International Journal of Epidemiology, 2023, 1448–1458
https://doi.org/10.1093/ije/dyad004
Advance Access Publication Date: 24 January 2023
Original article

Cancer

In-utero exposure to antibiotics and risk of colorectal cancer in a prospective cohort of 18 000 adult offspring

Caitlin C Murphy [1]* Piera M Cirillo,[2] Nickilou Y Krigbaum,[2] Amit G Singal [3] Dean P Jones,[4] Timothy Zaki,[3] and Barbara A Cohn[2]

[1]Department of Health Promotion and Behavioral Sciences, University of Texas Health Science Center at Houston (UTHealth Houston), School of Public Health, Houston, TX, USA, [2]Child Health and Development Studies, Public Health Institute, Berkeley, CA, USA, [3]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA and [4]Departments of Medicine and Biochemistry, Emory University School of Medicine, Atlanta, GA, USA

*Corresponding author. Department of Health Promotion & Behavioral Sciences, University of Texas Health Science Center at Houston (UTHealth Houston), School of Public Health, 7000 Fannin Street, Suite 2618, Houston, TX 77030, USA. E-mail: caitlin.c.murphy@uth.tmc.edu

Received 27 June 2022; Editorial decision 15 December 2022; Accepted 10 January 2023

Abstract

Background: Incidence rates of colorectal cancer (CRC) are increasing among younger adults and in mid-life, implicating exposures in early life as risk factors. We examined the association between *in-utero* exposure to antibiotics and risk of CRC in adult offspring.

Methods: The Child Health and Development Studies is a prospective cohort of women receiving prenatal care between 1959 and 1966 in Oakland, California, with deliveries through June 1967. Diagnosed conditions and all prescribed medications were abstracted from mothers' medical records beginning 6 months prior to pregnancy through delivery. We identified mothers who received antibiotics in pregnancy, including penicillins, tetracyclines, short-acting sulfonamides and long-acting sulfonamides. Diagnoses of CRC in adult (age $\geq$18 years) offspring were ascertained through 2021 by linkage with the California Cancer Registry. Cox proportional models were used to estimate adjusted hazard ratios (aHR), with follow-up accrued from birth through cancer diagnosis, death or last contact.

Results: Of 18 751 liveborn offspring, about 15% ($n=2635$) were exposed *in utero* to antibiotics: 5.4% ($n=1016$) to tetracyclines, 4.9% ($n=918$) to penicillins, 4.2% ($n=785$) to short-acting sulfonamides and 1.5% ($n=273$) to long-acting sulfonamides. Compared with offspring not exposed, associations between *in-utero* exposure and CRC in adult offspring were: aHR 1.03 (95% CI 0.32, 3.31) for tetracyclines; aHR 1.12 (95% CI 0.35, 3.58) for penicillins; aHR 0.83 (95% CI 0.20, 3.42) for short-acting sulfonamides; and aHR 4.40 (95% CI 1.63, 11.88) for long-acting sulfonamides.

Conclusion: Our findings support an association between *in-utero* exposure to long-acting sulfonamides and CRC in adulthood.

Key words: Colorectal cancer, young adult, antimicrobial agent, risk factor

Key Messages

- Incidence rates of colorectal cancer are increasing in younger adults and in mid-life, but the reason for this increase is not known. Early exposure to antibiotics has been suggested as a potential explanation.
- In this prospective study of more than 18 000 mother-child dyads, *in-utero* exposure to long-acting sulfonamides increased risk of colorectal cancer in adult offspring.
- There was no difference in risk associated with short-acting sulfonamides or other antibiotic classes, such as tetracyclines and penicillins.
- Although long-acting sulfonamides are no longer routinely prescribed in humans, their continued presence as environmental pollutants raise the possibility that long-term exposure may also increase risk of colorectal cancer.

Introduction

Incidence rates of colorectal cancer (CRC) have increased in young adults (age 18–49 years) in the USA, and more recently rates have increased in adults in their early 50s.[1] The shifting epidemiology of CRC has sparked interest in identifying novel risk factors.[2] Importantly, incidence rates of CRC have increased across birth cohorts,[3] starting with persons born in the 1960s and implicating exposures in early life as risk factors. Early life, beginning *in utero*, represents a critical window of susceptibility,[4] and exposures during this time can translate into large effects on risk of cancer in adulthood. A robust experimental and epidemiological literature supports the importance of gestation and infancy for several adult cancers.[5]

In the 1960s, antibiotics were frequently prescribed to pregnant women to treat urinary tract infections,[6,7] upper respiratory infections, sexually transmitted infections and skin infections.[8] Antibiotics cross the placental barrier via simple diffusion,[9] and exposure *in utero* may directly affect the developing gastrointestinal tract of the fetus through alterations in maternal microbiota or immune response.[10] Exposure *in utero* may also have prolonged antimicrobial effects in newborns.[11] This is consistent with embryo-fetal toxicity of several antibiotic classes (e.g. tetracyclines[12,13]), as well as epidemiological studies demonstrating long-term consequences of antibiotic exposure in early life for childhood obesity,[14–16] a well-established risk factor for CRC.[17]

We examined the association of *in-utero* exposure to antibiotics and CRC in adult offspring of the Child Health and Developments Studies (CHDS), a multi-generational cohort of more than 18 000 mother-child dyads followed for 60 years. The CHDS provides a unique opportunity to study impacts of early life by linking *in-utero* exposures with cancers ascertained from a population-based registry.[18,19]

Materials and methods

Study population

The CHDS was established in 1959 and recruited nearly all (98%) pregnant women receiving prenatal care from the Kaiser Foundation Health Plan (Oakland, CA) between June 1959 and September 1966, with deliveries through June 1967. Embedding the study in the Kaiser Foundation Health Plan provided several advantages: mothers received all care at centralized facilities; care was equally available; and medical services, including visits and prescribed medications provided by all physicians across specialties, were assembled for each mother into a single file. Additional details of the CHDS and methodology are available.[20]

CHDS participants are regularly monitored by linkage to the California Department of Motor Vehicles, California Department of Vital Statistics and California Cancer Registry. We supplement surveillance by additionally linking with the National Death Index. Mothers and their families are matched to these sources using an accumulated name and address history, successfully identifying more than 80% of families.

Primary outcome

We ascertained diagnoses of CRC in adult (age $\geq$18 years) offspring through 2021 by linkage with the California Cancer Registry (International Classification of Disease in Oncology, 3rd edition, codes C18.0–1, C19.9, C20.9). We used a rigorous protocol to verify cases, comparing fixed (e.g. birth date, sex, race) and changeable (e.g. address) identifiers by manual review.

In-utero exposure to antibiotics

Clinical information, including prenatal visits, diagnosed conditions and all prescribed medications, were abstracted from mothers' medical records beginning 6 months prior to pregnancy through labour and delivery. We identified mothers who received antibiotics during pregnancy, including the timing (first trimester: Days 0–90; second trimester: Days 91–180; third trimester: Days $\geq$181), frequency and indication. Because the mechanism of action differs across antibiotic classes, we examined *in-utero*

exposure to: (i) tetracyclines; (ii) penicillins; (iii) short-acting sulfonamides; and (iv) long-acting sulfonamides (Supplementary Table S1, available as Supplementary data at *IJE* online). We distinguished short- from long-acting sulfonamides because pharmacokinetics considerably differ.[21] We did not examine other classes, including cephalosporins and macrolides, because they were not available or rarely prescribed in the late 1950s and 60s.

Statistical analysis

We estimated incidence rates of CRC and 95% confidence intervals based on the discrete probability distribution for a binomial parameter, separately for any *in-utero* exposure to tetracyclines, penicillins, short-acting sulfonamides and long-acting sulfonamides.

We used Cox proportional hazards models to estimate hazard ratios (HR) and their 95% confidence intervals, accounting for correlation between observations from siblings ($n = 4244$) with robust sandwich estimators. Follow-up time was accrued from birth through date of cancer diagnosis, death or last contact. The majority of exposed offspring (87.6% of 2635 exposed, Supplementary Table S2, available as Supplementary data at *IJE* online) were exposed to only one antibiotic class. Therefore, we built separate models for any *in-utero* exposure to tetracyclines, penicillins, short-acting sulfonamides and long-acting sulfonamides, with offspring not exposed to any antibiotic ($n = 16\,116$) as the reference category. Those exposed to more than one antibiotic class (12.4% of 2635 exposed) contributed exposed person-time to more than one model, as appropriate. For example, offspring exposed to penicillins and tetracyclines contributed exposed person-time to the penicillin and tetracycline models.

We selected confounders a priori as maternal characteristics associated with both *in-utero* exposure to antibiotics and CRC in adult offspring,[19] year of birth, maternal smoking (current vs else), maternal race (non-Hispanic Black vs else) and maternal body mass index (overweight or obese vs else). Maternal smoking and race were reported by mothers during in-person interviews at enrollment. We used height and weight reported by mothers during in-person interviews at enrollment or recorded at the first prenatal visit to measure body mass index. We did not adjust for mediators (e.g. gestational age, birthweight), as recommended in the literature.[22]

We assessed the proportional hazards assumption in adjusted models by including an interaction term of log(time) and *in-utero* exposure to antibiotics. The assumption was not violated in any model (tetracyclines: $P = 0.55$; penicillins: $P = 0.72$; short-acting sulfonamides: $P = 0.66$; long-acting sulfonamides: $P = 0.45$).

Sensitivity analyses

To address the possibility of confounding by indication, we examined the association between upper respiratory infection, urinary tract infection, sexually transmitted infection and skin infection in mothers and CRC in offspring, using Cox proportional hazards models as detailed above. We also built adjusted models of *in-utero* exposure that included the indicating condition.

We calculated E-values[23,24] to address unmeasured confounding. E-values provide an estimate of the minimum strength of association that an unmeasured confounder must have with both the exposure and outcome to explain away the observed association. We applied the formula *RR + sqrt[RR x (RR-1)]* and report the limit of the confidence interval closest to the null.

Missingness ranged from 1.5% (maternal race) to 20.8% (maternal smoking) for variables included in adjusted models. We used multiple imputation by fully conditional specification to estimate associations with CRC in adult offspring, separately for any *in-utero* exposure to tetracyclines, penicillins, short-acting sulfonamides and long-acting sulfonamides. Fully conditional specification[25] relaxes assumptions of joint multivariate normality and linearity and is well suited for imputation of both categorical and continuous variables.

All analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC).

Patient and public involvement

The CHDS routinely engages its own cohort members in community-based participatory research. We meet quarterly with our Participant Advisory Council (PAC), a racially and sexually diverse representation of the cohort, to develop research questions, resolve ethical issues related to study participation, design innovative recruitment methods and improve dissemination of findings. At the beginning of this study, we met with PAC members to provide an overview of CRC and to discuss study concepts, rationale and approach; they asked questions and offered feedback.[19] Meetings will be scheduled in the future to present and interpret ongoing results, as well as plans to disseminate findings to the larger cohort.

Results

Of 18 751 liveborn offspring, about 15% ($n = 2635$) were exposed *in utero* to antibiotics: 5.4% ($n = 1016$) to tetracyclines, 4.9% ($n = 918$) to penicillins, 4.2% ($n = 785$) to short-acting sulfonamides and 1.5% ($n = 273$) to long-acting sulfonamides (Figure 1). Table 1 shows

characteristics of offspring by any *in-utero* exposure to tetracyclines, penicillins, short-acting sulfonamides and long-acting sulfonamides. Median and mean follow-up (in years) was similar by *in-utero* exposure to antibiotics [tetracyclines: median 50.5 (IQR 27.5–53.5), mean 40.4 (SD 18.5); penicillins: median 50.5 (IQR 29.5–52.5), mean 40.4 (SD 18.0); short-acting sulfonamides: median 49.5 (IQR 26.5–53.5), mean 39.0 (SD 18.7); long-acting sulfonamides: median 50.5 (IQR 27.5–53.5), mean 40.4 (SD 18.5); and not exposed: median 50.5 (IQR 26.5–53.5), mean 39.3 (SD 19.0)].

Timing and frequency of exposure was similar across antibiotic classes (Table 3). About 35% of exposed offspring were first exposed in the first trimester and most offspring were exposed to one prescription. There were some differences in indication by antibiotic class. For example, urinary tract infection was the most common indication for short-acting (84.8%) and long-acting (52.4%) sulfonamides, whereas upper respiratory infection was the most common indication for tetracyclines (66.4%) and penicillins (50.1%).

Over 739 177.5 person-years of follow-up, 80 adult offspring were diagnosed with CRC (Table 2). Offspring were diagnosed between ages 23 and 59 years (median: 50.0 years, IQR: 46.0 –53.0 years); 15% had a family history of CRC.

Incidence rates of CRC were 7.3 per 100 000 (95% CI 1.5, 21.4), 10.8 per 100 000 (95% CI 2.9, 27.6), 6.5 per 100 000 (95% CI 0.8, 23.6) and 54.4 per 100 000 (95% CI 20.0, 118.5) in offspring exposed to tetracyclines, penicillins, short-acting sulfonamides and long-acting sulfonamides, respectively (Table 4). By comparison, the incidence rate was 10.6 per 100 000 (95% CI 8.2, 13.4) in offspring not exposed to any antibiotics. Cumulative incidence of CRC is shown in Figure 2. Similar to incidence rates, cumulative incidence of CRC at age 55 years was 0.4%, 0.9%, 0.2% and 4.2% for *in-utero* exposure to tetracyclines, penicillins, short-acting sulfonamides and long-acting sulfonamides, respectively.

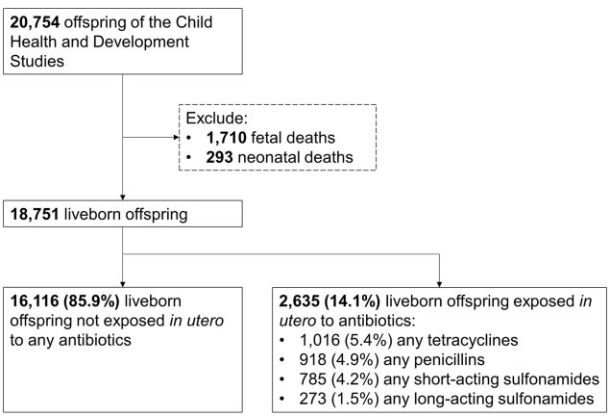

Figure 1 Study flow diagram

Adjusted associations between *in-utero* exposure and CRC in adult offspring were as follows: aHR 1.03 (95% CI 0.32, 3.31) for tetracyclines; aHR 1.12 (95% CI 0.35, 3.58) for penicillins; aHR 0.83 (95% CI 0.20, 3.42) for short-acting sulfonamides; and aHR 4.40 (95% CI 1.63, 11.88) for long-acting sulfonamides (Table 4). Associations were similar in the sensitivity analysis using multiple imputation by fully conditional specification (Supplementary Table S3, available as Supplementary data at *IJE* online).

Associations between CRC in offspring and conditions indicating antibiotics in mothers were as follows: HR 0.64 (95% CI 0.26, 1.56) for urinary tract infection; HR 1.02 (95% CI 0.64, 1.53) for upper respiratory infection; HR 1.50 (95% CI 0.37, 6.08) for sexually transmitted infection; and HR 0.85 (95% CI 0.45, 1.61) for skin infection (not shown). Similarly, the association between *in-utero* exposure to long-acting sulfonamides and CRC in offspring remained similar, although was less precise, in models additionally adjusted for urinary tract infection (aHR 8.40, 95% CI 2.76, 25.62), upper respiratory infection (aHR 4.81, 95% CI 1.82, 12.69) and skin infection (aHR 4.61, 95% CI 1.69, 12.62).

For *in-utero* exposure to long-acting sulfonamides, applying the E-value formula produced E = 9.17 for the estimate and E = 3.62 for the lower confidence limit (not shown).

Discussion

Our findings support an association between *in-utero* exposure to long-acting sulfonamides and CRC in adulthood. Incidence rates of CRC were more than five times higher in offspring exposed to long-acting sulfonamides compared with offspring exposed to other antibiotic classes or not exposed. Cases exposed to long-acting sulfonamides were all exposed to sulfadimethoxine, and our findings may reflect a specific effect of sulfadimethoxine or an effect of long-acting sulfonamides as a drug class. Experimental studies will be critical to understand mechanisms of risk, and additional, well-conducted epidemiological studies in other pregnancy cohorts (e.g. Finnish Maternity Cohort[26]) may substantiate the association between *in-utero* exposure to long-acting sulfonamides and CRC which we have reported here.

Exact mechanisms contributing to the higher risk of CRC in offspring exposed *in utero* to long-acting sulfonamides are not yet known, but our findings point to three possibilities. First, the pharmacokinetics of long-acting sulfonamides may play a role: long-acting sulfonamides have

Table 1 Characteristics of 18 751 liveborn offspring of the Child Health and Development Studies, 1959–67, by *in-utero* exposure to antibiotics

	No *in-utero* exposure ($n = 16\,116$)		*In-utero* exposure to tetracyclines ($n = 1016$)		*In-utero* exposure to penicillins ($n = 918$)		*In-utero* exposure to short-acting sulfonamides ($n = 785$)		*In-utero* exposure to long-acting sulfonamides ($n = 273$)	
Offspring characteristics, *n* (%)										
Sex										
Male	8228	(51.1)	519	(51.1)	463	(50.4)	411	(52.4)	155	(56.8)
Female	7888	(49.0)	497	(48.9)	455	(49.6)	374	(47.6)	118	(43.2)
Year of birth										
1959–61	4878	(30.3)	245	(24.1)	252	(27.5)	206	(26.2)	110	(40.3)
1962–64	7730	(48.0)	609	(59.9)	392	(42.7)	371	(47.3)	134	(49.1)
1965–67	3508	(21.8)	162	(15.9)	274	(29.8)	208	(26.5)	29	(10.6)
Race and ethnicity										
Non-Hispanic White	10544	(66.4)	737	(73.6)	558	(61.5)	477	(61.5)	184	(67.9)
Non-Hispanic Black	3645	(23.0)	195	(19.5)	291	(32.1)	240	(30.9)	62	(22.9)
Hispanic	538	(3.4)	25	(2.5)	20	(2.2)	33	(4.3)	8	(3.0)
Asian	674	(4.3)	20	(2.0)	14	(1.5)	8	(1.0)	6	(2.2)
Other	471	(3.0)	24	(2.4)	25	(2.8)	18	(2.3)	11	(4.1)
Missing	244		15		10		9		2	
Gestational age										
<37 weeks	1239	(7.8)	76	(7.5)	76	(8.3)	76	(9.7)	29	(10.6)
≥37 weeks	14579	(92.2)	940	(92.5)	842	(91.7)	709	(90.3)	244	(89.4)
Missing	298									
Birthweight (grams)										
<2500	913	(5.7)	61	(6.0)	57	(6.2)	55	(7.0)	23	(8.4)
2500–3999	13841	(85.9)	848	(83.5)	776	(84.5)	664	(84.6)	223	(81.7)
≥4000	1362	(8.5)	107	(10.5)	85	(9.3)	66	(8.4)	27	(9.9)
Maternal characteristics										
Maternal age at pregnancy (years)										
<24	1433	(9.0)	70	(7.0)	94	(10.4)	91	(11.7)	27	(10.0)
20–24	4849	(30.4)	287	(28.5)	282	(31.1)	249	(32.1)	85	(31.5)
25–29	4640	(29.1)	290	(28.8)	245	(27.0)	227	(29.2)	76	(28.2)
30–34	2822	(17.7)	222	(22.1)	160	(17.6)	137	(17.6)	45	(16.7)
35–39	1661	(10.4)	116	(11.5)	100	(11.0)	58	(7.5)	27	(10.0)
≥40	565	(3.5)	21	(2.1)	27	(3.0)	15	(1.9)	10	(3.7)
Missing	146		10		10		8		3	
Parity at pregnancy										
Primiparous	5056	(31.6)	227	(22.4)	245	(26.9)	240	(30.8)	75	(27.5)
Multiparous	10939	(68.4)	786	(77.6)	667	(73.1)	539	(69.2)	198	(72.5)
Missing			3		6		6		0	
Body mass index (kg/m^2)										
Underweight or healthy (<25)	10508	(75.4)	654	(75.7)	573	(70.7)	532	(74.8)	183	(78.5)
Overweight or obese (≥25)	3428	(24.6)	210	(24.3)	237	(29.3)	179	(25.2)	50	(21.5)
Missing	913		152		108		74		40	
Maternal education										
Less than high school	2459	(17.9)	150	(17.4)	152	(19.7)	149	(22.1)	57	(24.7)
High school or trade school	5299	(38.5)	360	(41.8)	309	(40.1)	276	(41.0)	76	(32.9)
Some college or college degree	5995	(43.6)	351	(40.8)	309	(40.1)	249	(36.9)	98	(42.4)
Missing	2363		155		148		111		42	
Annual family income[4]										
<Median	4182	(36.7)	220	(31.6)	218	(35.7)	208	(38.0)	76	(39.8)
≥Median	7221	(63.3)	477	(68.4)	393	(64.3)	339	(62.0)	115	(60.2)
Missing	4713		319		307		238		82	

(Continued)

Table 1 Continued

	No *in-utero* exposure ($n = 16\,116$)		*In-utero* exposure to tetracyclines ($n = 1016$)		*In-utero* exposure to penicillins ($n = 918$)		*In-utero* exposure to short-acting sulfonamides ($n = 785$)		*In-utero* exposure to long-acting sulfonamides ($n = 273$)	
Smoking										
Never	6203	(48.5)	306	(39.4)	297	(40.9)	283	(45.6)	96	(44.4)
Former	2190	(17.1)	133	(17.4)	110	(15.7)	107	(17.3)	35	(16.2)
Current	4404	(34.4)	337	(43.4)	304	(43.4)	230	(37.1)	85	(39.4)
Missing	3319		240		217		165		57	

In-utero exposure to tetracyclines, penicillins, short-acting sulfonamides and long-acting sulfonamides not mutually exclusive, and 327 offspring were exposed to more than one class of antibiotics (see Supplementary Table S2, available as Supplementary data at *IJE* online). Offspring exposed to intermediate-acting sulfonamides or sulfonamides not otherwise specified are not included in the 785 or 273 offspring exposed to short- and long-acting sulfonamides, respectively.

a serum half-life of up to 40 h, are highly protein bound and are slowly excreted.[21,27] This is consistent with evidence that long-acting sulfonamides cross the placental barrier and quickly establish equilibrium; fetal blood levels are sufficient to cause both antimicrobial and toxic effects.[28] Long-acting sulfonamides actively compete with bilirubin and can displace unconjugated bilirubin in the fetus. A small literature suggests unbound bilirubin is preferentially distributed to the gastrointestinal tract in newborns (vs to the liver in adults),[29] and this process *in utero* may programme the sensitivity of developing fetal tissue in the colorectum.

Second, long-acting sulfonamides bind and inhibit dihydropteroate synthase, an enzyme critical for bacterial synthesis of folate,[21,30] suggesting that the folate metabolic pathway frequently implicated in adverse neonatal outcomes[31] contributes to colorectal carcinogenesis later in life. Long-acting sulfonamides may additionally act as folic acid antagonists via independent effects on dihydrofolate reductase.[30] Folic acid antagonists in early pregnancy may increase risk of congenital anomalies,[31,32] and a recent meta-analysis[33] suggests that sulfonamide antibiotics are associated with adverse pregnancy outcomes. Experimental studies have similarly shown long-acting sulfonamides in early pregnancy can produce a range of abnormalities in offspring.[28,34]

A large body of observational studies demonstrates that inadequate folate consumption is a risk factor for CRC,[35] although effects of maternal consumption of folate during pregnancy on risk of CRC in offspring have not been studied in humans. Some studies suggest that folic acid supplementation protects against childhood cancers,[36] and rodent models show maternal but not post-weaning folic acid supplementation reduces risk of CRC by two-thirds in offspring.[37,38] Elsewhere, maternal serum levels of folate contribute to differential expression of *APC2* in newborn cord blood.[39] *APC2* is functionally redundant of *APC*,[40] and *APC* mutations are found in the very earliest stages of the adenoma–carcinoma sequence. Although these studies are not specific to long-acting sulfonamides, they support the concept that the fetus may be particularly susceptible to folate-related methylation during development.[41]

Third, the regulatory history of long-acting sulfonamides provides some insight into hypersensitivity-related mechanisms by which *in-utero* exposure may increase risk of CRC. Long-acting sulfonamides were first introduced in the USA in late 1957[42] for upper respiratory and urinary tract infections.[43] Several case reports subsequently linked sulfadimethoxine and sulfamethoxypyridazine with Stevens–Johnson syndrome, a rare but often fatal inflammatory skin condition. In December 1965, the U.S. Food and Drug Administration required that all long-acting sulfonamides carry a warning label, and they withdrew their approval of sulfadimethoxine in March 1966.[42,44] Other hypersensitivity reactions were later reported,[45] such as toxic epidermal necrolysis,[46] hypersensitivity vasculitis and granulomatous hepatitis.[47] There is very little information on the regulatory history of long-acting sulfonamides in countries other than the USA, and although still currently used in Russia,[48] it is not clear the extent to which these antibiotics may contribute to the growing burden of CRC worldwide.[49]

Incidence rates of CRC in offspring exposed *in utero* to tetracyclines or penicillins are suggestive of no association. It is possible that we were underpowered to detect modest associations with these antibiotic classes, as evidenced by the wide confidence intervals for the adjusted effect estimates. It is also possible that risk of CRC may be related to use of these antibiotics later in life rather than *in-utero* exposure. Several European studies demonstrate an

Table 2 Characteristics of 80 adult offspring diagnosed with colorectal cancer

	n	(%)
Sex		
Male	39	48.8
Female	41	51.3
Year of birth		
1959–61	32	40.0
1962–64	38	47.5
1965–67	10	12.5
Race and ethnicity		
Non-Hispanic White	40	52.0
Non-Hispanic Black	26	33.8
Hispanic	5	6.5
Asian	3	3.9
Other	3	3.9
Missing	3	
Year of diagnosis		
1980–89		
1990–99	2	2.5
2000–09	6	7.5
2010+	26	32.5
Age at diagnosis (years)	46	57.5
Median (IQR)	50 (46–53)	
18–29	2	2.5
30–39	3	3.8
40–49	17	21.3
50–59	58	72.5
Stage at diagnosis		
Local	21	30.0
Regional	31	44.3
Distant	18	25.7
Missing	10	
Tumour location		
Proximal colon	21	26.9
Distal colon	33	42.3
Rectum	24	30.8
Missing	2	
Family history of CRC[a]		
No	68	85.0
Yes	12	15.0

CRC, colorectal cancer; IQR, interquartile range.
[a]Family history of CRC defined as diagnosis in mother or father.

association between oral antibiotic use in adulthood and CRC, and particularly use of penicillins.[50–53] Importantly, these studies used national registries to establish links between antibiotic use and incident cancers. In the USA, a report from the Nurses' Health Study suggests that long-term antibiotic use, starting in early adulthood (age 20–39 years), is associated with colorectal adenoma.[54]

A strength of our study is the robust follow-up of the cohort, with detailed information on both mothers and offspring. The prospective design of the CHDS offers a unique opportunity to study exposures *in utero* and cancers diagnosed in the 60 years after offspring were born. Antibiotic prescriptions were prospectively collected from mothers' medical records, and we ascertained cancers in offspring by linkage with a high-quality, population-based cancer registry, minimizing the possibility of bias due to measurement error. Follow-up of offspring was similar by *in-utero* exposure to antibiotics, and it is unlikely that differential ascertainment of cancer in offspring explains our findings.

There are some limitations of our study. We could not examine effects of timing of antibiotic exposure because exposed offspring were similarly first exposed in the first, second and third trimester. It is not known whether mothers took antibiotics as prescribed, a common limitation in studies of medication use.[55] However, we do not expect this to be differential by antibiotic class. In observational studies of medication use, it is also possible that associations are related to the underlying medical conditions indicating the medication. Specifically, urinary tract infections accounted for about half of long-acting sulfonamides in our study, and infections during pregnancy may disrupt fetal development via effects of various metabolic byproducts, colonization or other bacterial activity.[56] We addressed this possibility by examining associations with urinary tract infection and other conditions indicating antibiotics, providing some confidence that observed associations are not explained by indications for use. Associations between *in-utero* exposure to antibiotics and CRC in offspring may be confounded by factors not measured in the CHDS. We addressed unmeasured confounding by calculating E-values. These results suggest that the observed aHR of 4.40 for the association with *in-utero* exposure to long-acting sulfonamides can only be explained away by an unmeasured confounder associated with both the exposure and outcome by a risk ratio of 9-fold each, scenarios that are highly unlikely. Finally, we did not examine some antibiotic classes used in current clinical practice (e.g. cephalosporins) because they were not available or rarely prescribed in the late 1950s and 60s.

In summary, we observed higher incidence rates of CRC in adult offspring exposed *in utero* to long-acting sulfonamides, but rates of CRC in offspring exposed to short-acting sulfonamides or other antibiotic classes, including tetracyclines and penicillins, were suggestive of no association. Although no longer routinely prescribed in humans, long-acting sulfonamides are used extensively in veterinary medicine and prophylactically in medicated feed for livestock.[57,58] As a consequence, long-acting sulfonamide residues are frequently detected in milk and soft-cheese products,[59] eggs[60] and beef, pork and chicken meat,[61,62] as well as in water and soil.[63,64] Their continued presence as

Table 3 Timing, frequency and indication of *in-utero* exposure to antibiotics

	Tetracyclines ($n = 1106$)		Penicillins ($n = 918$)		Short-acting sulfonamides ($n = 785$)		Long-acting sulfonamides ($n = 273$)	
	n	%	*n*	%	*n*	%	*n*	%
Timing of first exposure								
Gestational week—median (IQR)	18.4 (8.4–28.5)		18.3 (8.1–29.1)		18.9 (8.4–27.9)		16.9 (6.7–26.3)	
First trimester	372	(36.6)	342	(37.3)	285	(36.3)	109	(39.9)
Second trimester	325	(32.0)	279	(30.4)	265	(33.8)	92	(33.7)
Third trimester	319	(31.4)	297	(32.4)	235	(29.9)	72	(26.4)
Number of prescriptions								
1	846	(83.3)	596	(64.9)	663	(84.5)	248	(90.8)
≥2	170	(16.7)	322	(35.1)	122	(15.5)	25	(9.2)
Indication								
Upper respiratory infection	675	(66.4)	460	(50.1)	43	(5.5)	74	(27.1)
Urinary tract infection	70	(6.9)	35	(3.8)	666	(84.8)	143	(52.4)
Sexually transmitted infection	3	(0.3)	99	(10.8)	0	–	0	–
Skin infection	94	(9.3)	68	(7.4)	12	(1.8)	35	(12.8)
Other infection	174	(17.1)	256	(27.9)	64	(8.2)	21	(7.7)

IQR, interquartile range.

Table 4 Incidence rates (per 100 000 persons) and crude and adjusted hazard ratios for colorectal cancer in adult offspring with and without *in-utero* exposure to antibiotics

	Person-years	*n*	Incidence rate per 100 000	95% CI	Crude HR	95% CI	Adjusted HR[a]	95% CI
Tetracyclines								
Not exposed	633 985.0	67	10.6	8.2, 13.4	1.00	Ref.[b]	1.00	Ref.[b]
Any *in-utero* exposure	41 025.0	3	7.3	1.5, 21.4	0.69	0.22, 2.19	1.03	0.32, 3.31
Penicillins								
Not exposed	633 985.0	67	10.6	8.2, 13.4	1.00	Ref.[b]	1.00	Ref.[b]
Any *in-utero* exposure	37 067.0	4	10.8	2.9, 27.6	1.09	0.40, 3.00	1.12	0.35, 3.58
Short-acting sulfonamides								
Not exposed	633 985.0	67	10.6	8.2, 13.4	1.00	Ref.[b]	1.00	Ref.[b]
Any *in-utero* exposure	30 641.5	2	6.5	0.8, 23.6	0.69	0.17, 2.80	0.83	0.20, 3.42
Long-acting sulfonamides								
Not exposed	633 985.0	67	10.6	8.2, 13.4	1.00	Ref.[b]	1.00	Ref.[b]
Any *in-utero* exposure	11 021.5	6	54.4	20.0, 118.5	4.63	2.01, 10.67	4.40	1.63, 11.88

Observations with missing values ($n = 3965$ for tetracyclines; $n = 3938$ for penicillins; $n = 3886$ for short-acting sulfonamides; and $n = 3777$ for long-acting sulfonamides) not included in adjusted models; see Supplementary Table S3 (available as Supplementary data at *IJE* online) for results of multiple imputation by fully conditional specification.

HR, hazard ratio; CI, confidence interval; Ref., reference category.

[a]Adjusted for year of birth, maternal race (Black v. else), maternal smoking (current vs else), and maternal body mass index (overweight or obese v. else).

[b]Offspring not exposed *in utero* to any antibiotics ($n = 16 116$) are reference category for all models.

environmental pollutants raises the possibility that long-term exposure, albeit at lower levels, may also increase risk. Additional, well-conducted studies are needed to test for associations with long-term exposure from applications to livestock used for human consumption.

Ethics approval

Individual participants have not provided consent for this study. The requirement for informed consent was waived as the risk to participants was considered minimal. The Institutional Review Board at the Public Health Institute (#I92-004) and the University of

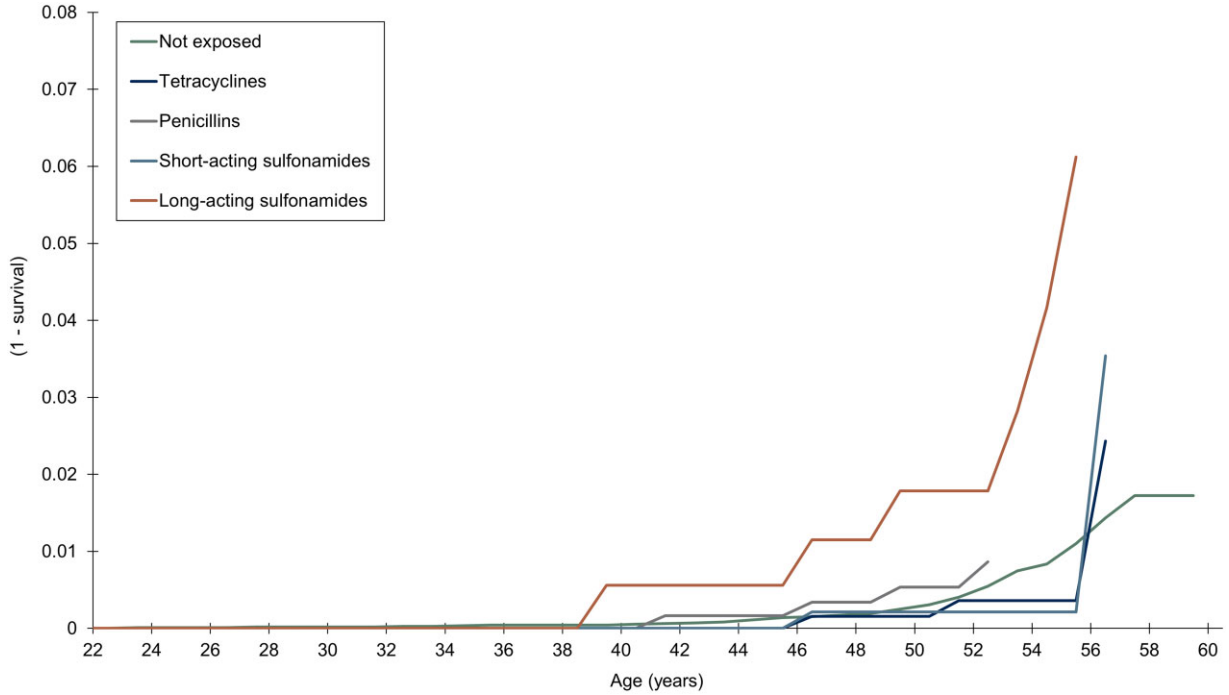

Figure 2 Cumulative incidence of colorectal cancer in adult offspring by *in-utero* exposure to antibiotics. X-axis begins at age 22 years to reflect youngest age at colorectal cancer diagnosis

Texas Health Science Center at Houston (#21–0271) approved this study.

Data availability

De-identified (anonymized) data are available upon request from Barbara A Cohn, PhD, Director of the Child Health and Development Studies. Requests will be reviewed by Dr Cohn, research staff and the Institutional Review Board at the Public Health Institute. Approval of requests for de-identified (anonymized) data requires execution of a data use agreement.

Supplementary data

Supplementary data are available at *IJE* online.

Author contributions

Study concept and design: C.C.M., B.A.C. Acquisition of data: B.A.C., P.M.C., N.Y.K. Analysis and interpretation of data: all authors. Statistical analysis: C.C.M., P.M.C. Drafting of manuscript: C.C.M. Critical revision: all authors. The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Funding

Research reported in this publication was supported by the National Cancer Institute at the National Institutes of Health under award number R01CA242558 (C.C.M.) and by the National Institute of Child Health and Development at the National Institutes of Health under contract number HHSN275201100020C (B.A.C.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Mention herein of trade names, commercial products or organizations does not imply endorsement by the US government.

Collection of cancer data used in this study was supported by the California Department of Public Health as part of the statewide cancer reporting programme mandated by California Health and Safety Code Section 103885; the National Cancer Institute's Surveillance, Epidemiology, and End Results Program under Contract HHSN261201000140C (awarded to the Cancer Prevention Institute of California), Contract HHSN261201000035C (awarded to the University of Southern California) and Contract HHSN261201000034C (awarded to the Public Health Institute); and the Centers for Disease Control and Prevention's National Program of Cancer Registries, under Agreement U58DP003862-01 (awarded to the California Department of Public Health).

Acknowledgements

A version of this manuscript was presented at Digestive Disease Week 2021, Virtual, 21–23 May 2021.

Conflict of interest

C.C.M. reports consulting for Freenome; A.G.S. reports consulting for Exact Sciences and Bayer; all other authors have no financial disclosures or competing interests.

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