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Antibiotics and fecundability among female pregnancy planners: a prospective cohort study

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STUDY QUESTION: To what extent is female preconception antibiotic use associated with fecundability?

SUMMARY ANSWER: Preconception antibiotic use overall was not appreciably associated with fecundability.

WHAT IS KNOWN ALREADY: Antibiotics are commonly used by women and are generally thought to be safe for use during pregnancy. However, little is known about possible effects of antibiotic use on fecundability, the per-cycle probability of conception. Previous research on this question has been limited to occupational rather than therapeutic exposure.

STUDY DESIGN, SIZE, DURATION: We analyzed data from an Internet-based preconception cohort study of 9524 female pregnancy planners aged 21–45 years residing in the USA and Canada who had been attempting to conceive for six or fewer cycles at study entry. Participants enrolled between June 2013 and September 2020 and completed baseline and bimonthly follow-up questionnaires for up to 12 months or until a reported pregnancy, whichever came first. The questions pertaining to antibiotic type and indication were added to the PRESTO questionnaires in March 2016.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We assessed antibiotic use in the previous 4 weeks at baseline and on each follow-up questionnaire. Participants provided the name of the specific antibiotic and the indication for use. Antibiotics were classified based on active ingredient (penicillins, macrolides, nitrofurantoin, nitroimidazole, cephalosporins, sulfonamides, quinolones, tetracyclines, lincosamides), and indications were classified by type of infection (respiratory, urinary tract, skin, vaginal, pelvic, and surgical). Participants reported pregnancy status on follow-up questionnaires. We used proportional probabilities regression to estimate fecundability ratios (FR), the per-cycle probability of conception comparing exposed with unexposed individuals, and 95% confidence intervals (CI), adjusting for sociodemographics, lifestyle factors, and reproductive history.

MAIN RESULTS AND THE ROLE OF CHANCE: Overall, women who used antibiotics in the past 4 weeks at baseline had similar fecundability to those who had not used antibiotics (FR: 0.98, 95% CI: 0.89–1.07). Sulfonamides and lincosamides were associated with slightly increased fecundability (FR: 1.39, 95% CI: 0.90–2.15, and FR: 1.58 95% CI: 0.96–2.60, respectively), while macrolides were associated with slightly reduced fecundability (FR: 0.70, 95% CI: 0.47–1.04). Analyses of the indication for antibiotic use suggest that there is likely some confounding by indication.

LIMITATIONS, REASONS FOR CAUTION: Findings were imprecise for some antibiotic classes and indications for use owing to small numbers of antibiotic users in these categories. There are likely heterogeneous effects of different combinations of indications and treatments, which may be obscured in the overall null results, but cannot be further elucidated in this analysis.

WIDER IMPLICATIONS OF THE FINDINGS: There is little evidence that use of most antibiotics is associated with reduced fecundability. Antibiotics and the infections they treat are likely associated with fecundability through differing mechanisms, resulting in their association with increased fecundability in some circumstances and decreased fecundability in others.

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Introduction

Antibiotic use among women of reproductive age is common, particularly in the USA. According to the Centers for Disease Control and Prevention, there were 916 oral antibiotic prescriptions per 1000 women in 2017, compared to 604 prescriptions per 1000 men (CDC, 2018). Most classes of antibiotics are generally deemed safe for use during pregnancy, but some registry studies have found an association between use of trimethoprim or clarithromycin and increased miscarriage risk (Andersen *et al.*, 2013a,b; Muanda *et al.*, 2018). While data are sparse on the association between antibiotics and time to pregnancy, prolonged time to pregnancy may actually reflect subclinical pregnancy loss, suggesting that these outcomes may have common etiologies, including exposures occurring prior to implantation (Gray and Becker, 2000). To date, the only study of antibiotic use and time to pregnancy in females showed an increase in time to pregnancy among Danish pharmacy assistants exposed to any class of antibiotics, although this exposure was from handling, packaging, and bottling the medication rather than ingesting it for treatment (Schaumburg and Olsen, 1989).

In certain circumstances, antibiotics may be beneficial for female fertility, as they treat conditions related to subfertility, such as bacterial vaginosis and pelvic inflammatory disease (Casari *et al.*, 2010; Emanuele Levi-Setti, 2016). However, prolonged antibiotic use or use for other indications may cause disruptions in the reproductive tract microbiome (Schaumburg and Olsen, 1989; Emanuele Levi-Setti, 2016). Such bacterial imbalances could contribute to increased genital tract acidity, which may impair sperm motility (Schaumburg and Olsen, 1989; Emanuele Levi-Setti, 2016). Additionally, some antibiotics have anti-inflammatory effects, which could disrupt the inflammatory process of implantation (Ng *et al.*, 2002). In the present work, we examined the association between preconception use of antibiotics and fecundability, the per-cycle probability of conception, in a large preconception cohort study of North American pregnancy planners.

Materials and methods

Pregnancy Study Online (PRESTO) is an ongoing, web-based preconception cohort study. The study methods have been described in detail elsewhere (Wise *et al.*, 2015). Briefly, women are eligible for participation if they are aged 21–45 years, residing in the USA or Canada, and not using contraception or fertility treatment. Participants complete an online baseline questionnaire with items on demographics, lifestyle, reproductive history, and medical history (including medication use). They complete follow-up questionnaires every 8 weeks for up to 12 months to ascertain pregnancy status. This study was approved by the Institutional Review Board at the Boston University Medical Campus, and online informed consent was obtained from all participants.

From June 2013 through September 2020, 11 970 eligible women completed the baseline questionnaire. We excluded 138 women whose date of last menstrual period (LMP) at baseline was more than 6 months in the past, and 31 women with missing/implausible LMP data. We then excluded 2500 women who had been trying to conceive for more than six cycles at baseline, to reduce the potential for reverse causation (e.g. subfertility causing changes in medication use). The final data set thus included 9524 women. The questions pertaining to antibiotic type and indication were added to the PRESTO questionnaires in March 2016; therefore, the antibiotic type and indication analyses are restricted to the 7111 participants completing the baseline questionnaire in March 2016 or later. This complete case analysis included 59% of the overall sample and 68% of antibiotic users.

Assessment of exposure

Women were asked at baseline and follow-up if they had taken antibiotics in the past 4 weeks. If they reported antibiotic use in March 2016 or later, they were asked to provide the name of the antibiotic in free text boxes. Free text antibiotic names were classified by active ingredient. Women were also asked the reason for their antibiotic prescription. Free text antibiotic indication responses were classified by type of infection (respiratory, urinary tract, vaginal, skin, pelvic, surgical, or other). Antibiotic indication categories were not mutually exclusive, and some women reported more than one indication for a single antibiotic prescription.

Assessment of outcome

At baseline, participants reported their LMP date, usual cycle length, and number of cycles they had attempted to conceive prior to enrollment. On each follow-up questionnaire, participants were asked for their most recent LMP date and whether they had become pregnant since the previous questionnaire. Total discrete cycles at risk were calculated as follows: cycles of attempt at study entry + [(LMP from most recent follow-up questionnaire−date of baseline questionnaire completion)/usual cycle length] +1. Participants contributed cycles of observation from baseline until they conceived or experienced a censoring event, defined as loss to follow-up, withdrawal, initiation of fertility treatment, no longer trying to conceive, or 12 cycles, whichever came first. Notably, 49 women reported taking an antibiotic for infection prophylaxis following or in preparation for a hysterosalpingogram, a procedure to assess whether the fallopian tubes are blocked (García-Velasco *et al.*, 2017). These women were censored at the cycle when the hysterosalpingogram was reported to avoid bias due to reverse causation.

Assessment of covariates

We selected potential confounders *a priori* based on available literature. Information collected at baseline included age, education, income, BMI, multivitamin use, current smoking, perceived stress score (PSS-10) (Cohen *et al.*, 1983), major depressive inventory (MDI) score (Bech *et al.*, 2001), efforts to improve the chances of conception (e.g. charting menstrual cycles, ovulation testing), intercourse frequency, recent irregular menstrual cycles when not using birth control, history of spontaneous abortion, history of infertility (attempting to conceive for ≥12 months without becoming pregnant), and history of sexually transmitted infections (STIs), including genital warts, herpes, chlamydia or pelvic inflammatory disease.

Data analysis

We used life-table methods to compute the proportion of participants who conceived during follow-up, accounting for censoring. We used proportional probabilities regression to estimate fecundability ratios (FR), the per-cycle probability of conception comparing exposed with unexposed individuals, and 95% confidence intervals (CI), adjusting for potential confounders. FR below 1.00 indicate reduced probability of conception (Weinberg *et al.*, 1989). We used the Anderson–Gill data structure to account for left truncation (Cox, 1972), and we accounted for the decline in baseline fecundability with increasing attempt time by including indicators for cycle at risk in regression models. Results were adjusted for age (<25, 25–29, 30–34, ≥35 years), education (≤12, 13–15, 16, ≥17 years), household income (<50 000, 50 000–99 000, 100 000–149 000, ≥$150 000 US dollars), BMI (<25, 25–30, 31–34, ≥35 kg/m^2), preconception multivitamin use (yes, no), PSS score (continuous), MDI score (continuous), any efforts to improve the chances of conception, (e.g. checking basal body temperature, charting menstrual cycles) (yes, no), intercourse frequency (<1, 1, 2–3, ≥4 times per week), current smoking (yes, no), and a history of STI (yes, no).

Antibiotic use was analyzed at baseline and as a time-varying exposure. As the potential effect of a single instance of antibiotic use on fecundability is likely to be transient, analyses with antibiotic use as a time-varying exposure are likely to be the most biologically relevant and were therefore considered to be the main analyses of interest. Analyses of antibiotic class and indication were similarly analyzed as time-varying exposures. To further isolate potential transient effects of antibiotic use on fecundability, we examined the effect of antibiotic use overall on fecundability, restricting to the first cycle of follow-up after baseline as a sensitivity analysis.

We conducted several stratified analyses to assess effect measure modification of the relation between antibiotic use and fecundability. We stratified by age (<30 years of age versus ≥30 years of age), to determine if any association of antibiotics with time to pregnancy is compounded by increasing age, a strong determinant of fertility (Wesselink *et al.*, 2017). We also stratified by pregnancy attempt time at study entry (0–2 versus 3–6 cycles at study entry), to assess reverse causation, wherein participants who have been trying to conceive for longer are more likely to have altered their behavior in response to their difficulty conceiving (Wise *et al.*, 2020). A stronger effect in the 0–2 cycles group may be more likely to be due to an effect of antibiotics rather than other behavioral changes undergone while trying to conceive. We also stratified by current smoking status, as smoking leads to increased oxidative stress and may compound the effect of antibiotic use or underlying infection on fecundability (Kamceva *et al.*, 2016; Wesselink *et al.*, 2019). We considered BMI (<30 vs ≥30 kg/m^2) as a potential effect measure modifier due to the association between obesity and the vaginal microbiome (Brookheart *et al.*, 2019). Finally, results were stratified by a composite variable representing history of reproductive health conditions (no history of STI, endometriosis, bacterial vaginosis, infertility, or spontaneous abortion versus a history of any of these conditions) to examine the effect of antibiotics among women for whom they may improve fecundability by treating an underlying pelvic infection.

We used multiple imputation to generate values for missing baseline and follow-up data on covariates and pregnancy status. Covariate information was missing for <1% of participants for all covariates except for income, which was missing for 3% of participants. Missingness for antibiotic use overall was also <1%. We assigned one cycle of observation to the 17% of women who did not complete any follow-up questionnaires and imputed the outcome of that cycle (pregnant vs not). We created five imputed datasets and statistically combined coefficient and standard error estimates from the five datasets.

In analyses of antibiotic class and indication for use, we excluded 2413 (25%) of the 9524 participants owing to their completion of the questionnaire prior to March 2016. Antibiotic class was unknown or could not be determined for 15% of included participants. Antibiotic class for these participants was imputed to the most common class reported for the indication they provided. Antibiotic indications were unknown or could not be classified for 24 participants (2%). These participants were excluded from antibiotic indication analyses and imputed to penicillins (the most commonly used antibiotic) for antibiotic class analyses.

Results

The analytic cohort included 9524 women with 5382 pregnancies and 36 353 menstrual cycles. During follow-up, 71% conceived based on life-table methods, 14% did not become pregnant within 12 cycles, 3% stopped trying to conceive, 7% began fertility treatment, 2% were still actively participating, and 21% were lost to follow-up. Loss to follow-up did not materially differ by antibiotic status (18% of antibiotic users and 22% of non-users). Of the 9524 participants in this study, 776 (8%) reported using antibiotics at baseline, and 758 (8%) reported using antibiotics during follow-up. A total of 1432 participants (15%) reported antibiotic use at any point during their participation in the study (baseline or follow-up), and 14% of these participants (n = 196) reported antibiotic use at multiple time points.

Baseline characteristics of antibiotic users and non-users (at any point during PRESTO participation) are presented in Table I. Antibiotic users more often had lower household income and lower educational attainment than non-users and were more likely to live in the southern USA.They were also slightly more likely to have ever smoked (27% vs 23%). A greater proportion of antibiotic users reported irregular menstrual cycles (20% vs 17%), used hormonal methods as their last method of contraception (42% vs 37%), and have a history of spontaneous abortion (29% vs 25%), infertility (12% vs 8%), or STI (18% vs 14%).

Table I Baseline characteristics of female 9524 female pregnancy planners, by antibiotic use, in PRESTO.

Characteristic[a]	Antibiotic use during study participation	
	No	Yes
Number of participants, N (%)	8092 (85.0)	1432 (15.0)
Age (years, mean)	29.8	30.1
Partners age (years, mean)	31.8	32.0
White, non-Hispanic (%)	84.7	82.4
Annual household income <US$50 000 (%)	19.5	22.7
<College degree (%)	27.5	30.1
Geographic region (%)		
US Midwest	21.7	21.5
US Northeast	22.5	20.7
US South	23.0	28.1
US West	16.1	16.1
Canada	16.5	13.4
Physical activity (MET-hours/week, mean)	34.2	32.3
BMI (kg/m^2, mean)	27.9	28.6
Multivitamin use (%)	80.0	80.0
Ever smoker (%)	22.8	26.9
Alcohol intake (drinks/week, mean)	3.2	3.2
Parous (%)	33.0	32.7
Irregular menstrual cycles (%)	16.8	20.2
Doing something to improve changes of conceiving (%)	77.3	74.6
Intercourse frequency <1 time/week (%)	20.9	21.5
Intercourse frequency >3 time/week (%)	15.8	18.5
Hormonal last method of contraception (%)	37.3	41.7
History of spontaneous abortion (%)	25.0	28.8
History of infertility (%)	8.3	12.2
History of STI (%)[b]	14.1	17.8

[a]All characteristics except for age are age-standardized to the cohort at baseline.
[b]Includes genital warts, herpes, chlamydia or pelvic inflammatory disease.
PRESTO, Pregnancy Study Online; STI, sexually transmitted infections; MET, metabolic equivalent of task.

Overall, antibiotic use was not associated with fecundability. As shown in Table II, when antibiotic use was analyzed as a time-varying exposure, women who used antibiotics in the past 4 weeks did not have appreciably lower fecundability than those who had not used antibiotics (FR: 0.98, 95% CI: 0.89–1.07). When we restricted to the first cycle after use, we similarly found no appreciable relation between antibiotic use and fecundability (FR: 1.07, 95% CI: 0.94–1.23).

Antibiotic class

The most commonly reported antibiotic class was penicillins, which accounted for 49% of total reported antibiotics. Macrolides, tetracyclines, and nitrofurantoin were also commonly reported, with each comprising approximately 9% of the reported antibiotics. The remaining antibiotics reported included (in order of frequency): cephalosporins (7%), quinolones (5%), nitroimidazole (5%), sulfonamides (4%), and lincosamides (2%).

The associations between use of specific antibiotic classes and fecundability are presented in Table III. The use of sulfonamides and lincosamides was associated with slightly increased fecundability (FR: 1.39, 95% CI: 0.90–2.15 and FR: 1.58, 95% CI: 0.96–2.60, respectively), while macrolide use was associated with slightly reduced fecundability (FR: 0.70, 95% CI: 0.47–1.04). There was little association between the use of other classes of antibiotics and fecundability. Estimates for use of several antibiotic classes were imprecise, owing to small numbers of women reporting use of these antibiotics.

Antibiotic indication

A total of 1284 indications for antibiotic use were reported, as some women reported more than one indication for antibiotic use and others did not provide an indication. The most common indications for antibiotic use overall were respiratory infections and urinary tract infections, which together accounted for approximately half of all antibiotic use. Respiratory infections comprised 30% (n = 384) of reported indications and urinary tract infections comprised 23% of reported indications (n = 289). Vaginal or pelvic infections were listed as an indication for 15% of antibiotic prescriptions (n = 187). The most common indications for recurrent antibiotic use were urinary tract infection treatment or prevention, skin conditions including recurrent

Table II Use of antibiotics and fecundability among 9524 female pregnancy planners.

Exposure	No. of cycles	No. of pregnancies	Unadjusted FR (95% CI)	Adjusted FR (95% CI)[a]
Females				
Baseline use				
No	33402	4975	Reference	Reference
Yes	2923	403	0.93 (0.85–1.02)	0.99 (0.90–1.08)
Time-varying use[b]				
No	33379	4974	Reference	Reference
Yes	2974	408	0.93 (0.85–1.02)	0.98 (0.89–1.07)
One cycle of follow up				
No	8733	776	Reference	Reference
Yes	776	184	1.00 (0.87–1.15)	1.07 (0.94–1.23)

[a]Models adjusted for age at baseline, education, income, BMI, multivitamin use, perceived stress score (PSS), major depressive inventory (MDI) score, doing anything to improve chances of conception, intercourse frequency, geographic region, current smoking, and history of STI.
[b]Total cycles and pregnancies are slightly higher in the time-varying analyses due to 28 women with missing data on baseline antibiotic use.
FR, fecundability ratio.

Table III Fecundability among 7111 female pregnancy planners, by antibiotic type.[a]

Exposure	No. of cycles	No. of pregnancies	Unadjusted FR (95% CI)	Adjusted[b] FR (95% CI)
No antibiotic	23080	3623	Reference	Reference
Penicillins	1081	153	1.04 (0.89–1.22)	1.09 (0.94–1.28)
Nitrofurantoin	255	38	0.86 (0.62–1.18)	0.83 (0.60–1.13)
Cephalosporins	175	25	0.95 (0.66–1.37)	0.98 (0.68–1.40)
Macrolides	249	24	0.63 (0.42–0.94)	0.70 (0.47–1.04)
Nitroimidazole	137	20	0.85 (0.57–1.27)	0.90 (0.60–1.34)
Sulfonamides	101	17	1.16 (0.75–1.80)	1.39 (0.90–2.15)
Quinolones	123	15	0.86 (0.54–1.37)	0.82 (0.52–1.30)
Tetracyclines	128	17	0.84 (0.51–1.37)	0.90 (0.55–1.48)
Lincosamides	40	11	1.47 (0.88–2.47)	1.58 (0.96–2.60)

[a]Time-varying analyses, among women completing the baseline questionnaire on or after 1 March 2016.
[b]Models adjusted for age at baseline, education, income, BMI, multivitamin use, PSS score, MDI score, doing anything to improve chances of conception, intercourse frequency, geographic region, current smoking, history of STI.

cysts, recurrent sinus infections, recurrent yeast infections, and malaria prophylaxis. Respiratory, surgical, skin, pelvic, and vaginal indications were most often treated with penicillins, while pelvic infections were most often treated with tetracyclines.

Generally, the use of an antibiotic for a given indication was not associated with fecundability. There was no substantial association between the use of an antibiotic for a respiratory, urinary tract, skin, or surgical infection when compared to no antibiotic use (Table IV). The use of an antibiotic for a vaginal or pelvic infection was associated with slightly reduced fecundability whether compared with use of an antibiotic for respiratory infection (FR: 0.80, 95% CI: 0.53–1.20, and FR: 0.75, 95% CI: 0.43–1.29, respectively (data not shown)) or compared with no antibiotic use (FR: 0.89, 95% CI: 0.58–1.37, and FR: 0.71, 95% CI: 0.40–1.27, respectively (Table IV)).

Results of the stratified analyses showed little evidence of effect measure modification by the stratification variables. Little difference in the effect of antibiotic use overall on time to pregnancy was found when stratifying by pregnancy attempt time at study entry or BMI (data not shown). We observed slightly lower fecundability among antibiotic users in women <30 years of age (FR: 0.88, 95% CI: 0.76–1.02), current smokers (FR: 0.84, 95% CI: 0.55–1.29), and women with a history of reproductive health conditions (FR: 0.86, 95% CI: 0.75–1.00), but not in women ≥30 years of age (FR: 1.08, 95% CI: 0.95–1.23), those who do not currently smoke (FR: 0.99, 95%

Table IV Fecundability among 7087 female pregnancy planners, by antibiotic indication.[a]

Exposure	No. of cycles	No. of pregnancies	Unadjusted FR (95% CI)	Adjusted[b] FR (95% CI)
No antibiotic	23053	3621	Reference	Reference
Respiratory	713	102	0.93 (0.77–1.12)	1.02 (0.85–1.23)
Urinary tract	532	84	0.98 (0.79–1.22)	0.99 (0.80–1.23)
Skin	163	29	1.14 (0.82–1.58)	1.09 (0.79–1.51)
Vaginal	155	21	0.88 (0.57–1.36)	0.89 (0.58–1.37)
Pelvic	121	13	0.63 (0.35–1.11)	0.71 (0.40–1.27)
Surgical	98	12	0.88 (0.49–1.58)	0.89 (0.49–1.63)

[a]Time-varying analyses, among women completing the baseline questionnaire on or after 1 March 2016.
[b]Models adjusted for age at baseline, education, income, BMI, multivitamin use, PSS score, MDI score, doing anything to improve chances of conception, intercourse frequency, geographic region, current smoking, history of STI.

CI: 0.90–1.09), and women without a history of reproductive health conditions (FR: 1.07, 95% CI: 0.93–1.22).

Discussion

Overall, we found little evidence of an association between antibiotic use and fecundability. The use of sulfonamides and lincosamides may be associated with slightly improved fecundability, while the use of macrolides or use of antibiotics for pelvic or vaginal indications may be associated with slightly reduced fecundability, although estimates were imprecise.

Macrolides and lincosamides, while chemically distinct, share a common mechanism of action (interruption of bacterial protein synthesis) (Leclercq and Courvalin, 1991). However, we found opposite associations between macrolides and fecundability (FR: 0.70, 95% CI: 0.47–1.05) and lincosamides and fecundability (FR: 1.58, 95% CI: 0.96–2.60). These opposite observed associations may be more attributable to chance or confounding by indication rather than actual effects of these specific types of antibiotics. Although there were not sufficient numbers to assess combinations of indication and antibiotic class, macrolides were more frequently used orally to treat illness, while lincosamides were more frequently used topically for skin conditions such as acne, which may partially explain the divergent findings.

Most lincosamides and macrolides are categorized as Food and Drug Administration (FDA) Category B for use in pregnancy, meaning that animal studies show no harm or animal studies show harm that has been unconfirmed in human studies (Sá Del Fiol *et al.*, 2005). Clarithromycin, a specific macrolide, has been associated with spontaneous abortion, which may share a common etiological pathway with prolonged time to pregnancy, and is classified as FDA category C, meaning that use during pregnancy should be avoided whenever possible (Sá Del Fiol *et al.*, 2005; Andersen *et al.*, 2013b). While there were too few clarithromycin users to examine clarithromycin independently in this sample, a strong association between clarithromycin and prolonged time to pregnancy or early spontaneous abortion could contribute to the lower fecundability observed in macrolide users.

Lower fecundability was observed among participants using antibiotics for vaginal or pelvic infections, but not among participants using antibiotics for respiratory infections, despite similar patterns of macrolide use (17% of participants with vaginal or pelvic infections and 24% of participants with respiratory infections used macrolides), suggesting potential confounding by indication. The reduced fecundability observed among women with a history of reproductive health conditions and women taking an antibiotic for pelvic or vaginal infections also provides evidence for confounding by indication. Residual confounding by indication is a concern, as we were not able to jointly stratify by antibiotic class and indication.

Bacterial vaginosis is commonly treated with nitroimidazole or lincomycin antibiotics. It is believed to be the most common vaginal infection among women of reproductive age (Casari *et al.*, 2010) and has been associated with subfecundity and infertility in several studies (Salah *et al.*, 2013; Haahr *et al.*, 2016; Lokken *et al.*, 2021). A recent prospective cohort study of 458 Kenyan pregnancy planners found that patients with bacterial vaginosis at the health care visit prior to pregnancy testing had 17% lower fecundability than those without bacterial vaginosis (FR: 0.83, 95% CI: 0.6–1.1). The effect was stronger in women who had persistent bacterial vaginosis, which was associated with a 43% reduction in fecundability (FR: 0.57, 95% CI: 0.4–0.8) (Lokken *et al.*, 2021). A 2013 cohort study found that women with infertility had over five times the odds of bacterial vaginosis than fertile women (odds ratio: 5.23, 95% CI: 3.06–8.12) (Salah *et al.*, 2013). When stratifying by cause of infertility, investigators found that the 6-month cumulative pregnancy rate was higher among those with treated bacterial vaginosis than those with untreated bacterial vaginosis, regardless of the underlying cause of fertility (hormonal or unexplained). A 2019 meta-analysis of 12 studies found that the prevalence of tubal factor infertility was greater in patients with bacterial vaginosis than in those with normal vaginal microbiota (45% vs 28%) (Haahr *et al.*, 2019). In addition to the vaginal microbiome, the microbiome of the upper reproductive tract may be important for fertility, as semen protects sperm from the acidic vaginal environment, but vaginal bacteria can ascend up the reproductive tract and adversely impact fertility (Suarez and Pacey, 2006; Moreno and Simon, 2019). These findings suggest a complex relation between the reproductive tract microbiome, hormonal disturbances, and fertility issues, which may be, at least in some cases, improved with antibiotic treatment (Salah *et al.*, 2013; Haahr *et al.*, 2019; Lokken *et al.*, 2021). While we were unable to specifically analyze use of an antibiotic for bacterial vaginosis, when analyzing use of nitroimidazole, lincomycins, and use of an antibiotic

for a vaginal infection overall, our study found mixed results. Given the differing mechanisms by which antibiotics may be helpful or detrimental to fertility, the overall null results may mask heterogeneous effects. While treating an active infection with antibiotics may improve the chances of conception, repeated use of antibiotics may interfere with conception. Some studies have provided evidence of an association between levels of reproductive hormones, vaginal pH, and relative bacterial abundance in the vagina (Casari *et al.*, 2010; Farage *et al.*, 2010; Wira *et al.*, 2015; Emanuele Levi-Setti, 2016). Alterations in the acidity of the genital tract may interfere with the mobility of sperm and, therefore, conception. While antibiotics can aid fertility by resolving an acute infection, excessive antibiotic therapy may alter the normal reproductive tract flora and disrupt the optimal bacterial environment for conception and implantation (Emanuele Levi-Setti, 2016). Repeated exposure to small doses of antibiotics, with subsequent superinfections and changes in vaginal acidity, was proposed as a mechanism for the observed effect of antibiotics on time to pregnancy in the Danish study of pharmacy assistants (Schaumburg and Olsen, 1989).

When we stratified the analysis of overall time-varying antibiotic use and fecundability, we observed slightly lower fecundability among antibiotic users in women <30 years of age, and current smokers. Distribution of medication class and indication were similar among younger and older women, although younger women were slightly less likely to use antibiotics for a pelvic infection than older women (8% vs 11% of ever antibiotic users). As antibiotic use for a pelvic infection was associated with slightly lower fecundability, fewer pelvic infections in this group would not explain the slightly lower fecundability observed among antibiotic users.

Current smokers and nonsmokers also had similar distributions of antibiotic class and indications, although current smokers were slightly more likely to use sulfonamides (8% vs 4%, respectively) and an antibiotic for a vaginal infection (9% vs 5%, respectively). As sulfonamides and use of an antibiotic for a vaginal infection were associated with fecundability in opposite directions, it is unlikely that differing antibiotic classes or indications explain the stronger findings among smokers.

Stratified analysis showed that antibiotic use may have a slightly different association with fecundability among different subgroups of pregnancy planners, although these associations were modest, further illustrating the potentially complex relation between antibiotic use, underlying health, and fecundability.

In addition to confounding by indication, potential sources of bias in this study include misclassification, selection bias, and unmeasured or residual confounding. Participants may misremember the timing or type of antibiotic taken for a particular infection. Such misclassification is likely to be non-differential and reduced by the 4-week recall period. As participants were surveyed every 8 weeks and asked about their medication use in the past 4 weeks, medication use in the 4 weeks immediately following a follow-up questionnaire was not assessed. This missing antibiotic use would be unlikely to materially impact our results, as singular instances of antibiotic use are likely to be transient exposures, and we did not have a sufficient number of women taking multiple antibiotics over follow-up to analyze the association between repeated antibiotic use and fecundability.

Misclassification of time to pregnancy is possible, particularly if participants incorrectly report their cycles of attempt time at study entry, although such misclassification is less likely in a study of pregnancy planners and likely non-differential. Selection bias due to differential loss to follow-up is unlikely, as antibiotic users were not substantially more or less likely to be lost to follow-up than non-users. Residual confounding by factors such as diet quality, health care quality, or health care accessibility is possible, although this study collected extensive data on participant demographic and lifestyle factors, as well as reproductive and medical history.

FR for several antibiotic classes and indications for use were imprecise, owing to the small numbers of participants reporting these use patterns. Small numbers also prevented more detailed analysis of the association between specific antibiotic and indication combinations and fecundability, which may aid in identifying how antibiotics may be associated with increased or decreased fecundability.

Our results do not support the hypothesis that antibiotic use overall is associated with fecundability, however, specific types of antibiotics and specific indications for their use may be associated with either increased or decreased fecundability. Several factors, including medical history, underlying health status, inflammatory responses, and the reproductive tract microbiome, likely contribute to these differences in observed effects, although stratified analyses show that antibiotic use may be slightly more likely to be associated with lower fecundability among younger women, smokers, and those with a history of reproductive health conditions.

Data availability

The data underlying this article cannot be shared publicly to protect the privacy of the individuals that participated in this study.

Authors' roles

H.M.C. contributed to the study design and data analysis and drafted the manuscript. A.K.W. contributed to the study design, data analysis, data interpretation, and revision of the manuscript. L.A.W. contributed to the study design, data interpretation, and revision of the manuscript. T.R.W. contributed to the study design, data analysis, and revision of the manuscript. C.R.H.J. contributed to the data interpretation and revision of the manuscript. E.M.M. contributed to the study design, data interpretation, and revision of the manuscript. E.E.H. contributed the study design, data interpretation, and revision of the manuscript. All authors approved the final version of this manuscript.

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Conflict of interest

L.A.W. has received in-kind donations from Swiss Precision Diagnostics, Sandstone Diagnostics, Fertility Friend, and Kindara for

primary data collection in PRESTO. The other authors have no conflicts of interest to disclose.

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