ORIGINAL ARTICLE

BACTERIOLOGY

Antibiotic use during pregnancy alters the commensal vaginal microbiota

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Abstract

Antibiotics may induce alterations in the commensal microbiota of the birth canal in pregnant women. Therefore, we studied the effect of antibiotic administration during pregnancy on commensal vaginal bacterial colonization at gestational week 36. Six hundred and sixty-eight pregnant women from the novel unselected Copenhagen Prospective Studies on Asthma in Childhood (COPSAC$_{2010}$) pregnancy cohort participated in this analysis. Detailed information on oral antibiotic prescriptions during pregnancy filled at the pharmacy was obtained and verified prospectively. Vaginal samples were obtained at pregnancy week 36 and cultured for bacteria. Women who received oral antibiotics during any pregnancy trimester had an increased rate of colonization by *Staphylococcus* species in the vaginal samples as compared with samples obtained from women without any antibiotic treatment during pregnancy (adjusted OR 1.63, 95% CI 1.06–2.52, p 0.028). Oral antibiotic administration in the third trimester were also associated with increased colonization by *Staphylococcus* species (adjusted OR 1.98, 95% CI 1.04–3.76, p 0.037). These bacteriological changes were associated with urinary tract infection antibiotics. Women treated in the third trimester of pregnancy were more often colonized by *Escherichia coli* than women without antibiotic treatment in the third trimester (adjusted OR 1.91, 95% CI 1.04–3.52, p 0.038). This change was associated with respiratory tract infection (RTI) antibiotics. We did not observe any significant changes in vaginal *Streptococcus agalactiae* (group B *streptoccocus*) or *Staphylococcus aureus* colonization following antibiotic treatment in pregnancy. Antibiotic administration during pregnancy leads to alterations in the vaginal microbiological ecology prior to birth, with potential morbidity, and long-term effects on the early microbial colonization of the neonate.

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Oral antibiotic administration during pregnancy leads to alterations in the vaginal bacterial microbiota before birth, with potential effect on the woman and fetus.

Introduction

Across various cultural and healthcare settings, antibiotics are among the most widely used drugs in pregnancy [1]. Prescribing antibiotics during pregnancy presents a challenge, as infections need to be treated, but the fetus needs to be protected from possible side effects of the drugs [2].

The composition of the commensal vaginal microbiota is not constant [3]. The commensal microbiota protects against the proliferation of pathogenic bacteria [4,5]. Numerous factors may contribute to changes in the vaginal composition, including antibiotic administration [3]. A few studies have addressed the effects of local antibiotics on the vaginal microbiota. However, these had a focus on alterations related to treatment for bacterial vaginosis and whether the antibiotic treatment was preventive for preterm birth [3,6,7].

The study objective was to analyse the effect of antibiotic use during pregnancy on vaginal colonization at week 36 of pregnancy. We hypothesized that maternal antibiotic use in pregnancy is an environmental risk factor for both short-term and long-term changes in the vaginal microbial composition.

Materials and Methods

Ethics

The study followed the principles of the Declaration of Helsinki, and was approved by the Ethics Committee for Copenhagen (H-B-2008-093) and the Danish Data Protection Agency (2008-41-2599). Written informed consent was obtained from all participants.

The study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [8].

Study population

The novel Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC$_{2010}$) is an ongoing Danish cohort study of 738 pregnant women and their children followed prospectively from pregnancy week 24 in a protocol designed from the first COPSAC birth cohort (COPSAC$_{2000}$) [9–11]. Participants were recruited unselectively during 2009–2010, and the only exclusion criteria were chronic cardiac, endocrinological, nephrological or lung disease other than asthma. Data validation and quality control followed the guidelines for Good Clinical Practice. Data were collected during visits to the clinical research unit, and stored in a dedicated online database, double-checked against source data, and locked.

Bacterial samples

Vaginal samples from asymptomatic pregnant women were characterized by culture at pregnancy week 36. Swabs were sampled from the fornix posterior of the vagina with flocked swabs (ESWAB flocked regular; SSI Diagnostica, Hillerød, Denmark), and were cultured within 24 h according to standard methods on non-selective and selective media (SSI Diagnostica). One set of blood agar plates (5% horse blood)

and chocolate agar plates (including lysed blood cells) were used for general culture. These were incubated aerobically at 37°C for 18–20 h. The other set of blood agar and chocolate agar plates were incubated under micro-aerophilic conditions (5% CO_2, 3% H_2, 5% O_2, and 87% N_2) at 37°C for 48 h. Additionally, one HBT plate was used for selection of *Gardnerella vaginalis* incubated under micro-aerophilic conditions at 37°C for 48 h. Subsequently, microbial identification was performed according to growth on selective media, characteristics of colonies, and cellular morphology. All bacterial identifications were confirmed biochemically with VITEK-2 (BioMérieux, Marcy l'Etoile, France). The isolates were characterized at the species level, and grouped in the analyses at the genus level. We used a cut-off value of <5% women colonized for a genus to be analysed. Group B streptococcus (GBS; *Streptococcus agalactiae*), *Escherichia coli* and *Staphylococcus aureus* were analysed at the species level.

Information on antibiotic use

Detailed information on oral antibiotic ingestion during pregnancy was obtained by interviews with the participants at the COPSAC research clinic at pregnancy weeks 24 and 36, and 1 week postpartum. This information was validated against data in the Danish Medical Agency's Register, which included records on all drug prescriptions filled at the pharmacy, to minimize recall bias and exclude antibiotics collected but not ingested.

Oral antibiotic ingestion was analysed both as a dichotomized (yes/no) and as a categorized variable by treatment indication (A—urinary tract infection (UTI) antibiotics (J01CA08, J01EBxx, and J01XExx); B—respiratory tract infection (RTI) antibiotics (J01CAxx, excluding J01CA08, J01CExx, and J01FAxx); C—other antibiotics (J01CFxx, D06BXxx, J01AAxx, and P01ABxx)). Only treatments with oral antibiotics administered before the vaginal sampling date were used in this analysis. Analyses were performed on antibiotic use at any time-point during pregnancy and on use in the third trimester of pregnancy.

Covariates

Information on race, maternal age at birth, parity, number of older siblings at home, asthma, alcohol intake, smoking, cat or dog in the home and household income during pregnancy was obtained by the study physicians during the scheduled visits to the COPSAC clinics at gestational weeks 24 and 36 and 1 week postpartum.

Statistical analysis

The chi-square test, Student's *t*-test or the Wilcoxon rank sum test was used for simple associations in the demographic

characteristics. The associations between antibiotics and bacterial cultures were analysed by multiple logistic regression with each of the other vaginal bacteria identified in the samples and socio-economic confounders found in the baseline characteristics (Table 1) as covariates. Estimates were expressed as ORs, with the untreated group as reference and with corresponding 95% CIs. A significance level of 0.05 was used in all types of analysis. Missing data were treated as missing observations. Data processing was conducted with SAS version 9.3 for Windows (SAS Institute, Cary, NC, USA).

Diversity and composition analyses of the microbiology and the association with antibiotic treatment were performed with data-driven methods by pattern recognition (partial least-squares discriminant analysis) and evaluated from receiver operating characteristic curves showing specificity as compared with sensitivity. Data processing was performed with MATLAB R2009b 7.9.

Results

Demographics

The women were categorized on the basis of their use of antibiotics in pregnancy. There were no significant differences between the groups with respect to ethnicity, maternal age, household income, alcohol intake, previous pregnancies, or number of older siblings in the home. We observed significantly higher rates of asthmatic (31% vs. 23%) and smoking mothers (11% vs. 5%), and of mothers living with a cat or a dog in the home (44% vs. 31%), in the group treated with antibiotics (Table 1). Therefore, all risk analyses were adjusted for maternal asthma, smoking, and cat and dog in the home, as well as all other vaginal bacteria identified in the samples.

TABLE 1. Baseline characteristics for the study cohort, and grouped according to antibiotic treatment during pregnancy

| | | Antibiotic treatment | | |
	All	Yes	No	p
All, % (N)	668	34 (226)	66 (442)	—
Caucasian, % (n)	96 (637)	96 (214)	96 (423)	0.91
Age (years) at birth, mean (SD)	32.3 (4.4)	32.2 (4.8)	32.4 (4.2)	0.61
Asthma history, % (n)[a]	26 (174)	31 (70)	23 (104)	**0.04**
Smoking, % (n)	7 (48)	11 (24)	5 (24)	**0.01**
Alcohol >1 unit/week, % (n)	5 (30)	4 (10)	5 (20)	0.96
Household income—high, % (n)[b]	37 (242)	33 (72)	40 (170)	0.09
Nulliparity, % (n)	47 (316)	45 (102)	48 (214)	0.42
Older children (no.), mean (SD)	0.8 (0.8)	0.8 (0.9)	0.8 (0.8)	0.22
Cat or dog in the household, % (n)	35 (236)	44 (99)	31 (137)	**<0.01**

SD, standard deviation.
[a]History of doctor-diagnosed asthma.
[b]>€110 000/year.
Significant values are in bold.

Baseline characteristics

Complete data on antibiotic administration and week 36 vaginal cultures were available for 668 pregnant women. Of these women, 226 (34%) received antibacterial drugs prior to sampling (369 treatments in total). The most frequently administered antibacterial agents during pregnancy were UTI antibiotics (19% of the women). Eighteen per cent of the women were treated with RTI antibiotics. Only 1% received other antibacterial drugs, so we did not analyse this group further (Table 2).

Vaginal cultures

Gram-positive bacteria dominated the colonization. Among 668 samples, we found *Staphylococcus* species in 79% (526), *Corynebacterium* species in 41% (276), *Lactobacillus* species in 40% (264), yeast in 29% (197), *Enterococcus* species in 24% (162), *Micrococcus* species in 17% (112), *Streptococcus* species in 18% (117), *E. coli* in 12% (78), and *Kocuria* species in 5% (34) (see Table S1 for more details). Ten per cent (64) of the women were colonized by GBS. Eighteen per cent (117) of the women were colonized by *S. aureus*. For 4% (24) of the samples, we did not obtain any visual growth.

Oral antibiotic use and vaginal colonization

Oral antibiotic administration at any pregnancy trimester was associated with a significantly increased rate of colonization (83%) by *Staphylococcus* species in the vaginal samples as compared with women without antibiotic treatment (76%). No other bacterial genus was significantly affected by overall treatment in pregnancy. Eighty-six per cent of women treated with antibiotics in the third trimester, and 77% of women without antibiotic treatment in the third trimester, were colonized by *Staphylococcus* species. Nineteen per cent of women treated with oral antibiotics in the third trimester, and

TABLE 2. Prevalence of antibiotic administration in pregnancy prior to vaginal sampling at pregnancy week 36; 668 women

Drug group	Any trimester, % (n)	First trimester, % (n)	Second trimester, % (n)	Third trimester, % (n)
Antibacterial	34 (226)	13 (85)	16 (110)	14 (95)
UTI antibiotic	19 (129)	7 (44)	10 (65)	8 (55)
Pivmecillinam	17 (111)	5 (35)	8 (55)	7 (47)
Sulphamethizole	3 (22)	2 (12)	1 (7)	1 (5)
Nitrofurantoin	2 (10)	0 (2)	1 (5)	1 (4)
RTI antibiotic	18 (123)	7 (44)	8 (52)	7 (45)
Penicillin	14 (96)	5 (34)	6 (39)	4 (30)
Ampicillin derivate	5 (33)	2 (11)	2 (14)	2 (13)
Macrolide	2 (11)	0 (3)	0 (3)	1 (5)
Other antibiotic	1 (8)	1 (4)	0 (2)	0 (2)
Dicloxacillin	1 (5)	0 (2)	0 (2)	0 (1)
Metronidazole	0 (2)	0 (1)	0 (0)	0 (1)
Tetracycline	0 (1)	0 (1)	0 (0)	0 (0)

RTI, respiratory tract infection; UTI, urinary tract infection.

TABLE 3. Multivariate ORs with 95% CIs for each bacterial genus cultured from the vaginal samples for women treated with any type of oral antibacterial drug either in the third trimester (pregnancy weeks 26–36) or in any trimester (pregnancy weeks 0–36). Estimates are adjusted for all other vaginal bacteria identified in the samples, maternal asthma, smoking, and cat or dog in the home

Microorganism	Antibiotic use in third trimester (pregnancy weeks 26–36) N = 95 (668)		Antibiotic use in any trimester (pregnancy weeks 0–36) N = 226 (668)	
	Adjusted OR (95% CI)	p	Adjusted OR (95% CI)	p
Staphylococcus spp.	1.98 (1.04–3.76)	**0.037**	1.63 (1.06–2.52)	**0.028**
Corynebacterium spp.	0.84 (0.53–1.33)	0.467	1.02 (0.73–1.44)	0.901
Lactobacillus spp.	1.06 (0.67–1.68)	0.808	0.99 (0.70–1.39)	0.936
Enterococcus spp.	0.79 (0.46–1.37)	0.407	1.23 (0.83–1.80)	0.301
Micrococcus spp.	0.82 (0.44–1.52)	0.524	0.93 (0.59–1.45)	0.749
Streptococcus spp.	0.75 (0.40–1.38)	0.352	0.74 (0.47–1.17)	0.194
Escherichia coli	1.91 (1.04–3.52)	**0.038**	0.93 (0.55–1.56)	0.773
Kocuria spp.	0.89 (0.30–2.64)	0.826	0.88 (0.40–1.91)	0.739

10% of women without antibiotic treatment in the third trimester, were colonized by *E. coli* (Table 3).

The increase in vaginal *Staphylococcus* species was mainly associated with treatment with UTI antibiotics. Eighty-five per cent 85% of women treated with UTI antibiotics at any point of pregnancy, and 76% of women without any antibiotic treatment in pregnancy, were colonized with *Staphylococcus* species (adjusted OR 1.90, 95% CI 1.08–3.33, p 0.026). Ninety-three per cent of women treated with UTI antibiotics in the third trimester, and 77% of women without any antibiotic treatment in the third trimester, were colonized with *Staphylococcus* species (adjusted OR 3.97, 95% CI 1.38–11.43, p 0.011). There were no significant effects on *Staphylococcus* species of other antibiotic groups (Fig. 1).

The increase in vaginal *E. coli* after treatment in the third trimester was mainly associated with RTI antibiotics. Twenty-two per cent of women treated with RTI antibiotics in the third trimester, and 10% of women without any antibiotic treatment in the third trimester, were colonized with *E. coli* (adjusted OR 2.47, 95% CI 1.12–5.46, p 0.025). UTI antibiotics administered in the third trimester also showed a tendency to increase vaginal *E. coli*, but not significantly (Fig. 2).

We did not observe any significant changes in the rate of vaginal GBS colonization following antibiotic treatment. Nine per cent of antibiotic-treated women, and 10% of women without any antibiotic treatment in pregnancy, were colonized with GBS.

We did not observe any significant changes in the rate of vaginal *S. aureus* colonization in women treated with antibiotics in pregnancy.

No changes in the overall diversity and composition of the commensal vaginal microbiota were observed. We were not able to differentiate between the treated and the untreated women by evaluating the receiver operating characteristic curves (from partial least-squares discriminant analysis).
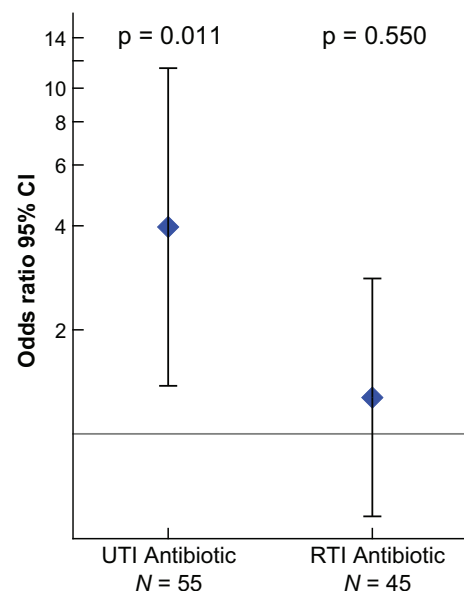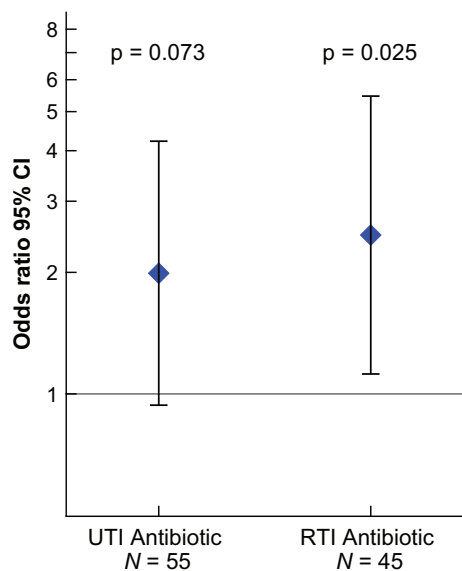

FIG. 1. Associations between the use of oral urinary tract infection (UTI) antibiotics and respiratory tract infection (RTI) antibiotics in the third trimester, pregnancy weeks 26–36, and vaginal colonization by *Staphylococcus* species. Estimates are adjusted for all other vaginal bacteria identified in the samples, maternal asthma, smoking, and cat or dog in the home.

Discussion

Principal findings

Antibiotic use was associated with increased vaginal colonization by *Staphylococcus* species, especially after UTI treatment. Vaginal colonization by *E. coli* increased after antibiotic administration during the third trimester, RTI antibiotics contributing most to this. Antibiotic administration did not significantly influence the vaginal GBS or *S. aureus* colonization

FIG. 2. Associations between the use of oral urinary tract infection (UTI) antibiotics and respiratory tract infection (RTI) antibiotics in the third trimester; pregnancy weeks 26–36, and vaginal colonization by *Escherichia coli*. Estimates are adjusted for all other vaginal bacteria identified in the samples, maternal asthma, smoking, and cat or dog in the home.

rates. Approximately one-third of the pregnant women were treated with antibiotics, which is quite a high proportion in a country such as Denmark, which has low consumption of antibiotics in general.

Strengths and limitations

The main strength of this study is the design of the COPSAC$_{2010}$ pregnancy cohort, which is a replication and extension of the COPSAC$_{2000}$ cohort design. The pregnant women were recruited and closely monitored at the dedicated COPSAC clinical research centre from gestational week 24.

The high reliability of the data obtained on maternal history of antibiotic administration is a further strength of this study. This information was obtained through interviews with the participants during their visit to the COPSAC clinic, and validated against recordings from the Danish Central Medical Register. This is a highly accurate register for data validation, as antibiotics may only be prescribed by an authorized physician, and can only be purchased from authorized pharmacies.

A limitation of the study is that we relied solely on culture, with which only a percentage of bacterial species can be successfully identified [12]. Bacteriological culture methods are vulnerable to the time from sampling to analysis, as well as bacterial growth conditions. This poses a limitation for assessment of the composition of the commensal vaginal

microbiota, and may also explain the samples without growth, as these were all handled in the same manner as the other samples, and did not differ regarding time of transport; also, none of the women was receiving ongoing antibiotic treatment. The culture methods used were not able to identify *Mycobacterium* species and *Ureaplasma* species, which would also have been of clinical interest.

Another limitation of our study is that bacterial samples were analysed at the genus level. However, subclassification and analyses at the species level would result in loss of power and the issue of multiple testing, which was controlled for in the current study by adjusting the analyses for all other vaginal bacteria identified in the samples and socio-economic confounders.

Interpretation

Staphylococcus species were found to colonize 79% of the women. This number conforms with previous studies in pregnant women. We found less *Lactobacillus* species colonization than expected; only 40% of the women were colonized. We believe that this is atributable to overgrowth of other bacteria, which makes the detection of *Lactobacillus* less successful. However, the remaining bacteria represented in the samples matched the expected values [13].

Only 10% of women carried GBS, which is a low frequency as compared with other reports [14,15]. This percentage was not significantly affected by any antibiotic treatment. Even treatment with RTI antibiotics in the third trimester did not eradicate the vaginal GBS at week 36 of pregnancy. GBS screening is not applied in Denmark, and, in our study, oral antibiotic use during pregnancy did not affect the GBS colonization rate, confirming previous reports [16,17]. This is interpreted as recolonization from the gastrointestinal tract, which serves as the primary reservoir for GBS [18].

The increase in the Gram-positive *Staphylococcus* species is mainly a result of treatment with UTI antibiotics. These drugs are effective against Gram-negative bacteria, and have previously been shown to increase the amount of staphylococci found in non-pregnant women [19]. A similar mechanism may explain the increase in *E. coli* following treatment with RTI antibiotics in the third trimester. Most antibiotics in this group are effective against Gram-positive bacteria; hence the increase in Gram-negative *E. coli*. No long-term changes were found after treatment in the first or second trimester. Thus, it seems that the microbial ecology returns to equilibrium in the months following treatment.

Women living with a cat or a dog in the household were at higher risk of being colonized by vaginal *E. coli* and of being treated with antibiotics in pregnancy, as we previously reported [20]. All estimates remained significant after adjustment for this

confounder, suggesting a different mechanism for this observation.

Ascending pathogens from the vagina constitute the most likely source of intrauterine infection, and abnormal colonization of the genital tract has been associated with subsequent preterm birth [21]. Vaginal *E. coli* has likewise been described as an independent risk factor for preterm birth [22,23] and a major contributor to neonatal infection [24,25]. Preventive strategies leading to increased antibiotic use in pregnancy may therefore indirectly increase adverse pregnancy outcomes and early-onset neonatal sepsis, caused by an increased rate of vaginal colonization by *E. coli*.

Ultimately, the newborn child may receive a different and perhaps non-favourable composition of microbiota through vertical transmission during birth. We have previously reported that reduced diversity of the neonatal faecal microbiota and overgrowth with staphylococci is associated with atopic sensitization at school age [26]. Likewise we reported that both the use of antibiotics and vaginal colonization by staphylococci in pregnancy were associated with wheezy disorders in childhood [27,28]. In this way, alterations in the vaginal microbiota may act as a vector for atopic disease in childhood. It is plausible that general alterations in the bacterial composition on all colonized human surfaces might occur, as observed for the vaginal microbiota following antibiotic treatment.

The overall composition of bacteria showed no differences in a data-driven model. Even though the levels of some bacteria were significantly increased after treatment, the remaining bacteria seem to equalize the differences in composition.

Conclusion

Antibiotic administration during pregnancy may lead to alterations in the vaginal microbiological ecology prior to birth. We found preferential increases in *Staphylococcus* species and *E. coli*. A changed vaginal microbiota in pregnant women may lead to infections, with potential morbidity, preterm delivery, neonatal infection, and long-term effects on the early microbial colonization of the neonate.

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Authorship

All authors listed contributed sufficiently to the project to be included as authors, and all those who are qualified to be authors are listed in the author byline. The guarantor of the study is H. Bisgaard, who was responsible for the integrity of the work as a whole, from conception and design to the acquisition of data, analysis and interpretation of data, and writing of the manuscript. J. Stokholm contributed to the acquisition of data, data analyses, and interpretation and writing of the manuscript. S. Schjørring contributed to the identification of bacteria, interpretation of the results, and writing of the manuscript. K. A. Krogfelt and B. Jacobsson contributed to the interpretation of the results and writing of the manuscript. L. Pedersen, A. L. Bischoff, N. Følsgaard, C. G. Carson, B. L. K. Chawes, K. Bønnelykke and A. Mølgaard contributed to data collection. C. E. Eskildsen contributed to the statistical analyses. All authors made important intellectual contributions and contributed critically to the final revision of this manuscript.

Transparency Declaration

To the best of our knowledge, no conflict of interest, financial or other, exists.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Microbial distribution in the vaginal samples. Genus level.

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