnancy body mass index (BMI), education level, ethnicity, household monthly income per capita, smoking status and alcohol use, was collected. We collected electronic medical records during three trimesters and at delivery, including pregnancy complications, biochemical indices, gestational week, delivery mode and neonatal gender. The selection of covariates was based on univariate analysis results and published literature. We considered these covariates based on a directed acyclic graph (DAG) (Figure S2): maternal education level, residence, household monthly income per capita, smoking during pregnancy, gestational week and neonatal gender.

2.5. Statistical Analysis

The demographic characteristics were presented as the means ± standard deviations (SDs) or numbers (percentages), and Student's *t* tests and chi-square tests were used to test the differences between groups with and without ASD symptoms. The demographic characteristics of the exposed and unexposed groups were also compared. The presence of one or more antibiotics above the LOD is considered positive in a urine sample. We calculated descriptive statistics for creatinine-adjusted biomarker concentrations (μg/g) and the percentage of values above the LOD. Given the short half-lives of antibiotics, we averaged the natural log-transformed biomarker concentrations of multiple urine samples collected during three trimesters to estimate the mean exposure in the pregnancy window. A detection rate of antibiotics less than 10% was combined into one variable, and detection rates of antibiotics greater than 10% were analyzed separately as independent variables. Least absolute shrinkage and selection operator (LASSO) regression was used to screen for independent covariates associated with ASD symptoms in children. The associations between log10-transformed creatinine-adjusted antibiotic concentrations and the risk of ASD symptoms in children were evaluated by using the odds ratio (OR) and 95% confidence interval (CI) in Modified Poisson regression models.[29] In addition, we stratified the participants by trimester to explore exposure and outcome in each trimester, and analyzed the trimester differences using a multi-information level model.[30,31] To explore whether the association between antibiotic exposure and ASD symptoms is influenced by neonatal gender, we analyzed the interaction between antibiotics and gender. To investigate the possible dose−response relationship between antibiotics and ASD symptoms, we used a restricted cubic spline (RCS). To assess whether the CABS critical value is available for ASD classification, we also adjusted the CABS critical value to 15, which enhanced the stability of the results according to sensitivity analyses.

All the statistical analyses were conducted using SPSS 23.0 (SPSS Inc., Chicago, IL, USA), the R statistical package (R 4.0.3, R Core Team), the SAS (version 9.4; SAS Institute Inc.) and GraphPad Prism 6.0 (GraphPad Software Inc.). A p value <0.05 was considered to indicate statistical significance for two-tailed tests.

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3. RESULTS

3.1. Characteristics of the Study Population

The descriptive statistics of the study variables are displayed in Table 1. In our study, 5.45% (156/2860) of the children had ASD symptoms. In the ASD symptoms group, the mean age of mothers was 26.22 ± 3.71 years, 68.59% had lived in urban areas for the past six months, and 17.31% had a university degree. Household monthly income per capita is mainly concentrated between 2500 and 4000 CNY. Approximately 89% did not smoke or drink during pregnancy. Prenatal education level, residence status, and neonatal gender distribution were significantly different between the no ASD symptoms group and the ASD symptoms group. In the exposed and no-nexposed groups, prenatal education level and residence, household monthly income per capita and gestational week were significantly different (Table S1).

Table 1. Demographic Characteristics of the Participants (N = 2860) from Ma'an-shan, China (2013/05−2014/09)[a]

Characteristics	Children without ASD symptoms (n = 2704)	ASD symptoms children (n = 156)	p-value
Maternal characteristic			
Age (years)	26.44 ± 3.59	26.22 ± 3.71	0.458
Prepregnancy BMI (kg/m^2)	20.62 ± 2.81	20.65 ± 2.95	0.920
Education			**<0.01**
Middle school or below	497 (18.38)	47 (30.13)	
High school	599 (22.15)	45 (28.85)	
Junior college	859 (31.77)	37 (23.72)	
University or above	749 (27.70)	27 (17.31)	
Ethnicity			0.815
Han	2663 (98.48)	154 (98.72)	
Other	41 (1.52)	2 (1.28)	
Residence (last half year)			**<0.01**
Urban	2175 (80.44)	107 (68.59)	
Rural	529 (19.56)	49 (31.41)	
Household monthly income per capita (CNY)			0.963
2500 or less	737 (27.26)	44 (28.21)	
2500−4000	1133 (41.90)	64 (41.03)	
4000 or more	834 (30.84)	48 (30.77)	
Smoking during pregnancy			**0.016**
Yes	69 (2.55)	9 (5.77)	
No	2635 (97.45)	147 (94.23)	
Drinking during pregnancy			0.231
Yes	221 (8.17)	17 (10.90)	
No	2483 (91.83)	139 (89.10)	
Infant characteristic			
Gestational week			0.634
Full-term birth	2587 (95.67)	148 (94.87)	
Preterm or postterm birth	117 (4.33)	8 (5.13)	
Delivery mode[b]			0.651
Delivery	1332 (49.30)	74 (47.44)	
Cesarean delivery	1370 (50.70)	82 (52.56)	
Neonatal gender			**0.024**
Male	1361 (50.33)	93 (59.62)	
Female	1343 (49.67)	63 (40.38)	

[a]BMI: Body mass index. [b]Missing n = 2.

3.2. Detection Frequencies and Concentrations of Urinary Antibiotics

Our analysis included 2860 mothers who had at least one urinary antibiotic measurement during pregnancy. The detection rates of the sum of antibiotics in the first trimester, second trimester, and third trimester were 97.13%, 92.78%, and 90.84%, respectively (Table S2). Among the nine antibiotic concentrations with detection rates greater than 10%, both trimethoprime (49.72%) and Ofloxacin (46.78%) were the most common single antibiotics (Figure 1A). The detection rate of PVAs (84.51%) was greater than that of VAs (61.84%), PHAs (25.33%), and HAs (13.42%) across pregnancy. The concentrations of five antibiotics with concentrations greater than 1 μg/g in the 90th percentile were Trimethoprime (1.33 μg/g), Penicillin G (1.63 μg/g), Doxycycline (1.19 μg/g), Ciprofloxacin (1.59 μg/g) and Florfenicol (1.24 μg/g) (Figure 1B). The creatinine-adjusted urinary concentrations of most antibiotics increased during pregnancy, except for HAs and PHAs (Table S2).

3.3. Prenatal Antibiotic Exposure and ASD Symptoms among 3-Year-Old Children

LASSO regression was used to screen prenatal Tetracycline and Ofloxacin concentrations associated with ASD symptoms in children (Figure 2 and Table S3). After stratification by trimester, meaningful results were not obtained for the second trimester. The effects of tetracycline and Ofloxacin were significant in the first and third trimesters (Figure S3). The associations between prenatal antibiotic exposure and ASD symptoms among 3-year-old children are shown in Table S4 and Table S5. After adjusting for confounding factors, the adjusted RR for the risk of ASD symptoms was 1.66 (95% CI: 1.14, 2.40) for the mean Tetracycline concentration during pregnancy. When the four single-antibiotic volume fractions of the Tetracyclines category were combined, the total Tetracyclines category concentration also increased the risk of ASD symptoms in children (adjusted RR: 1.41, 95% CI: 1.01, 1.97).

The results of trimester-stratified analyses are shown in Table 2 and Table S4. In the first trimester, after adjusting for confounding factors, Tetracycline (adjusted RR: 1.55, 95% CI: 1.12, 2.14) and Ofloxacin (adjusted RR: 1.36, 95% CI: 1.11, 1.67) increased the risk of ASD symptoms in children. In the third trimester, for every one log-unit increase in the concentration of Tetracycline, the risk of ASD symptoms in children increased by 1.73 times (95% CI: 1.22, 2.46). However, there were no associations during the second trimester. The Type 3 tests revealed that the associations between Tetracycline (Type 3 p = 0.023) and Ofloxacin (Type 3 p = 0.036) exposure and ASD symptoms differed across trimesters. Tetracyclines exposure only increased the risk of ASD symptoms in first trimester, and not found the difference during three trimesters (Table S6).

On the basis of trimester stratification, neonatal gender stratification was further analyzed between prenatal antibiotic exposure and ASD symptoms in children (Table 3). The incidence of ASD symptoms was 6.40% for boys and 4.48% for girls. The mean value of Tetracycline concentration during pregnancy significantly increased the risk of ASD symptoms in boys (adjusted RR = 1.90, 95% CI: 1.16, 3.14), but this association was not observed in girls. The association was also found only in boys who were exposed to Tetracycline during

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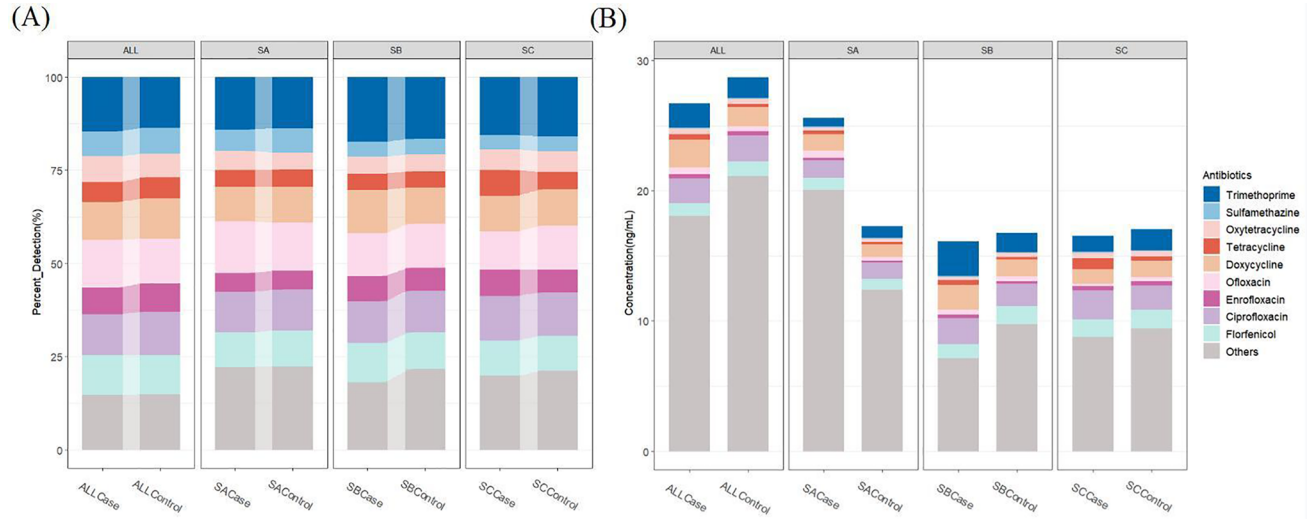

Figure 1. Composition profiles of urinary antibiotics: (A) percentage, %; (B) 90th concentration, μg/g, in the 2860 mother−child pairs. Abbreviations: All: Mean value of the three trimesters; SA: First trimester; SB: Second trimester; SC: Third trimester; urinary concentration of florfenicol was considered as the sum of its prototype and metabolite (florfenicol amine). Others: Antibiotics with a detection rate of less than 10% were combined as one variable.

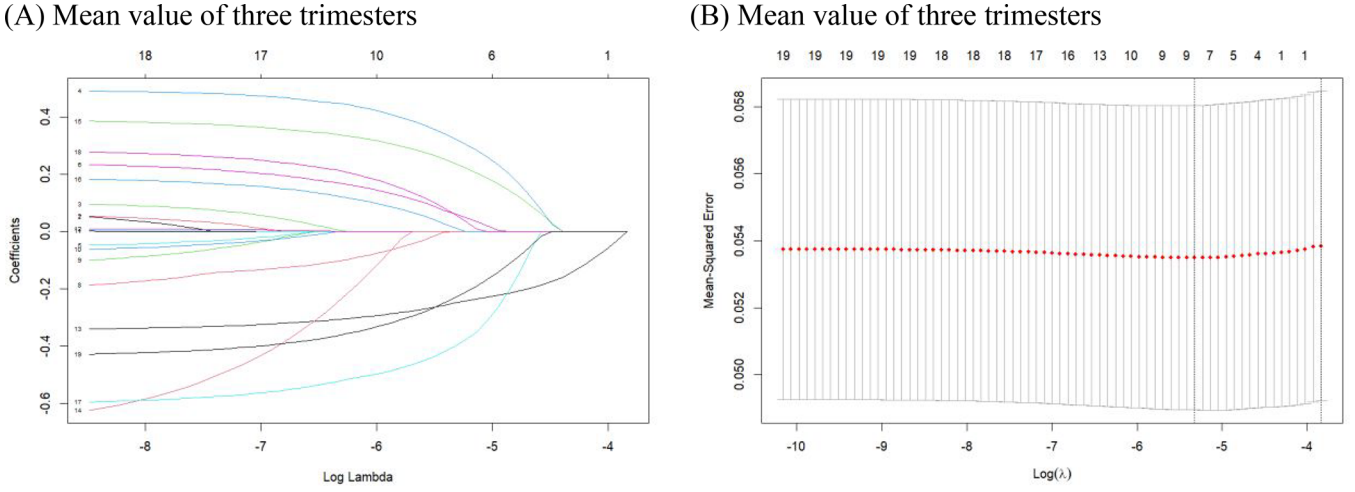

Figure 2. LASSO regression was used to select individual antibiotics of detection rates greater than 10% and covariates. Individual antibiotic concentrations were the mean value of three trimesters. (A) Change trajectory of each independent variable coefficient in the regression model. (B) Cross Validation was used to select the model.

Table 2. Associations between Log-Transformed Creatinine-Adjusted of Prenatal Urinary Antibiotic Concentrations and Risk of ASD Symptoms by Trimester

	First trimester		Second trimester		Third trimester		
Antibiotics	RR (95% CI)[a]	*p*-value	RR (95% CI)[a]	*p*-value	RR (95% CI)[a]	*p*-value	Type 3 *p*-value[b]
Tetracycline	**1.55 (1.12, 2.14)**	**0.007**	1.14 (0.78, 1.67)	0.503	**1.73 (1.22, 2.46)**	**0.002**	**0.023**
Ofloxacin	**1.36 (1.11, 1.67)**	**0.003**	1.05 (0.84, 1.31)	0.675	0.90 (0.69, 1.17)	0.418	**0.036**

[a]Adjusted for maternal education, residence, household monthly income per capita, smoking during pregnancy, gestational week, and neonatal gender. [b]Based on the multi-informant model, type 3 tests were conducted by generalized estimation equations of log-link function and Poisson distribution; Type 3 $p < 0.10$: the association was significantly different between trimesters.

the first and third trimesters. In contrast to Tetracycline, Ofloxacin in the first trimester increased the risk of ASD symptoms in girls (adjusted RR = 1.47, 95% CI: 1.07, 2.02). There was no statistically significant interaction between Tetracycline and Ofloxacin exposure and neonatal gender, suggested that gender difference was not found in these associations (Table 3). Similar results were found in the association analysis of the mean prenatal Tetracycline concentration (adjusted RR = 1.65, 95% CI: 1.08, 2.52). Ofloxacin belongs to the Fluoroquinolones category, and not found significant results for Fluoroquinolones exposure (Table S7).

The dose−response relationships between prenatal antibiotic exposure and ASD symptoms among 3-year-old children are shown in Figure 3. After stratification by trimester and gender, the Tetracycline concentration was linearly associated

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Table 3. Association between Log-Transformed Creatinine-Adjusted of Prenatal Urinary Antibiotic Concentrations and Risk of ASD Symptoms by Trimester and Gender

	Boys		Girls		
Antibiotics	Adjusted RR (95% CI)[a]	*p*-value	Adjusted RR (95% CI)[a]	*p*-value	*p*-value for interaction
First trimester					
Tetracycline	**1.74 (1.13, 2.68)**	**0.011**	1.42 (0.87, 2.31)	0.162	0.650
Ofloxacin	1.26 (0.96, 1.65)	0.096	**1.47 (1.07, 2.02)**	**0.017**	0.398
Second trimester					
Tetracycline	1.41 (0.86, 2.34)	0.177	0.90 (0.49, 1.66)	0.741	0.290
Ofloxacin	0.98 (0.73, 1.33)	0.896	1.12 (0.80, 1.57)	0.514	0.600
Third trimester					
Tetracycline	**1.86 (1.16, 3.00)**	**0.010**	1.60 (0.95, 2.70)	0.078	0.807
Ofloxacin	0.93 (0.66, 1.31)	0.675	0.82 (0.53, 1.24)	0.343	0.697
Mean value of three trimesters					
Tetracycline	**1.90 (1.16, 3.14)**	**0.012**	1.49 (0.85, 2.62)	0.161	0.600
Ofloxacin	1.22 (0.89, 1.65)	0.224	1.19 (0.82, 1.73)	0.360	0.978

[a]Adjusted for maternal education, residence, household monthly income per capita, smoking during pregnancy, and gestational week.

with ASD symptoms (nonlinear p value >0.05). The dose−response relationship between Ofloxacin in the first trimester and ASD symptoms was weak (nonlinear p value <0.10). In the sensitivity analyses, we also adjusted the CABS critical value to 15, and the association between average antibiotic exposure during pregnancy and increased risk of ASD in children was largely unchanged (Figure S4). Excluding the children of preterm births or postterm births, we still found these associations between Tetracycline and Ofloxacin and ASD symptoms (Table S8).

4. DISCUSSION

In this MABC study, we assessed the relationship between prenatal urine antibiotic concentrations during pregnancy and ASD symptoms evaluated at 3 years of age in children. Exposure to Tetracycline and Ofloxacin in pregnant women increased the risk of ASD symptoms in children. Trimester seem to play a role in this relationship. Overall, the first and third trimesters may be the key window periods for prenatal environmental antibiotic exposure to affect children's neurobehavior. With respect to neonatal gender, prenatal Tetracycline exposure increased the risk of ASD in boys, and prenatal Ofloxacin exposure increased the risk of ASD in girls.

In our study, the total detection rate of antibiotics in pregnant women was 93.58% in Anhui Province, which is higher than the detection rates in pregnant women residing in eastern China (41.60%), Jiangsu (30.95%), and Xizang (34.66%),[6,32,33] similar to the detection rates in Shanghai (98.56%).[8] Several possible reasons account for these differences. First, our study detected 41 antibiotics and 2 metabolites, which were the largest categories and greatest number of antibiotics. Second, this study focused on pregnancy, and samples were collected from all three trimesters. Third, the antibiotic concentrations were measured repeatedly, and the results were robust. Consistent with the above studies, PVAs and VAs were the most commonly detected antibiotics. PVAs and VAs were antibiotics with detection rates greater than 10%. These two antibiotics are used on a large scale in animal and aquatic products and are widely distributed in the natural environment.[34,35] In this study, the concentration of creatinine-adjusted antibiotics in urine was mostly −16.48 μg/g, indicating low-dose exposure during pregnancy. The 90th percentile of the sum of antibiotic concentrations was greater than that in Shanghai (0.44 μg/g) and Xizang (3.21 μg/g).[6,33] There were significant differences in the concentrations of various antibiotics, which were related to antibiotic consumption and dietary habits in different regions.[6,8,32] In addition, the concentration of antibiotics increases gradually with pregnancy, especially for patients with PVAs and VAs. Previous studies have also shown that antibiotics pose a cumulative health risk during pregnancy.[7] This further suggests that food and water may be long-term pathways for pregnant women to be exposed to antibiotics.

In this study, the incidence of ASD symptoms was 5.45%, which was greater than the prevalence of ASD in children worldwide.[36−38] First, we used a screening scale to test for ASD-positive children, not children confirmed to be positive, and the sensitivity was amplified. However, there are few studies on ASD-positive early childhood children at present, and these findings cannot be compared with those of other studies. Third, although ASD symptoms were identified from the screening scale in our study, the high positivity rate suggested that we should pay attention to ASD symptoms in young children to effectively prevent and reduce the incidence of ASD.

Based on repeated measurements of biological monitoring data, we found that Tetracycline exposure during pregnancy increased the risk of ASD symptoms in 3-year-old children. Previous studies on the influence of antibiotic exposure during pregnancy on children's ASD symptoms and their results were inconsistent. Only two studies have shown that prenatal antibiotic use increases the risk of ASD in children. The adjusted HR for the risk of ASD symptoms was 1.10 (95% CI: 1.01, 1019) in Canada.[39] Other studies have reported no association between prenatal antibiotic exposure and ASD.[40] Previous studies have used antibiotic prescriptions extracted from the electronic medical records of pregnant patients to represent antibiotic exposure levels, but the dosage and specific use of antibiotics are not known, making it impossible to estimate antibiotic exposure in pregnant women. In addition, pregnant women are exposed to antibiotics not only in medicines but also from food animals and the water environment. Our study compensates for this shortcoming to some extent by using a biological monitoring technique. In our study, we repeatedly measured urine samples during three trimesters and described in detail the exposure levels of various antibiotics in urine samples. Moreover, we classified antibiotics according to their usage, such as HAs, PHAs, VAs, and PVAs,

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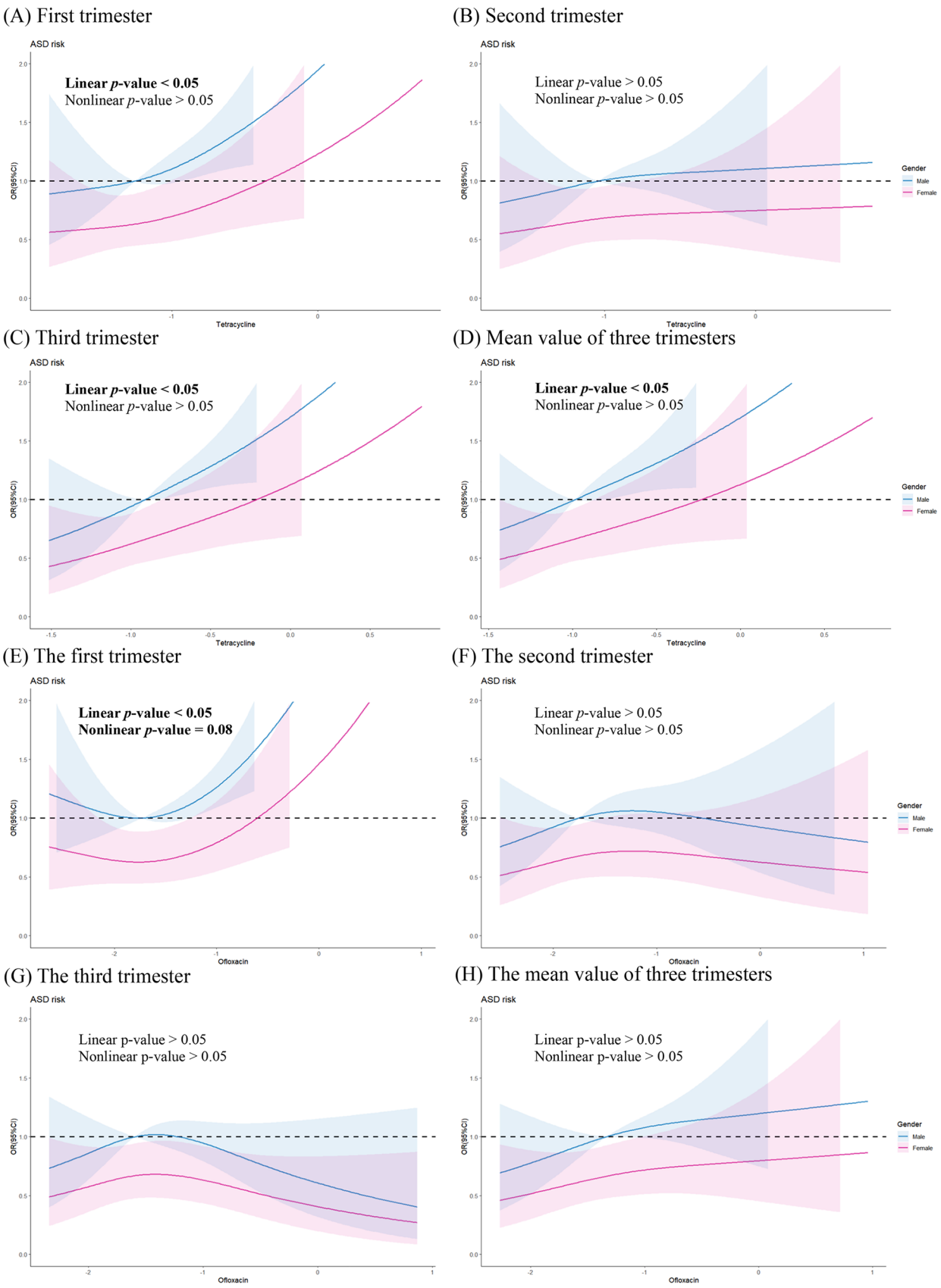

Figure 3. RCS between log-transformed creatinine-adjusted of prenatal urinary Tetracycline and Ofloxacin concentration with ASD symptoms in children. (A–D) Tetracycline concentrations; (E–H) Ofloxacin concentration.

to determine the risk of ASD resulting from exposure to different prenatal antibiotics. Previous animal studies have demonstrated the reliability of our mechanistic results. These studies revealed that perinatal antibiotic exposure affects

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cortical development, has long-term effects on the brain function of offspring, and leads to changes in anxiety-like and aggressive behavior and decreased social skills.[41−44]

We found that the first and third trimesters are the key window periods for Tetracycline, and the first trimester is the key window period for Ofloxacin. The first trimester is a critical period for the formation of neural tubes and neuronal cells. In addition, neuronal axons, glial cells, and oligodendrocytes are integrated into neural circuits during the third trimester. The fetal brain is particularly sensitive to ambient stimuli that affect the development of the central nervous system during the first and third trimesters.[45] Antibiotic exposure can disrupt the prenatal environment, potentially leading to neurodevelopmental changes that subsequently lead to ASD symptoms.[46] The potential gender differences between the effects of Tetracycline exposure and the risk of ASD symptoms remain unsolved. In our study, we found that Tetracycline exposure in the first and third trimesters was associated with an increased risk of ASD symptoms in boys but not in girls. The effect of antibiotics on hormones and neurotransmitters may explain these associations.[47] There are only a few studies on this topic, and further research is clearly needed.

The association between prenatal antibiotic exposure and ASD symptoms in children may reflect direct effects on the offspring's gut-brain axis. In mouse experiments, pregnant mice exposed to antibiotics exhibited changes in the intestinal microbiota and microbiota composition of the offspring, which subsequently affected the social behavior and motor activity of the offspring.[48] Moreover, animal experiments revealed disruption of the gut microbiota in pregnant mice exposed to antibiotics, resulting in memory retention deficits and a significant reduction in the production of BDNF in the hippocampus.[49] This finding also confirms the link and transfer between maternal and embryonic microbiota. Tetracycline can inhibit microglia in the central nervous system during development.[50,51] This also explains the importance of the first trimester as a critical window period. Tetracycline can affect maternal thyroid hormone levels in the first trimester, which may be another potential mechanism.[52] The specific mechanisms underlying the relationship between prenatal antibiotic exposure and children's ASD symptoms need to be explored in the future.

Our research has the following advantages. This is the first prospective birth cohort study to assess the effects of environmental antibiotic exposure during pregnancy effect on ASD symptoms in children by biomonitoring data. Moreover, repeated measurements of urine antibiotics at multiple time points during pregnancy can reduce exposure misclassification and more accurately describe exposure to multiple antibiotics during pregnancy.

However, there are also certain disadvantages in our study. First, we did not measure antibiotic exposure during childhood, which is what we will do next. Second, we only screened for ASD by one assessment tool and did not make further definitive diagnoses. Third, we did not collect data on antibiotic prescriptions consumed before pregnancy. Fourth, the study population all came from the same region, and the results should be extrapolated to other regions with caution. Finally, the effect of some unmeasured confounders cannot be completely ruled out in our study.

5. CONCLUSIONS

We found that prenatal exposure to environmental Tetracycline and Ofloxacin increased the risk of ASD symptoms in children at 3 years of age. The first and third trimesters are key window periods for antibiotic exposure. Tetracycline exposure increased the risk of ASD in boys, and Ofloxacin exposure increased the risk of ASD in girls.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00032.

Research flowchart; directed acyclic graph; LASSO regression; sensitivity analysis; distribution of antibiotic concentrations; demographic characteristics, modified Poisson regression, and other materials (PDF)

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