The impact of therapeutic antibiotic use in childhood on the risk of precocious puberty

Çocuklukta terapötik antibiyotik kullanımının erken ergenlik riskine etkisi

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Abstract

Aim: Early pubertal development (precocious puberty) in girls is associated with genetic, metabolic, and environmental factors. Although it is thought that antibiotics used in early childhood may affect pubertal timing due to their impact on the gut microbiota, there is no definitive evidence on this subject. The aim of this study is to investigate the relationship between antibiotic use characteristics (age at first use, frequency of use, types of antibiotics) in girls with and without early pubertal development.

Methods: The study included 122 girls aged 6–8 years. Participants were divided into Early Puberty, Premature Thelarche, and Control groups based on clinical, hormonal, and radiological assessments. Antibiotic use data were obtained from national prescription records. Differences in antibiotic use between groups and correlations with pubertal parameters were analyzed statistically.

Results: The pubertal group showed higher body weight, BMI, basal luteinizing hormone (LH) levels, and advanced bone age compared to the control group ($p < 0.05$). No significant differences were found between groups in terms of age at first antibiotic use, total number of antibiotic courses, or types of antibiotics used ($p > 0.05$). A weak positive correlation was found between age at first antibiotic use and bone age in the Premature Thelarche group. Amoxicillin-clavulanic acid was the most frequently prescribed antibiotic, and all participants had a history of antibiotic use.

Conclusion: In this study, no differences were observed in antibiotic use characteristics between girls with and without early pubertal development. Genetic and metabolic factors are believed to play a more decisive role in precocious puberty. Longitudinal studies including microbiome analyses and environmental exposures are necessary to better understand these complex relationships.

Keywords: Antibiotics; bone age; environmental exposure; microbiome; pediatrics; precocious puberty

Öz

Amaç: Kız çocuklarında erken pubertal gelişim (erken ergenlik) genetik, metabolik ve çevresel faktörlerle ilişkilidir. Erken çocukluk döneminde kullanılan antibiyotiklerin bağırsak mikrobiyotası üzerindeki etkileri nedeniyle pubertal zamanlamayı etkileyebileceği düşünülmekle birlikte, bu konuda kesin kanıtlar bulunmamaktadır. Bu çalışmanın amacı, erken pubertal gelişim gösteren ve göstermeyen kız çocuklarında antibiyotik kullanımı özellikleri (ilk kullanım yaşı, kullanım sıklığı, antibiyotik türleri) arasındaki ilişkiyi araştırmaktır.

Yöntemler: Çalışmaya 6-8 yaş arası 122 kız çocuğu dahil edilmiştir. Katılımcılar klinik, hormonal ve radyolojik değerlendirmelere göre Erken Puberte, Prematür Telarş ve Kontrol gruplarına ayrılmıştır. Antibiyotik kullanımı, ulusal reçete kayıtlarından elde edilmiştir. Gruplar arasında antibiyotik kullanımı açısından farklar ve pubertal parametrelerle ilişkiler istatistiksel olarak incelenmiştir.

Bulgular: Pubertal grup, kontrol grubuna kıyasla daha yüksek vücut ağırlığı, BMI, bazal luteinize hormon (LH) düzeyi ve ileri kemik yaşı göstermiştir ($p < 0,05$). Antibiyotik kullanım yaşı, toplam kullanım sayısı ve antibiyotik türleri açısından gruplar arasında anlamlı fark bulunmamıştır ($p > 0,05$). Prematür Telarş grubunda antibiyotiklerin ilk kullanım yaşı ile kemik yaşı arasında zayıf pozitif bir korelasyon tespit edilmiştir. Amoksisilin-klavulanik asit en sık reçete edilen antibiyotik olup, tüm katılımcıların antibiyotik kullanma geçmişi vardır.

Sonuç: Bu çalışmada erken pubertal gelişim gösteren ve göstermeyen kız çocuklarında antibiyotik kullanımı özellikleri açısından fark gösterilmedi. Erken ergenlikte genetik ve metabolik faktörlerin daha belirleyici olduğu düşünülmektedir. Mikrobiyom analizleri ve çevresel maruziyetlerin de dahil edileceği uzunlamasına çalışmalar, bu karmaşık ilişkilerin daha iyi anlaşılması için gereklidir.

Anahtar Sözcükler: Antibiyotikler; çevresel sorunlara maruziyet; erken puberte; kemik yaşı; mikrobiyom; pediatri

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INTRODUCTION

Antibiotics are the most commonly prescribed drugs in the pediatric age group, reducing morbidity and mortality associated with bacterial infections. Antibiotic use has increased, and bacterial resistance and microbiota dysbiosis have become substantial concerns (1).

Precocious puberty in girls is characterized by breast development and pubic hair growth before the age of eight, with idiopathic central precocious puberty being the most common type. In these cases, the hypothalamic-pituitary-gonadal (HPG) axis has activated prematurely (2). Although the exact reason for the early activation of the HPG axis is not clearly understood, a complex interaction of metabolic, nutritional, and hormonal factors resulted (3). The timing of puberty is influenced by the complex interplay of genetic and environmental factors, particularly the pervasive presence of environmental endocrine disruptors (EEDs). In recent years, the association between EEDs and precocious puberty (PP) has been documented. Endocrine-disrupting chemicals (EDCs) constitute a heterogeneous class of chemicals of increasing concern, possessing the capacity to emulate the mechanisms underlying the biosynthesis, transport, and metabolism of hormones (4).

The human microbiota is influenced by diet and antibiotics. The metabolites of gut microbiota can impact the secretion of sex hormones through pathways involving the immune system, chronic inflammation, and the gut-brain axis, which are some neuroendocrine axis (6). The girls with precocious puberty have an overproduction of bacterial species in their gut microbiota that produce metabolites capable of activating the HPG axis (6,7). Furthermore, microbiota diversity induces an upregulation of Beta-glucuronidase expression, which facilitates the deconjugation of estrogen, thereby promoting its metabolism. Non-conjugated estrogen can then enter the systemic circulation and continue its metabolic activities (8).

Antibiotic therapy reduces the diversity of gut microbiota species, leading to a condition known as dysbiosis, which causes antibiotic resistance and diarrhoea in the short term and obesity, asthma, and inflammatory bowel disease in the long term (9). Hu and colleagues have reported that children exposed to antibiotics, particularly fluoroquinolones and tetracyclines, are positively associated with the occurrence of precocious puberty. The authors have suggested a longitudinal study to understand the relationship between antibiotic use and precocious puberty (10).

In our country, the use of antibiotics and antibiotic prescriptions is frequent. The incidence of precocious puberty is also increasing (11). While the role of genetic and environmental factors is partially known, the impact of antibiotics used for treatment purposes is not clear in pubertal maturation.

This study aims to investigate whether there are differences in antibiotic use characteristics—such as the number of antibiotics used, types of antibiotics, and age at first antibiotic exposure—between girls who develop signs of puberty before the age of eight and those who do not. Specifically, the potential effects of early antibiotic exposure on the onset of pubertal development will be evaluated to better understand the role of antibiotics in pubertal timing. However, due to the cross-sectional design of the study, causal relationships cannot be established.

METHODS

A total of 122 girls aged 6 to 8 years who attended the Pediatric Endocrinology Clinic at Kayseri City Hospital were enrolled in this cross-sectional study. All participants underwent a comprehensive clinical examination conducted by a single pediatric endocrinologist to ensure consistency.

Inclusion criteria:

- **Pubertal Group:** Girls exhibiting Tanner stage ≥ 2 breast development (n=69). and consists of both the Precocious Puberty and Premature Thelarche groups.
- **Precocious Puberty Group:** Girls with breast development who met the diagnostic criteria for central precocious puberty (CPP), defined by estradiol levels >12 pg/ml, advanced bone age (bone age/chronological age ratio >1), and a positive gonadotropin-releasing hormone (GnRH) stimulation test (1-hour LH ICMA ≥ 5 mIU/ml) (n=14) (12).
- **Premature Thelarche Group:** Girls with breast enlargement who did not meet CPP criteria,

characterized by normal bone age and a negative GnRH stimulation test (1-hour LH ICMA <5 mIU/ml) (n=55) (13).

- **Control Group:** Girls with no signs of breast development (n = 53), representing healthy peers without any clinical evidence of pubertal onset.

Exclusion criteria:

Participants with malnutrition (body mass index (BMI) <-2 SD), chronic illnesses requiring ongoing treatment, organic causes of precocious puberty, and male gender were excluded.

Anthropometric and clinical assessments:

Height, weight, BMI, and corresponding standard deviation scores (SDS) were retrospectively obtained from medical records (14). Additional variables collected included birth weight, breastfeeding duration, maternal age at menarche, maternal BMI, and parental heights. Target height was calculated using the formula: [(maternal height + paternal height)/2] – 13 cm.

Anthropometric measurements were conducted in the morning under fasting conditions with the child barefoot and lightly clothed. Height was measured using a Harpenden stadiometer (Holstein Limited, UK) with a precision of 0.1 cm, and weight was measured with a SECA scale (Germany) with 0.1 kg accuracy. Pubertal development was staged according to the Marshall and Tanner criteria by the examining pediatric endocrinologist (15). Bone age was assessed through left wrist radiographs and interpreted using the Greulich and Pyle atlas (16). The difference between bone age and chronological age (ΔBA-CA) was calculated.

Laboratory and imaging studies:

For participants with breast development, pelvic ultrasonography was performed, and serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E2) were measured. Hormone assays were performed using electrochemiluminescence immunoassay (ECLIA) on the Cobas 8000 e602 analyzer (Roche Diagnostics, Germany).

Antibiotic exposure assessment:

Data regarding antibiotic use—including age at first prescription, number and types of antibiotics prescribed—were extracted from prescription records within the national personal health system. Confirmation of antibiotic usage was ensured through these records.

Ethical considerations:

Informed consent was obtained from all participants' legal guardians prior to enrollment. The study protocol was approved by the Kayseri City Hospital Clinical Research Ethics Committee (date: 28.11.2023, decision no: 949) and conducted by the Helsinki Declaration.

For all groups, the age at first antibiotic prescription, the number and types of antibiotics prescribed, and confirmation of their use were also recorded with personal health system. These data were based on derived from prescription records. Informed consent was obtained.

Statistical analysis

Data were analyzed using SPSS software version 25 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation (SD) for normally distributed continuous variables, for non-normally distributed data, and frequencies (percentages) for categorical variables.

Normality of continuous variables was assessed using the Shapiro-Wilk test. Comparisons between two groups were performed using the Student's t-test for normally distributed variables or the Mann-Whitney U test for non-parametric variables. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for normally distributed data, and the Kruskal-Wallis test followed by Dunn's post hoc test for non-normally distributed data.

Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate.

Correlations between antibiotic exposure variables (e.g., number of antibiotics, age at first use) and pubertal parameters were assessed using Pearson's or Spearman's correlation coefficients, depending on data distribution. BMI and age were included as confounding factors in the analysis. A p-value <0.05 was considered statistically significant.

A power analysis was performed using G*Power software, which determined that, to achieve a 95%

confidence level (α = 0.05), 81% statistical power (β = 0.19), and a medium effect size of 0.50 (based on Cohen's criteria), a minimum sample size of 52 participants per group was required for independent samples t-tests. In Precocious Puberty Group, the sample size did not reach the target determined by the power analysis, which may have limited the ability to detect statistically significant differences.

RESULTS

The mean age of all participants included in the study was 7.18 ± 0.87 years, the mean body BMI was 18.11 ± 3.90 kg/m^2, and the mean BMI standard deviation score (BMI SDS) was 0.66 ± 1.48. Amoxicillin-clavulanic acid was the most frequently prescribed antibiotic. All participants had a history of antibiotic use, with an average of 20.4 ± 12.9 antibiotic prescriptions per child throughout their lifetime.

Clinical, laboratory, and radiological characteristics of the Pubertal (n = 69) and Control (n = 53) groups are presented in Table 1. The groups were statistically compared, and the following significant differences were identified: Body weight was higher in the pubertal group (p = 0.015). Weight SDS was also significantly higher in the Pubertal group than in the Control group (p = 0.032). BMI and BMI SDS were both greater in the pubertal group (p = 0.030, p = 0.039). Basal LH levels were significantly higher in the pubertal group (p = 0.031). ΔBA-CA (difference between bone age and chronological age) was also greater in this group (p = 0.021). No statistically significant differences were observed between the two groups in terms of age, birth weight, breastfeeding duration, maternal and paternal anthropometrics, target height, basal FSH, estradiol levels, or bone age (all p>0.05). These findings suggest that increased weight, BMI, basal LH, and advanced bone maturation (ΔBA-CA) are associated with early pubertal development (Table 1).

The participants were classified into Precocious Puberty (n = 14, 14%), Premature Thelarche (n = 55, 55%), and Control groups (n = 53, 31%). The Premature Thelarche group showed a significantly higher BMI standard deviation score (BMI SDS) compared to the Control group (1.00 ± 1.29 vs. 0.40 ± 1.32, p = 0.049). Basal FSH levels were significantly elevated in the Precocious Puberty group compared to both the Premature Thelarche (2.45 ± 0.45 vs. 1.52 ± 1.03 mIU/L) and Control groups (2.45 ± 0.45 vs. 1.46 ± 0.87 mIU/L) (p = 0.010). Bone age was significantly advanced in the Precocious Puberty group compared to Controls (8.54 ± 1.03 vs. 7.31 ± 1.13 years, p = 0.004), and the difference between bone age and chronological age (ΔBA–CA) was also significantly higher in the Precocious Puberty group (1.19 ± 0.87 vs. –0.04 ± 1.13 years, p = 0.001) (Table 2).

When the patients were divided into the Control and Pubertal groups, no statistically significant differences were observed in the timing of antibiotic initiation (median age 0.73 vs. 0.72 years; p = 0.943) and in the number of antibiotic courses used (median 17 vs. 17; p = 0.896) (Table 3). Moreover, Chi-square tests showed no significant differences between groups in the proportions of cases starting antibiotics before 6 months (p = 0.471), first year (p = 0.880), or second year of age (p = 0.926). Similarly, there were no significant group differences in the frequency of use across antibiotic classes (Penicillin, Macrolides, Cephalosporins, Metronidazole, Trimethoprim-sulfamethoxazole), with all p-values>0.05 (Table 3).

When patients were divided into the Precocious Puberty (n=14), Premature Thelarche (n=55), and Control (n=53) groups, no statistically significant differences were found in the timing of first antibiotic use (median [range]: 0.79 [0.12-3.97], 0.72 [0.08-5.64], and 0.77 [0.20-6.15] years, respectively; p = 0.834) or in the number of antibiotic courses per case (median [range]: 16.5 [5-53], 18 [2-50], and 17 [3-78], respectively; p = 0.930). Similarly, no significant differences were observed in the proportions of cases starting antibiotics before 6 months, first year, or second years of age (p > 0.05 for all). The distribution of antibiotic types (penicillin, macrolides, cephalosporins, metronidazole, and trimethoprim-sulfamethoxazole) also did not significantly differ between groups (all p>0.05) (Table 4).

Correlation analyses were performed to assess the relationships between the age at first antibiotic prescription, the total number of antibiotics prescribed, and hormonal and radiological parameters in the three groups (Table 5). BMI and age were controlled as confounding factors in all analyses.

Table 1. Clinical laboratory and radiological data of subjects

	Pubertal (n:69)	Control (n:53)	*p*
Age, years *	7.4 (6-7.99)	7.39 (6-7.99)	0.395
Birthweight, gr	3125±542	3068 ±625	0.644
Duration of breastfeeding, months *	18 (0-36)	12.5 (0-30)	0.718
First maternal menarch, years *	13 (10-140)	12 (9-16)	0.167
Maternal BMI, kg/m²	27.8 ±5.5	29.2 ±5.9	0.358
Maternal height, cm	160.7 ±6.5	159.8 ±6.3	0.555
Paternal height, cm	173.9 ±8.9	173.5 ±6.1	0.822
Target height, cm	160.3 ±5.5	161.2 ±5.43	0.549
Target height, SD	-0.46 ±0.94	-0.30 ±0.91	0.562
Weight, kg *	30 (15.4-44.8)	25 (15.1-51.15)	**0.015**
Weight, SD *	1.40 (-2.15-3.79)	0.44 (-2.54-4.90)	**0.032**
Height, cm	126.1 ±6.9	124.2±7.9	0.162
Height, SD	0.59 ±1.12	0.34 ±1.28	0.250
BMI, kg/m² *	18.5 (13.2-26.3)	16 (13.1-30.2)	**0.030**
BMI, SD	0.9 ±1.27	0.41 ±1.31	**0.039**
Basal FSH, mIU/L *	1.53 (0.33-6.57)	1.22 (0.1-4.38)	0.158
Basal LH, mIU/L	0.3 (0.1-3.4)	0.3 (0-0.5)	**0.031**
Bazal E2, pg/ml*	5 (5-42)	5 (0-26.3)	0.911
Bone Age, years*	8 (5.8-11)	7.8 (4-8.8)	0.053
ΔBA-CA, years*	0.61 (-1.55-3.07)	0.4 (-2.62-1.27)	**0.021**

* Non-parametric Test, BMI: Body mass index; SD: Standard deviation; FSH: Follicule stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; BA: Bone age; CA: Calendar age, n: Number, %: Percent

In the Premature Thelarche group (n=55), a weak but statistically significant positive correlation was found between age at first antibiotic prescription and bone age (r = 0.307, p = 0.040). No other significant correlations were observed in this group.

In the Control group (n=53), there was a significant weak negative correlation between the number of antibiotics prescribed and age at first antibiotic prescription (r =-0.335, p = 0.014).

No statistically significant correlations were found between the hormonal levels (LH, FSH, E2), bone age parameters, and antibiotic usage variables in the Precocious Puberty group (n=14) (Table 5).

DISCUSSION AND CONCLUSION

In this study, girls exhibiting pubertal signs before age eight were compared with healthy controls regarding clinical characteristics, laboratory findings, and antibiotic use history. The pubertal group showed significantly higher body weight, BMI, BMI SDS, basal LH levels, and the difference between bone age and chronological age (ΔBA-CA), indicating that early puberty is associated with increased body mass, hormonal activity, and accelerated skeletal maturation. These findings align with prior studies suggesting obesity may accelerate puberty via activation of the hypothalamic-pituitary-gonadal (HPG) axis (2,17,18).

The Premature Thelarche group also exhibited higher BMI SDS compared to controls, implying that both central and peripheral hormonal mechanisms may contribute to early breast development (17).

Regarding antibiotic use, no significant differences were found between groups in age at first antibiotic exposure, number of courses, or antibiotic types, suggesting that antibiotic use during prenatal and early childhood periods may not directly influence pubertal development. This is consistent with findings from large cohort studies such as Hu et al. (18). Notably, an earlier age at first antibiotic use correlated with a higher number of antibiotic courses; however, no causal effect on puberty was established.

Table 2. Clinical laboratory and radiological data of three groups

	Precocious puberty (n:14)	Premature thelarche (n:55)	Control (n:53)	*p*
Age, years	7.35 ±0.50	7.32 ±0.54	7.18 ± 0.60	0.359
Birthweight, gr	2983 ±707	3072 ± 523	3068 ±625	0.396
Duration of breastfeeding, months	13.33 ± 11.21	15.94 ± 10.34	13.24 ± 8.33	0.497
Age at mother's first menstruation, years *	12.82 ± 1.47	12.95 ± 1.32	12.55± 1.47	0.483
Maternal BMI, kg/m²	28.83 ± 5.44	27.67 ±5.43	29.35 ±6.23	0.592
Maternal height, cm	160.50 ±7.09	159.65 ± 5.90	161.75 ±6.91	0.341
Paternal height, cm	173.35 ±14	174.10± 7.89	173.75 ±5.99	0.953
Target height, cm	160.58 ± 6.11	160.43 ± 5.28	161.18 ±5.59	0.821
Target height, SD	-0.38 ±1.06	-0.45 ± 0.89	-0.30 ±0.94	0.800
Weight, kg *	28.58 ± 6.98	30.28 ± 7.54	26.82± 7.61	0.061
Weight, SD *	0.83 ± 1.12	1.10 ± 1.46	0.50 ±1.51	0.101
Height, cm	127.85 ± 6.31	125.99 ±7.33	123.78 ±7.59	0.113
Height, SD	0.88 ± 0.82	0.56 ± 1.19	0.29 ±1.27	0.223
BMI, kg/m² *	17.30 ± 3.07	18.85 ± 3.54	17.30 ± 3.73	0.062
BMI, SD	0.47 ± 1.13	1.00 ± 1.29[a]	0.40 ±1.32[a]	**0.049**
Basal FSH, mIU/L *	2.45 ± 0.45 [a,b]	1.52 ± 1.03[b]	1.46 ± 0.87 [a,b]	**0.010**
Basal LH, mIU/L	0.45 ± 0.28	0.39 ± 0.44	0.27 ± 0.13	0.207
Bazal E2, pg/ml*	9.15 ± 6.20	7.77 ± 6.39	7.75 ± 5.27	0.728
Bone Age, years*	8.54 ± 1.03[a]	7.88 ± 1.03	7.31 ± 1.13[a]	**0.004**
ΔBA-CA, years*	1.19 ± 0.87[a]	0.53± 0.87	-0.04 ± 1.13[a]	**0.001**

BMI: Body mass index; SD: Standard Deviation; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; BA: Bone age; CA: Calendar age, n: Number, %: Percent

Early puberty prevalence is rising rapidly, influenced by genetic predisposition, increasing obesity rates, and exposure to endocrine disruptors (19). The effects of antibiotics on early pubertal development remain contentious (10,20-22). In Turkey, a national program implemented between 2014 and 2017 restricted over-the-counter antibiotic sales and promoted awareness, reducing outpatient antibiotic consumption from 42.2 to 35.25 defined daily doses per 1,000 patients (23). In our study, the average annual antibiotic prescription rate was 2.8 per patient (2.7 for the pubertal group, 2.9 for controls).

Animal studies suggest antibiotics may impact puberty via gut microbiota alterations and metabolic pathways (24). However, a large cohort study found no association between maternal antibiotic use during pregnancy and pubertal timing in offspring (22).

In our study, cases were divided into premature thelarche and true precocious puberty groups based on hormone levels and bone age; no association with antibiotic use was found in either group. A weak but significant positive correlation emerged between age at first antibiotic use and bone age in the Premature Thelarche group, possibly indicating an indirect antibiotic effect on bone development, though causality remains unproven. The link between microbiota dysbiosis and bone metabolism requires further investigation (25).

Metabolic alterations in precocious puberty include changes in lipid, steroid, and amino acid metabolism, with increased levels of pregnenolone sulfate, estrogen receptor alpha agonist propyl pyrazole triol, 3-methylhistamine, and triglycerides reported. Zhao et al. observed a 5.2-fold increase in ciprofloxacin levels in such cases (26). Ciprofloxacin, a fluoroquinolone antibiotic, inhibits topoisomerase IV and DNA gyrase, causing DNA strand breaks. Its use is contraindicated in pregnancy and restricted in children. No fluoroquinolone use was detected in our cohort; however, possible environmental exposures via food or water could not be ruled out.

Antibiotic types used before age eight were similar between girls with and without breast development. All participants had received common antibiotics, in-

Table 3. Antibiotic usage data of subjects

	Pubertal (n:69)	Control (n:53)	*p*
Timing of exposure to any antibiotics			
First of antibiotic use, years *	0.73 (0.08-5.64)	0.72 (0.2-6.15)	0.943
Number of antibiotic courses per case *	17 (2-53)	17 (3-78)	0.896
Number of case starting antibiotics before 6 months, %**	21 (30)	13 (24)	0.471
Number of case starting antibiotics before 1 year,% **	29 (42)	23 (43)	0.880
Number of case starting antibiotics before 2 years, % **	59 (85)	45 (84)	0.926
Type of antibiotics			
Penicillin using case, (%)**			
None case	-	-	
1-3 times	7 (10.3)	7 (13)	0,740
4-6 times	12 (17.3)	11 (20.7)	
>6 times	50 (72.4)	35 (66)	
Macrolides using case, (%)**			
None	10 (14.4)	10 (18.9)	
1-3 times	37 (53.6)	25 (47.1)	0.876
4-6 times	13 (18.8)	10 (18.9)	
>6 times	9 (13.2)	8 (15.1)	
Cephalosporins using case, (%)**			
None	11 (16)	6 (11.3)	
1-3 times	27 (39)	21(39.7)	0.162
4-6 times	14 (20.4)	19 (35.8)	
>6 times	17 (24.6)	7 (13.2)	
Metronidazole using case, (%)**			
None	32 (46.3)	29 (54.7)	
1-3 times	31(44.9)	19 (35.9)	0.580
4-6 times	5 (7.3)	5 (9.4)	
>6 times	1 (1.5)	-	
Trimethoprim sulfamethoxazole using case, (%)**			
None	54 (78.3)	46 (86.8)	
1-3 times	13 (18.8)	7 (13.2	0.304
4-6 times	2 (2.9)	-	
>6 times	-	-	

*Non-parametric test (Mann-Whitney U test), ** Chi-square test, n: Number, %: Percent

cluding penicillins, macrolides, cephalosporins, metronidazole, and trimethoprim-sulfamethoxazole at least once. This uniformity complicates establishing a direct link between specific antibiotic types and early puberty. Since antibiotic classes variably affect gut microbiota, this raises questions about their distinct roles in microbiota-mediated pubertal development. Future studies should detail dosage, duration, and antibiotic classes to better elucidate these relationships.

Antibiotics are widely used in medicine and agriculture, with residual antibiotics present in environmental and animal-derived foods, leading to human exposure through the food chain (27). Elevated fluoroquinolone and tetracycline levels have been found in urine samples of children with precocious puberty, indicating environmental exposure (10). Our study focused on therapeutic antibiotic use and did not assess environmental antibiotic exposure.

Table 4. Antibiotic usage data of three groups

	Precocious puberty (n:14)	Premature thelarche (n:55)	Control (n:53)	*p*
Timing of exposure to any antibiotics				
First of antibiotic use, years *	0.79 (0.12-3.97)	0.72 (0.08-5.64)	0.77 (0.20-6.15)	0.834
Number of antibiotic courses per case *	16.5 (5-53)	18 (2-50)	17 (3-78)	0.93
Number of case starting antibiotics before 6 months, %**	5 (35)	16 (29)	13 (24)	0.683
Number of case starting antibiotics before 1 year,% **	9 (64)	31 (55)	30 (53)	0.857
Number of case starting antibiotics before 2 years, % **	5 (35)	16 (29)	13 (24)	0.995
Type of antibiotics				
Penicillin using case, (%)**				
None	-		-	
1-3 times	1 (7.1)	6 (10.9)	7 (13)	0,722
4-6 times	4 (28.5)	8 (14.5)	11 (20.7)	
>6 times	9 (64.2)	41 (74.5)	35 (66)	
Macrolides(%)**				
None	2 (14.2)	8 (14.5)	10 (18.9)	
1-3 times	7 (50)	30 (54.5)	25 (47.1)	0.992
4-6 times	3 (21.4)	10 (18.1)	10 (18.9)	
>6 times	2 (14.2)	7 (12.7)	8 (15.1)	
Cephalosporins (%)**				
None	4 (28.5)	7 (12.7)	6 (11.3)	
1-3 times	5 (35.7)	22 (40)	21(39.7)	0.226
4-6 times	3 (21.4)	11 (20)	19 (35.8)	
>6 times	2 (14.2)	15 (27.2)	7 (13.2)	
Metronidazole (%)**				
None	6 (42)	26 (47.2)	29 (54.7)	
1-3 times	6 (42)	25 (45.4)	19 (35.9)	0.175
4-6 times	1 (7.1)	4 (7.2)	5 (9.4)	
>6 times	1 (7.1)		-	
Trimethoprim sulfometaksazol (%)**				
None	11 (78.5)	43 (78)	46 (86.8)	
1-3 times	2 (14.2)	11 (20)	7 (13.2	0.338
4-6 times	1 (7.1)	1 (1.8)	-	
>6 times	-		-	

*Non-parametric test (Mann-Whitney U test), ** Chi-square test, n: Number, %: Percent

Gonadotropin-releasing hormone agonists are used to treat true precocious puberty. In our cohort, 25% of patients began treatment at initial evaluation, consistent with literature reports. Pubertal disorders exist along a spectrum, with premature thelarche variants defined by ΔBA-CA less than one year and no Tanner stage progression over six months (28). No significant difference in antibiotic use was observed between premature thelarche and precocious puberty groups.

This study's strengths lie in its comprehensive assessment of clinical, hormonal, and anthropometric parameters alongside a detailed evaluation of antibiotic exposure, providing a multifaceted understanding of early pubertal development. The inclusion of a well-matched

Tablo 5. Correlation between the age at first antibiotic prescription and the number of antibiotics prescribed

Precocious puberty (n:14)		
	Age at first antibiotic prescription, years	**Number of antibiotics prescribed, n**
LH, mIU /L	r=0.255 p=0.379	r=-0.488 p=0.077
FSH, mIU /L	r=-0.208 p=0.476	r=-0.298 p=0.301
E2, pg/ml	r=-0.010 p=0.973	r=0.258 p=0.373
Bone Age, years*	r=-0.215 p=0.461	r=0.189 p=0.518
ΔBA-CA, years*	r=0.030 p=0.919	r=0.023 p=0.937
Number of antibiotics prescribed, n	r=-0.263 p=0.363	-
Premature thelarche (n:55)		
	Age at first antibiotic prescription, years	**Number of antibiotics prescribed, n**
LH, mIU /L	r=-0.079 p=0.572	r=-0.075 p=0.594
FSH, mIU /L	r=0.265 p=0.058	r=-0.093 p=0.511
E2, pg/ml	r=0.029 p=0.839	r= -0.039 p=0.782
Bone Age, years*	**r=0.307*** **p=0.040**	r= -0.007 p=0.962
ΔBA-CA, years*	r=0.283 p=0.059	r=-0.307 p=0.105
Number of antibiotics prescribed, n	r=-0.118 p=0.392	-
Control (n:53)		
	Age at first antibiotic prescription, years	**Number of antibiotics prescribed, n**
LH, mIU /L	r=0.109 p=0.553	r=-0.097 p=0.597
FSH, mIU /L	r=0.251 p=0.167	r=-0.083 p=0.650
E2, pg/ml	r=-0.076 p=0.677	r=0.069 p=0.709
Bone Age, years*	r=0.278 p=0.200	r=-0.361 p= 0.091
ΔBA-CA, years*	r=0.283 p=0.191	r=-0.307 p=0.105
Number of antibiotics prescribed, n	**r=-0.335*** **p=0.014**	-

BMI: Body mass index; SD: Standard Deviation; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; BA: Bone age; CA: Calendar age, n: Number, %: Percent

healthy control group and the stratification of cases into premature thelarche and true precocious puberty based on bone age and hormone levels allowed for nuanced analysis of heterogeneous pubertal presentations. Additionally, by situating the findings within the context of recent national antibiotic stewardship efforts, the study offers relevant epidemiological insights.

This study has several limitations. The cross-sectional design precludes causal inference. Antibiotic data derived from medical records may omit non-prescription or inappropriate usage. Microbiota composition was not directly analyzed, limiting insights into antibiotic-microbiota-puberty interactions. Other potential confounding factors—such as exposure to environmen-

tal endocrine disruptors, socioeconomic status, dietary habits, and physical activity—were not evaluated. Age and BMI were controlled as confounders in some analyses, which may have influenced the results. All children included in the study had a history of antibiotic use, which may limit the generalizability of the findings to antibiotic-naïve populations. Given the established impact of antibiotics on the gut microbiota, this constitutes a potential source of bias. Although some findings were statistically significant, their clinical relevance remains uncertain and should be interpreted with caution. Future studies should incorporate antibiotic-naïve control groups to allow for more robust and comparable evaluations. Additionally, the limited sample size in the precocious puberty group is restrictive in terms of statistical power and generalizability due to the low prevalence of the condition and the retrospective design of the study. Moreover, the structure of the control group and the lack of long-term follow-up have the potential to cause classification bias. The selection of the control group based solely on specific criteria and the limited follow-up duration may limit the generalizability of the results. Nevertheless, these results provide important preliminary data and emphasize the need for prospective studies with larger cohorts.

In conclusion, no significant association was found between antibiotic use and early pubertal development in this cohort. Genetic and metabolic factors appear to be the primary determinants of early puberty, while the roles of microbiota and antibiotics remain complex and incompletely understood. Larger, longitudinal studies incorporating microbiome analyses and environmental exposure assessments are warranted to clarify these relationships.

Conflict-of-interest and financial disclosure

The author declares that she has no conflict of interest to disclose. The author also declares that she did not receive any financial support for the study.

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