ORIGINAL ARTICLE

Long-term use of antibiotics and risk of colorectal adenoma

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ABSTRACT

Objective Recent evidence suggests that antibiotic use, which alters the gut microbiome, is associated with an increased risk of colorectal cancer. However, the association between antibiotic use and risk of colorectal adenoma, the precursor for the majority of colorectal cancers, has not been investigated.

Design We prospectively evaluated the association between antibiotic use at age 20–39 and 40–59 (assessed in 2004) and recent antibiotic use (assessed in 2008) with risk of subsequent colorectal adenoma among 16 642 women aged ≥60 enrolled in the Nurses' Health Study who underwent at least one colonoscopy through 2010. We used multivariate logistic regression to calculate ORs and 95% CIs.

Results We documented 1195 cases of adenoma. Increasing duration of antibiotic use at age 20–39 (p_{trend}=0.002) and 40–59 (p_{trend}=0.001) was significantly associated with an increased risk of colorectal adenoma. Compared with non-users, women who used antibiotics for ≥2 months between age 20 and 39 had a multivariable OR of 1.36 (95% CI 1.03 to 1.79). Women who used ≥2 months of antibiotics between age 40 and 59 had a multivariable OR of 1.69 (95% CI 1.24 to 2.31). The associations were similar for low-risk versus high-risk adenomas (size ≥1 cm, or with tubulovillous/villous histology, or ≥3 detected lesions), but appeared modestly stronger for proximal compared with distal adenomas. In contrast, recent antibiotic use within the past four years was not associated with risk of adenoma (p_{trend}=0.44).

Conclusions Long-term antibiotic use in early-to-middle adulthood was associated with increased risk of colorectal adenoma.

Significance of this study

What is already known on this subject?

- Increasing data have supported a role for the gut microbiota in colorectal carcinogenesis.
- Limited studies from cancer registries and healthcare claims with short-term follow-up suggest an association between antibiotic exposure and colorectal cancer.
- The association between antibiotic use and risk of colorectal adenoma, the precursor for the majority of colorectal cancers, has not been investigated.

What are the new findings?

- Exposure to antibiotics earlier in life (age 20–39 and 40–59) was significantly associated with an increased risk for colorectal adenoma after age 60.
- More recent antibiotic use (within 4 years) was not associated with risk of colorectal adenoma.
- These data provide additional support for the association of antibiotics with colorectal cancer and the potential mediating role of the gut microbiome in carcinogenesis.

How might it impact on clinical practice in the foreseeable future?

- The findings, if confirmed by other studies, suggest the potential need to limit the use of antibiotics and sources of inflammation that may drive tumour formation.

INTRODUCTION

In recent years, antibiotic use has increased dramatically in the USA.[1] Accumulating evidence suggests that exposure to antibiotics may be associated with risk for chronic illnesses such as IBD,[2 3] coeliac disease[4] and obesity.[5 6] It is hypothesised that the link between antibiotics and disease pathogenesis may be mediated by their effect on the taxonomic, genomic and functional capacity of the gut microbiota.[7–9] Similarly, increasing data have supported a role for the gut microbiota in colorectal carcinogenesis.[10–12] Thus, antibiotics and their effects on the gut microbiome may lead to the promotion of biological pathways that initiate or promote colorectal neoplasia.[13–15]

Limited studies from cancer registries and healthcare claims in Europe with short-term follow-up suggest an association between antibiotic exposure and colorectal cancer.[16–18] Although these data are intriguing, limitations of these studies influence their interpretation. First, the associations of antibiotics with colorectal cancer, particularly with shorter term follow-up, may be due to residual confounding or reverse causality. For example, antibiotics may be more likely to be prescribed for symptoms associated with colorectal cancer or conditions that predispose to cancer prior to a formal

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diagnosis. Second, the association of recent antibiotic exposure with a higher likelihood of colorectal cancer diagnosis may also be indicative of closer or more frequent medical surveillance among these antibiotic users. Third, prior studies were based on cohorts that had limited information on potential lifestyle factors influencing the risk of colorectal cancer or the use of antibiotics. Fourth, given that colorectal cancer is believed to typically develop over at least a decade, the short-term follow-up data compiled by these studies are unlikely to capture the role of antibiotics in the initiation of colorectal neoplasia. Finally, these studies are derived from prescription records, which may not reflect actual use of these agents.

To address these limitations, we examined the association of both past and recent antibiotic use with risk of colorectal adenoma, the precursor of the majority of colorectal cancers, among women enrolled in the Nurses' Health Study (NHS), which has collected detailed information on antibiotic use and lifestyle risk factors for colorectal cancer and endoscopic screening practices since 2004 and prospectively documented cases of adenoma through 2010. Because colorectal adenomas are largely asymptomatic and detected only during a colonoscopy, an association between antibiotic use and adenoma among a cohort of individuals uniformly undergoing colonoscopy would be less likely to be confounded by symptoms associated with colorectal cancer or differential exposure to medical care.

METHODS

Study population

The NHS is an ongoing prospective cohort study of 121 700 US female nurses aged 30–55 at enrolment in 1976. Participants have been mailed questionnaires every two years since baseline to collect data on demographics, lifestyle factors, medical history and disease outcomes, and every four years to collect dietary data. In this analysis, we excluded participants with a diagnosis of cancer (except non-melanoma skin cancer), UC or colorectal polyp before 2004. To reduce the potential for detection bias, we restricted the analysis to 16 642 women aged ≥60 in 2004 who reported their history of antibiotic use through age 59 via the 2004 questionnaire and subsequently reported having undergone at least one colonoscopy between 2004 and 2010 on biennial follow-up questionnaires.

Ascertainment of colorectal adenoma cases and controls

On each biennial questionnaire, we asked whether participants had undergone a colonoscopy; what the indications for these procedures were; whether colon or rectal polyps had been diagnosed in the past two years; and if they had, the date of diagnosis. When a diagnosis was reported, we obtained informed consent to acquire medical records and pathology reports. Investigators blinded to any exposure information reviewed all records and extracted data on histological type, anatomic location, size and number of the polyps. If more than one adenoma was diagnosed, the subject was classified according to the largest and most advanced histological adenoma. Adenomas in the caecum, ascending colon, hepatic flexure, transverse colon or splenic flexure were classified as proximal; adenomas in the descending or sigmoid colon were classified as distal and adenomas in the rectum or rectosigmoid junction were classified as rectal. We also grouped adenoma cases according to their features and subsequent likelihood of developing future advanced neoplasia: high risk, defined as at least one adenoma ≥1 cm in diameter, or with advanced histology (tubulovillous/villous histological features or high grade or severe dysplasia), or ≥3 adenomas regardless of histology or size versus low risk, which included all other adenomas,[19] size (large: ≥1 cm vs small: <1 cm), histology (tubulovillous/villous vs tubular) and multiplicity (≥3 vs <3). Cases and controls were separately defined in each 2-year period: all newly diagnosed adenomas were considered as cases and all the participants who reported colonoscopy but without a diagnosis of adenoma were defined as controls.

Assessment of antibiotic use

In 2004, participants reported their total time using antibiotics (excluding skin creams, mouthwash or isoniazid) for the time periods between age 20–39 and 40–59. The responses were recorded in eight categories (ranging from none to 5+ years). In 2008, participants recalled their total amount of time of antibiotic use (excluding skin creams, mouthwash or isoniazid) during the past four years in seven categories (ranging from none to 3+ years). They also reported the most common reason that an antibiotic was used, including respiratory infection, urinary tract infection, acne/rosacea, chronic bronchitis, dental and other reason.

Statistical analysis

We evaluated the association between antibiotic use at age 20–39 and 40–59 (assessed in 2004) with risk of colorectal adenoma among women who reported a colonoscopy between 2004 and 2010 as the main analyses. The minimum detectable OR was 1.45 when comparing individuals who used antibiotics for 2 months+ at age 20–39 to non-users, and 1.52 for individuals who used antibiotics for 2 months+ compared with non-users at age 40–59. We also examined exposure to antibiotics over the preceding four years (assessed in 2008) and risk of colorectal adenoma among women who had a colonoscopy between 2008 and 2010. In secondary analyses, we investigated the association between antibiotic exposure during each time period and risk of high-risk versus low-risk adenoma, as well as according to anatomic location, size, histological type and number of adenomas. As an exploratory analysis, we examined the association between the most common reason for antibiotic use (assessed in 2008) and risk of colorectal adenoma among women who had a colonoscopy between 2008 and 2010.

To account for the possibility that a single individual may have undergone multiple endoscopies between 2004 and 2010 and to handle time-varying exposure and covariates efficiently, we used an Andersen-Gill data structure with a new record for each 2-year follow-up period during which a participant underwent a colonoscopy. Exposure and covariates were set to their values at the time that the questionnaire was returned. Once a participant was diagnosed with adenoma, she was censored in all later follow-up cycles. Age and multivariable-adjusted logistic regressions for clustered data (PROC GENMOD) were used to account for repeated observations (ie, multiple endoscopies) and to calculate ORs approximating relative risks. Tests for trend were conducted using the median of the duration of antibiotic therapy as a continuous variable.

In age-adjusted models, we controlled for age in 5-year intervals, time period of colonoscopy (*in 2-year intervals*); number of endoscopies (*continuous*); time in years since the most recent endoscopy (*continuous*) and reason for the current colonoscopy (*screening*/*symptoms*/*missing*). In the multivariable models, we additionally adjusted for the following potential confounders (cumulatively updated when applicable): history of colorectal cancer in a first-degree relative (*yes*/*no*); personal history of diabetes (*yes*/*no*); use of menopausal hormone therapy (MHT) (*never*/*past*/*current*); body mass index (*kg*/*m*^{2} *in quintiles*); height (*continuous*); regular aspirin use (*yes*/*no*); current use of

multivitamin (*yes/no*); physical activity (metabolic equivalent task (MET)-hours/week in quintiles); smoking (*pack-years in categories: never-smoker, 1–4.9, 5–19.9, 20–39.9, 40+*); alcohol intake (*g/day in categories: <5, 5–9.9, 10–14.9, 15–29.9, 30+*); total calories (*kcal/day in quintiles*); folate intake (*µg/day in quintiles*); calcium intake (*mg/day in quintiles*); and red and processed meat intake (*servings/day in quintiles*). To control for potential confounding by multiple dietary factors, we adjusted

Table 1 Characteristics of participants according to antibiotic use at age 20–39, Nurses' Health Study 2004

	None	<15 days	15 days to 2 months	2 months+
Age, years*	71.9 (6.2)	68.9 (5.8)	68.0 (5.5)	67.2 (5.1)
Family history of cancer, %	21	19	18	20
History of diabetes, %	8.1	8.7	9.5	8.8
Height, cm	164 (6)	164 (6)	164 (6)	164 (6)
Body mass index, kg/m²	25.4 (4.2)	25.6 (4.4)	25.7 (4.4)	25.4 (4.4)
Current hormone therapy use, %	16	19	21	25
Current endoscopy due to symptoms, %	15	19	21	25
Number of previous endoscopies	2.8 (1.7)	3.0 (1.8)	3.0 (1.8)	3.1 (1.8)
Regular use of aspirin, %	39	42	45	47
Current use of multivitamin, %	74	77	79	79
Physical activity, MET-hours/week	19.9 (16.3)	18.9 (16.4)	18.0 (15.8)	19.4 (16.8)
Pack-years among ever-smokers	18.8 (18.7)	21.1 (19.3)	19.0 (17.5)	18.5 (17.7)
Alcohol intake, g/day	2.4 (1.0)	2.3 (0.9)	2.3 (0.9)	2.3 (0.9)
Total calorie intake, kcal/day	1674 (380)	1708 (400)	1774 (404)	1809 (410)
Folate intake, µg/day	492 (160)	499 (170)	500 (166)	530 (175)
Calcium intake, mg/day	1103 (337)	1095 (345)	1110 (327)	1159 (358)
Red meat intake, servings/week	5.4 (2.5)	5.6 (2.7)	5.9 (2.7)	5.8 (2.7)
Alternate Healthy Eating Index 2010†	48.8 (8.6)	48.6 (8.6)	47.8 (8.9)	48.8 (9.3)

*All values other than age have been directly standardised to age distribution (in 5-year age group) of all the participants. Mean (SD) was presented for continuous variables.
†Without alcohol intake.
MET, metabolic equivalent task.

Table 2 Antibiotic use at age 20–39 and risk of colorectal adenoma, Nurses' Health Study 2004–2010

	Antibiotic use at age 20–39				
	None	1–14 days	15 days to 2 months	2 months+	p_{trend}
Total adenoma					
No. of cases (n=1195)	141	653	296	105	
Age-adjusted* OR (95% CI)	1 (referent)	1.13 (0.93–1.37)	1.40 (1.13–1.74)	1.36 (1.04–1.79)	0.001
Multivariable† OR (95% CI)	1 (referent)	1.12 (0.92–1.36)	1.41 (1.13–1.75)	1.36 (1.03–1.79)	0.002
High risk‡					
No. of cases (n=436)	51	251	100	34	
Age-adjusted* OR (95% CI)	1 (referent)	1.25 (0.92–1.71)	1.40 (0.99–2.00)	1.35 (0.86–2.11)	0.22
Multivariable† OR (95% CI)	1 (referent)	1.23 (0.90–1.68)	1.43 (1.00–2.05)	1.37 (0.86–2.16)	0.14
Low risk					
No. of cases (n=630)	73	331	167	59	
Age-adjusted* OR (95% CI)	1 (referent)	1.08 (0.83–1.40)	1.47 (1.10–1.96)	1.40 (0.97–2.00)	0.002
Multivariable† OR (95% CI)	1 (referent)	1.08 (0.82–1.41)	1.47 (1.09–1.97)	1.42 (0.98–2.05)	0.002
Proximal					
No. of cases (n=709)	82	391	176	60	
Age-adjusted* OR (95% CI)	1 (referent)	1.18 (0.92–1.51)	1.46 (1.11–1.92)	1.36 (0.96–1.93)	0.02
Multivariable† OR (95% CI)	1 (referent)	1.17 (0.91–1.51)	1.46 (1.10–1.93)	1.43 (1.00–2.04)	0.01
Distal					
No. of cases (n=509)	67	271	128	43	
Age-adjusted* OR (95% CI)	1 (referent)	0.99 (0.75–1.30)	1.29 (0.94–1.76)	1.20 (0.81–1.79)	0.04
Multivariable† OR (95% CI)	1 (referent)	0.98 (0.74–1.30)	1.31 (0.96–1.81)	1.18 (0.78–1.78)	0.04
Rectal					
No. of cases (n=163)	13	96	40	14	
Age-adjusted* OR (95% CI)	1 (referent)	1.88 (1.03–3.43)	2.21 (1.15–4.24)	2.16 (0.99–4.71)	0.12
Multivariable† OR (95% CI)	1 (referent)	1.84 (1.00–3.38)	2.23 (1.16–4.30)	1.95 (0.87–4.37)	0.17

*Adjusted for age, time period of colonoscopy, number of reported endoscopies, time since most recent endoscopy and reason for current colonoscopy.
†Additionally adjusted for family history of colorectal cancer, history of diabetes, menopausal status and postmenopausal hormone use, body mass index, height, regular use of aspirin, current use of multivitamin, alcohol intake, smoking, total calorie, folate, calcium intake, red and processed meat intake and Alternate Healthy Eating Index 2010.
‡High-risk adenomas include adenoma ≥1 cm, or with tubulovillous/villous histology, or ≥3 adenomas.

for Alternate Healthy Eating Index (AHEI)-2010 (*in quintiles*),[20] which features greater consumption of vegetables (excluding potatoes), fruits (excluding juices); whole grains; nuts, legumes and vegetable protein, long chain omega-3 fatty acids and polyunsaturated fatty acids; and a lower consumption of sugar-sweetened beverages, red/processed meat, sodium, *trans* fat and moderate alcohol consumption. Adherence to the AHEI-2010 has been associated with reduced risk of cardiovascular disease, diabetes and cancer in our cohorts.[21] Because alcohol was included as a separate term in our model, we used a modified AHEI-2010 without alcohol consumption. All the analyses were performed using SAS V.9.4 (SAS Institute, Cary, North Carolina, USA). All the statistical tests were two-sided and p values <0.05 were considered statistically significant.

RESULTS

We documented 1195 newly diagnosed adenomas among 16 642 women aged ≥60 who had at least one colonoscopy between 2004 and 2010 and reported information on antibiotic use. We calculated the distribution of potential risk factors for adenomas according to duration of antibiotic use during age 20–39 (table 1). Women who used antibiotics for longer duration were generally similar to women who did not have any antibiotic treatment in terms of family history of colorectal cancer, personal disease/screening history and lifestyle factors, but were more likely to regularly use MHT and aspirin and undergo colonoscopy for symptoms (eg, abdominal pain, diarrhoea, constipation) rather than routine screening.

An increasing total exposure to antibiotics at age 20–39 was significantly associated with a higher risk of colorectal adenoma. Compared with non-users, women who used antibiotics for ≥2 months during age 20–39 had a multivariable OR for adenoma of 1.36 (95% CI 1.03 to 1.79) (p_{trend}=0.002) (table 2). The associations were similar for high-risk (size ≥1 cm, or with tubulovillous/villous histology, or ≥3) compared with low-risk adenomas (see table 2 and online supplementary table S1). In contrast, a somewhat stronger association was observed for adenomas located in the proximal compared with distal colon (table 2).

Similarly, antibiotic use during age 40–59 was associated with an increased risk of colorectal adenoma. Women who used ≥2 months of antibiotics during age 40–59 had a multivariable OR for adenoma of 1.69 (95% CI 1.24 to 2.31) (see table 3 and online supplementary table S2). The associations were similar for low-risk versus high-risk adenomas (table 3). Longer duration of antibiotic treatment appeared to be more strongly associated with proximal adenomas (table 3). Compared with non-users of antibiotics between age 20–39 and 40–59, women who used antibiotics for >15 days between both age 20 and 39 and >15 days between age 40 and 59 had a multivariable OR for adenoma of 1.73 (95% CI 1.19 to 2.51) (see online supplementary table S3).

In contrast, recent antibiotic use did not appear associated with risk of colorectal adenoma. Among women who had a colonoscopy between 2008 and 2010, antibiotic use in the past four years was not associated with risk of adenoma (p_{trend}=0.44) (table 4). In addition, none of the indications for

Table 3 Antibiotic use at age 40–59 and risk of colorectal adenoma, Nurses' Health Study 2004–2010

	Antibiotic use at age 40–59				
	None	1–14 days	15 days to 2 months	2 months+	p_{trend}
Total adenoma					
No. of cases (n=1195)	66	637	357	135	
Age-adjusted* OR (95% CI)	1 (referent)	1.35 (1.04–1.75)	1.53 (1.17–2.01)	1.74 (1.28–2.37)	<0.001
Multivariable† OR (95% CI)	1 (referent)	1.32 (1.01–1.72)	1.51 (1.14–1.99)	1.69 (1.24–2.31)	0.001
High risk‡					
No. of cases (n=436)	26	241	123	46	
Age-adjusted* OR (95% CI)	1 (referent)	1.34 (0.89–2.02)	1.42 (0.92–2.19)	1.64 (1.00–2.67)	0.11
Multivariable† OR (95% CI)	1 (referent)	1.32 (0.87–2.02)	1.43 (0.92–2.22)	1.60 (0.97–2.65)	0.12
Low risk					
No. of cases (n=630)	32	332	194	72	
Age-adjusted* OR (95% CI)	1 (referent)	1.41 (0.98–2.05)	1.66 (1.13–2.43)	1.82 (1.19–2.80)	0.01
Multivariable† OR (95% CI)	1 (referent)	1.37 (0.95–1.99)	1.61 (1.10–2.37)	1.74 (1.13–2.70)	0.01
Proximal					
No. of cases (n=709)	29	382	228	70	
Age-adjusted* OR (95% CI)	1 (referent)	1.84 (1.25–2.71)	2.24 (1.51–3.34)	2.07 (1.32–3.22)	0.01
Multivariable† OR (95% CI)	1 (referent)	1.89 (1.28–2.79)	2.29 (1.53–3.42)	2.13 (1.35–3.35)	0.01
Distal					
No. of cases (n=509)	34	269	142	64	
Age-adjusted* OR (95% CI)	1 (referent)	1.11 (0.78–1.60)	1.20 (0.82–1.76)	1.67 (1.09–2.55)	0.01
Multivariable† OR (95% CI)	1 (referent)	1.04 (0.72–1.50)	1.15 (0.78–1.68)	1.49 (0.96–2.29)	0.02
Rectal					
No. of cases (n=163)	11	92	44	16	
Age-adjusted* OR (95% CI)	1 (referent)	1.20 (0.64–2.28)	1.20 (0.61–2.36)	1.34 (0.61–2.95)	0.64
Multivariable† OR (95% CI)	1 (referent)	1.16 (0.61–2.21)	1.14 (0.57–2.29)	1.37 (0.62–3.04)	0.56

*Adjusted for age, time period of colonoscopy, number of reported endoscopies, time since most recent endoscopy and reason for current colonoscopy.
†Additionally adjusted for family history of colorectal cancer, history of diabetes, menopausal status and postmenopausal hormone use, body mass index, height, regular use of aspirin, current use of multivitamin, alcohol intake, smoking, total calorie, folate, calcium intake, red and processed meat intake, and Alternate Healthy Eating Index (AHEI) 2010.
‡High-risk adenomas include adenoma ≥1 cm, or with tubulovillous/villous histology, or ≥3 adenomas.

Table 4 Antibiotic use in the past four years and risk of colorectal adenoma, Nurses' Health Study 2008–2010

	Antibiotic use in the past four years				
	None	1–14 days	15 days to 2 months	2 months+	p_{trend}
Total adenoma					
No. of cases (n=180)	45	68	51	16	
Age-adjusted* OR (95% CI)	1 (referent)	0.84 (0.57–1.23)	0.98 (0.65–1.47)	1.06 (0.59–1.91)	0.54
Multivariable† OR (95% CI)	1 (referent)	0.84 (0.56–1.26)	1.02 (0.66–1.56)	1.10 (0.60–2.02)	0.44
High risk‡					
No. of cases (n=67)	16	29	14	8	
Age-adjusted* OR (95% CI)	1 (referent)	1.00 (0.54–1.86)	0.74 (0.36–1.52)	1.43 (0.60–3.40)	0.76
Multivariable† OR (95% CI)	1 (referent)	0.96 (0.52–1.80)	0.72 (0.36–1.47)	1.59 (0.66–3.81)	0.62
Low risk					
No. of cases (n=94)	26	29	31	8	
Age-adjusted* OR (95% CI)	1 (referent)	0.62 (0.36–1.06)	1.04 (0.61–1.78)	0.95 (0.43–2.13)	0.35
Multivariable† OR (95% CI)	1 (referent)	0.63 (0.36–1.11)	1.14 (0.64–2.01)	0.99 (0.42–2.31)	0.27
Proximal					
No. of cases (n=107)	21	42	36	8	
Age-adjusted* OR (95% CI)	1 (referent)	1.12 (0.66–1.91)	1.53 (0.89–2.63)	1.17 (0.51–2.68)	0.29
Multivariable† OR (95% CI)	1 (referent)	1.11 (0.64–1.92)	1.64 (0.93–2.87)	1.25 (0.54–2.89)	0.18
Distal					
No. of cases (n=77)	25	27	20	5	
Age-adjusted* OR (95% CI)	1 (referent)	0.60 (0.35–1.04)	0.69 (0.38–1.26)	0.59 (0.22–1.56)	0.47
Multivariable† OR (95% CI)	1 (referent)	0.60 (0.34–1.06)	0.69 (0.37–1.31)	0.63 (0.22–1.77)	0.59
Rectal					
No. of cases (n=25)	5	12	5	3	
Age-adjusted* OR (95% CI)	1 (referent)	1.32 (0.46–3.82)	0.81 (0.23–2.85)	1.61 (0.38–6.74)	0.93
Multivariable† OR (95% CI)	1 (referent)	1.43 (0.50–4.10)	0.86 (0.25–2.91)	1.77 (0.43–7.39)	0.87

*Adjusted for age, time period of colonoscopy, number of reported endoscopies, time since most recent endoscopy and reason for current colonoscopy.
†Additionally adjusted for family history of colorectal cancer, history of diabetes, menopausal status and postmenopausal hormone use, body mass index, height, regular use of aspirin, current use of multivitamin, alcohol intake, smoking, total calorie, folate, calcium intake, red and processed meat intake and Alternate Healthy Eating Index 2010.
‡High-risk adenomas include adenoma ≥1 cm, or with tubulovillous/villous histology, or ≥3 adenomas.

antibiotic use appeared to be significantly associated with risk of adenoma (see online supplementary table S4).

DISCUSSION

In this prospective analysis nested in a large cohort of women with well-characterised risk factors for colorectal neoplasia, exposure to antibiotics earlier in life (age 20–39 and 40–59) was significantly associated with an increased risk for colorectal adenoma after age 60. In contrast, more recent antibiotic use (within 4 years) was not associated with risk. To the best of our knowledge, this study is the first to link duration of antibiotic use, in a dose-dependent fashion, to colorectal adenoma, the primary precursor of colorectal cancer.

Our results are supported by prior studies of antibiotics and risk of colorectal cancer. A cohort study in Finland found that compared with people with ≤1 prescription for antibiotics, people who had ≥6 prescriptions had a 15% increased risk of developing colon cancer during up to 9 years of follow-up.[16] With a median follow-up of 6.2 years, a nested case-control study in The Health Improvement Network of the UK suggested that the first antibiotic exposure to penicillins, cephalosporins, trimethoprim/sulfamethoxazole and nitroimidazoles was associated with an increased risk of colorectal cancer within 1–5 years. Although no association was noted for exposure to most antibiotics >5 years before diagnosis, initial use of penicillin >10 years prior to diagnosis was associated with an increased risk of colorectal cancer, suggesting a possible role in the earliest stage of initiation of colorectal neoplasia.[17] A nested case-control study from the Netherlands also observed an increased risk of colorectal cancer associated with antibiotic use within 1–6 years before diagnosis.[18] Finally, in a nested case-control study among diabetic patients in Taiwan, prescriptions for antibiotics with anaerobic coverage, but not antiaerobic coverage, were linked to an elevated risk of colorectal cancer.[22] Our study significantly extends the findings of these prior studies by demonstrating an association of antibiotics with colorectal adenoma and its location, providing additional support that the association of antibiotics with colorectal cancer may be causal.

The proposed link between exposure to antibiotics and development of colorectal neoplasia is biologically plausible. Antibiotics shift the gut microbiota to temporally quasi-stable or alternative stable states.[23 24] Although it is unknown what factors influence either the recovery of gut microbiota to its native state or the development of alternative states after antibiotic exposure,[25] this dysbiosis is generally marked by a loss of diversity, alternations in the abundance of specific taxa, shifts in metabolic capacity and reduced resistance to colonisation by invading pathogens.[14 26–28] Studies have observed depletion of the phyla Bacteroidetes, Firmicutes (Clostridia) and Proteobacteria (Enterobacteriaceae), but enrichment in Fusobacteria in patients with colorectal cancer.[29 30] The interactions of these dysbiotic microbiota with mucosal immune and epithelial cells may be critical in the initiation and/or promotion of colorectal carcinogenesis.[10 12 14 31] The higher bacterial concentration and fermentation in the proximal colon[32] may also explain the observed stronger link between antibiotics and proximal adenomas. Finally, it is worth noting that pathogens that necessitate the use of antibiotics may induce

inflammation, a known risk for colorectal cancer.[33] Thus, it is possible that the observed link between antibiotics and adenoma may be mediated by inflammation.

Strengths of our study include detailed assessment of antibiotic use in early and middle adulthood, as well as recent antibiotic exposure and prospective follow-up to examine the influence of long-term and short-term impact of antibiotics on colorectal carcinogenesis with minimal recall bias. We were also able to control for a range of important potential confounders, (eg, dietary factors and physical activity), which were not included in previous analyses. In addition, although we have limited power to evaluate the association with adenomas defined by their anatomic location, it is worth noting that earlier life antibiotic exposure was more strongly associated with proximal adenomas, the subtype of adenoma that is less likely to be detected by screening colonoscopy.[34] Finally, our data, collected from health professionals, are more likely to reflect actual use of these agents in contrast to prior studies that relied on prescription information.

Our study also had several limitations. First, we did not access information on spectrum and route of administration of antibiotics. As a result, we are not able to investigate the differential effects of distinct antimicrobial classes. Nonetheless, if the effect of antibiotics on cancer is specific to only specific subtypes of antibiotics, our findings would be expected to be diluted. Moreover, it has been hypothesised that the influence of antibiotics on carcinogenesis is not dependent on the antimicrobial spectrum of the agent since the gut microbial ecosystem is co-dependent; thus perturbations specific to certain strains would be expected to have a broader impact on overall gut microbial composition and function.[14 28] Nonetheless, the long-term impact of recently more commonly used antibiotics (eg, rifaximin) should be examined in future studies. Second, measurement errors associated with recall of exposure to antibiotics during early life periods may be present. However, they would be expected to be non-differential to adenoma diagnosis. Third, as an observational study, the potential for residual confounding cannot be ruled out. Fourth, because age of exposure is closely correlated with duration of exposure and time since last exposure, we were unable to disentangle these factors to examine the independent association of these variables on risk. The specific timing of antibiotic exposure relative to development of adenoma is unclear since the diagnosis of adenoma was dependent on undergoing colonoscopy. Thus, some adenomas may have been prevalent at the time of antibiotic exposure but not yet detected. However, we observed an association of antibiotic use several decades before colonoscopy, minimising this likelihood. Finally, the generalisability of our data to other populations, particularly men and other racial or ethnic groups, is not known. Thus, further research is needed to confirm these findings.

In conclusion, early-to-middle adulthood antibiotic use was associated with increased risk of colorectal adenoma, especially in the proximal colon. These data provide additional support for the association of antibiotics with colorectal cancer and the potential mediating role of the gut microbiome in carcinogenesis. Additional studies investigating the impact of antibiotic exposure with gut microbial composition and function, particularly in relation to the mechanisms underlying colorectal carcinogenesis, are warranted.

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