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Article

Antibiotics Use and Subsequent Risk of Colorectal Cancer: A Swedish Nationwide Population-Based Study

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Abstract

Background: Antibiotics use may increase colorectal cancer (CRC) risk by altering the gut microbiota, with suggestive evidence reported. Our study aims to investigate antibiotics use in relation to subsequent CRC risk. **Methods:** This is a nationwide, population-based study with a matched case-control design (first primary CRC cases and 5 matched, cancer-free controls). Complete-population data, extracted from Swedish national registers for the period 2005-2016, were used to calculate odds ratios and 95% confidence intervals. **Results:** We included 40 545 CRC cases and 202 720 controls. Using the full dataset, we found a positive association between more frequent antibiotics use and CRC, excluding antibiotics prescribed within 2 years of diagnosis attenuated results toward the null. In site-specific analyses, excluding the 2-year washout, the positive association was confined to the proximal colon (adjusted odds ratio for very high use vs no use = 1.17, 95% confidence interval = 1.05 to 1.31). For rectal cancer, an inverse association, which appears to be driven by women, was observed. Quinolones and sulfonamides and/or trimethoprims were positively associated with proximal colon cancer, whereas a more general inverse association, across antibiotics classes, was observed for rectal cancer. We found no association between methenamine hippurate, a urinary tract antiseptic not affecting the gut microbiota, and CRC risk. **Conclusions:** This register-based study covering the entire population of Sweden found a robust association between antibiotics use and higher risk of proximal colon cancer and an inverse association with rectal cancer in women. This study strengthens the evidence from previous investigations and adds important insight into site-specific colorectal carcinogenesis.

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Colorectal cancer (CRC) is a multifactorial disease. Extensive epidemiological research has identified several lifestyle and medical risk factors for CRC (1,2), but the etiology is still partly unknown. A continued effort to identify risk factors for CRC is imperative, because reducing even minor risk factors at the population level could have a substantial impact on the incidence of CRC (3,4).

The composition and function of the gut microbiome are believed to have a role in CRC development (5). A structural segregation of the gut microbiome between colorectal carcinoma and benign colorectal mucosa has been reported (6,7) and evidence supports a pathogenic role of certain microbes, such as *Fusobacterium nucleatum*, in colorectal carcinogenesis (8-10). Mima et al. (11) reported that the proportion of colorectal cancers enriched with *F. nucleatum* decreases gradually from caecum to rectum, suggesting a site-specific effect of the gut microbiome in carcinogenesis.

Many established CRC risk factors, including excess body fat and dietary factors, could alter the gut microbiome (12,13). However, use of antibiotics can have a more disruptive effect

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(14,15). For example, treatment with antibiotics can alter the microbial balance in the gut resulting in intestinal overgrowth of toxin-producing *Clostridium difficile* bacteria (16), causing diarrhea and inflammation. Antibiotic-induced dysbiosis may disrupt the anti-inflammatory effects of some microbiota and increase pathogenic bacteria, influencing CRC tumorigenesis (7,17). Previous investigations of antibiotics use and CRC have generally indicated a positive association (18-23). However, most studies had limited information or insufficient power for extensive analyses of aspects such as type, dose, or duration of antibiotics and tumor stage and site. Recently, a large-scale study conducted in the United Kingdom reported that antibiotics use was associated with a higher risk of colon cancer but a lower risk of rectal cancer (24). These observations warrant swift validation.

In this study, we used data from the comprehensive Swedish national population registers to investigate antibiotics use in relation to CRC risk. The large sample size made it possible to conduct well-powered subgroup analyses on antibiotics type and clinical factors such as disease stage and tumor site.

Methods

Study Design

A matched case-control study was conducted using data from Swedish population registers (study period July 1, 2005, to December 31, 2016) (see Figure 1). Sweden's unique personal identity numbers allow for multiregister linkage and matching (25). In brief, CRC cases were identified using the Swedish Colorectal Cancer Register, controls were matched using the Total Population Register, data on antibiotics use were extracted from the Swedish Prescribed Drug Register, and other variables of interest were taken from the Swedish Inpatient Register and the Longitudinal Integration Database for Health Insurance and Labor Market Studies (LISA by Swedish acronym). Full descriptions of the Swedish national registers included in the study can be found in the Supplementary Methods (available online).

Selection of Cases and Controls

All primary CRC cases (*International Classification of Diseases*, 10th edition, codes: C18.0, C18.2-18.9, C19.9, and C20.9) diagnosed between January 1, 2010, and December 31, 2016, were selected from the Swedish Colorectal Cancer Register. Cases were classified as proximal colon cancer (caecum, ascending colon, hepatic flexure, transverse colon, or splenic flexure), distal colon cancer (descending or sigmoid colon), or rectal cancer (rectosigmoidal junction or rectum). Stages of CRC were categorized as early stage (stage I-II) and late stage (stage III-IV) based on *TNM Classification of Malignant Tumours*, 8th edition (26).

For each CRC case, 5 controls were selected from the Total Population Register. Incidence density sampling was used to minimize bias when using cases and controls with different follow-up times (27). The final dataset included 40 545 CRC cases and 202 720 controls (see Figure 1). A full description of the case and control selection can be found in the Supplementary Methods (available online).

Exposure Variables and Covariates

The study population was linked to the Swedish Prescribed Drug Register to extract information on dispensed antibiotics under Anatomical Therapeutic Chemical codes J01 and J04 (anti-infective agents for systemic use) from July 1, 2005 (start of the register), until December 31, 2016. We also obtained data on other drug groups with antibiotic effects under Anatomical Therapeutic Chemical codes: A07A (intestinal anti-infective agents) and P01 (anti-protozoal that could have antibacterial effects). Antibiotics use reported as defined daily doses, a unit of comparison for drug statistics (28), was categorized as no use (no reported use of antibiotics during the study period), low (1-10 days), moderate (11-60 days), high (61-180 days), and very high (>180 days) use. A binary variable for antibiotics use "no use" vs "any use" and a variable for total number of prescriptions were also constructed. Classification of antibiotics can be found in the Supplementary Methods (available online) and Supplementary Table 1 (available online).

Additional covariates were considered based on previously established associations with CRC risk and their availability in the registers. They included socioeconomic factors (level of education, country of birth, and marital status retrieved from the LISA database) and health-care utilizations (number of specialist visits and hospitalizations from the Swedish Inpatient Register), the latter as a surrogate for potentially relevant comorbidities and health-care–seeking behavior. Detailed descriptions of these covariates can be found in the Supplementary Methods (available online).

Statistical Analysis

Tests for differences in characteristics between cases and controls were performed using a Pearson χ^2 test and 2-sample *t* test. Conditional logistic regression, adjusting for selected covariates as potential confounders, was used to investigate associations between antibiotics use and risk of CRC, reported as odds ratios (ORs) with 95% confidence intervals (CIs). The reference category for antibiotics use was "no use." To evaluate any potential dose-response effect, we conducted tests for trends in which categorical antibiotics exposures were expressed as a continuous variable. Trend tests were conducted for all analyses except the analyses with binary antibiotics exposures. Multiplicative interaction terms were introduced to assess effect modification by sex, and Q-statistics with 1 degree of freedom were used to test for heterogeneity of estimates between men and women (using binary categories of antibiotics use). Based on the study hypothesis, a number of prespecified subgroup analyses (sex, age, and anatomical tumor site) and sensitivity analyses (excluding the 2 years prior to case diagnosis) were performed, which are described in detail in the Supplementary Methods (available online). All statistical tests, 2-sided with a statistical significance level of .05, were performed using Stata/MP 16.1 (Stata Corp, College Station, TX).

Ethical Approval

The study was approved by the regional ethical review board in Umeå, Sweden (Dnr: 2017/338-31), and was conducted in accordance with the Declaration of Helsinki.

Results

The study population consisted of 40 545 cases and 202 720 matched controls (52.9% men and 47.1% women; Table 1). During the study period, 18.7% of the cases and 22.4% of the controls had no antibiotics prescribed, and 20.8% of cases and

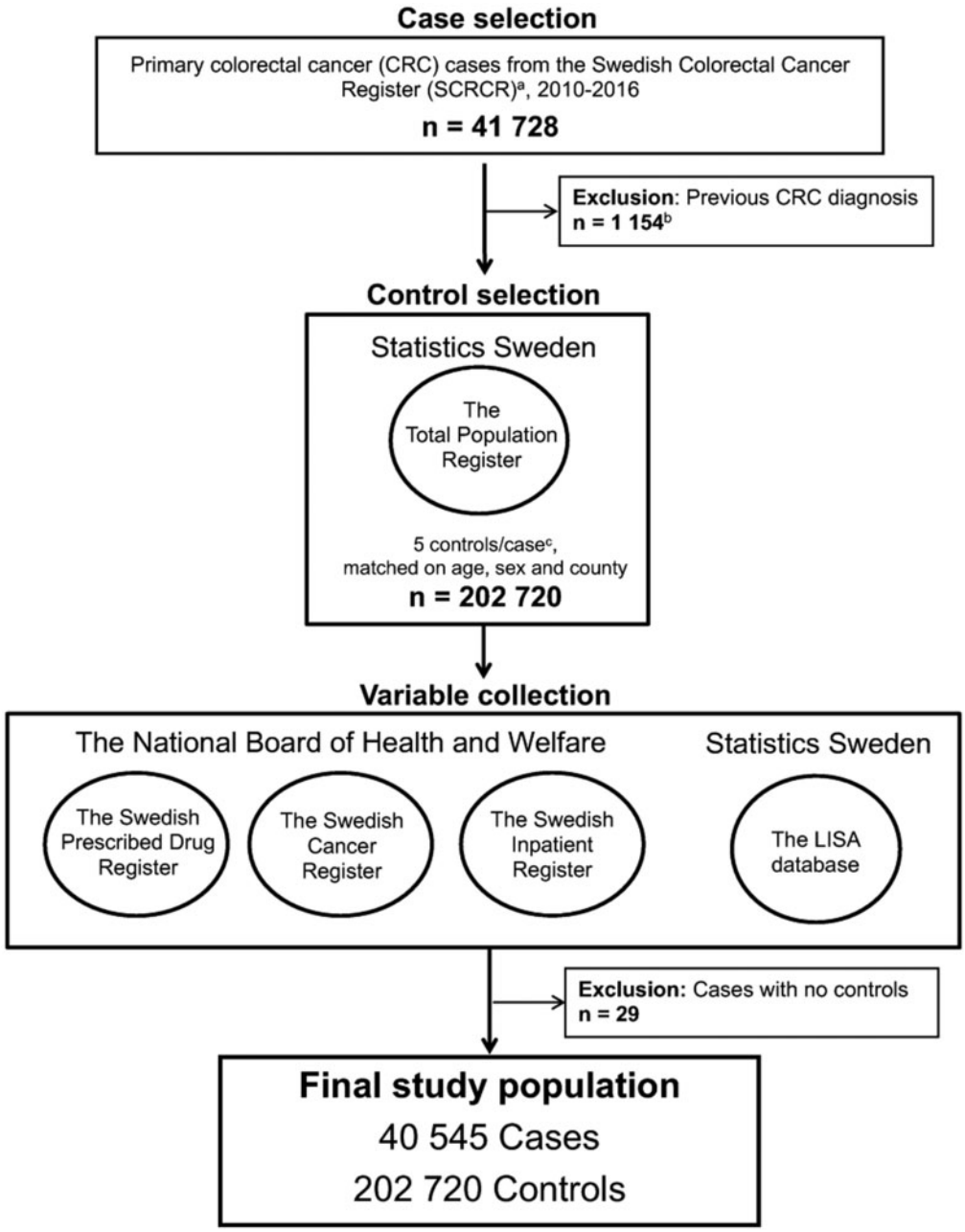

Figure 1. Flowchart of case and control selection. [a]The Swedish colorectal cancer register was initiated in 1995 for rectal cancer and in 2007 for colon cancer. [b]Case first diagnosed before the start of the Swedish Colorectal Cancer Register. [c]Three cases had fewer than 5 eligible controls.

19.3% of controls had used antibiotics for more than 2 months ($P < .001$). The mean age at CRC diagnosis was 72 years (Supplementary Table 2, available online). Among all cases, 36.4% had proximal colon cancer, 29.3% had distal colon cancer, and 33.0% had rectal cancer. The median follow-up time was 8 years.

Antibiotics use was positively associated with CRC for moderate use (OR = 1.15, 95% CI = 1.12 to 1.18) and very high use (OR = 1.17, 95% CI = 1.10 to 1.24) vs no use ($P_{trend} < .001$; Table 2). However, in the analysis excluding all antibiotics use occurring 2 years before CRC diagnosis (and for the controls, the 2 years before diagnosis of their index case) to account for reverse causation, the association was attenuated and not statistically significant for very high vs no use (OR = 1.02, 95% CI = 0.95 to 1.09; $P_{trend} = .97$). Consequently, this 2-year exclusion was applied to all analyses.

In analyses stratified by tumor site in the colorectum (Table 2), the dose-response association between antibiotics use and CRC risk was mostly confined to proximal colon cancer for moderate use (OR = 1.09, 95% CI = 1.05 to 1.14) and for very high use (OR = 1.17, 95% CI = 1.05 to 1.31) vs no use ($P_{trend} < .001$). Associations were close to null for distal colon cancer and slightly inverse for rectal cancer (Table 2), with odds ratios of 0.96 (95% CI = 0.84 to 1.10; $P_{trend} = .56$) and 0.91 (95% CI = 0.80 to 1.04), respectively, for very high use vs no use ($P_{trend} < .001$). Stratification by tumor subsites, in the analysis of any use vs no

Table 1. Characteristics of the study population

Characteristics	Cases (n = 40 545)	Controls (n = 202 720)	P[a]
Sex, No. (%)[b]			
Men	21 458 (52.9)	107 285 (52.9)	
Women	19 087 (47.1)	95 435 (47.1)	1.00
County, No. (%)[b]			
Region Stockholm	6 995 (17.3)	34 974 (17.3)	
Region Skåne	5 481 (13.5)	27 403 (13.5)	
Region Västra Götaland	7 060 (17.4)	35 300 (17.4)	
Other regions[c]	21 009 (51.8)	105 043 (51.8)	1.00
Country of birth, No. (%)			
Sweden	35 391 (87.3)	175 288 (86.5)	
Rest of Europe	4 090 (10.1)	20 662 (10.2)	
Non-European country	936 (2.3)	6 757 (3.3)	<.001
Unknown	128 (0.3)	13 (<0.01)	
Education, No. (%)			
Primary school up to 9 years	15 031 (37.1)	74 912 (37.0)	
Secondary school	16 028 (39.5)	78 030 (38.5)	
Postsecondary school	8 672 (21.4)	44 618 (22.0)	<.001
Unknown	814 (2.0)	5 160 (2.5)	
Marital status, No. (%)			
Married/Living with partner	21 382 (52.7)	105 802 (52.2)	
Widower/Widow	7 653 (18.9)	38 702 (19.1)	
Unmarried	5 018 (12.4)	25 740 (12.7)	
Divorced	6 492 (16.0)	32 475 (16.0)	.14
Unknown	0 (<0.01)	1 (<0.01)	
Specialist visits, mean (SD)			
All specialist visits within the study period	28.6 (29.5)	15.6 (25.5)	<.001
Specialist visits up to 2 years before case diagnosis[d]	4.2 (7.9)	3.0 (7.1)	<.001
Specialist visits more than 2 years before case diagnosis[d]	8.2 (15.6)	7.2 (13.5)	<.001
Hospitalizations, mean (SD)			
All hospitalizations within the study period	5.6 (5.1)	2.7 (4.4)	<.001
Hospitalizations up to 2 years before case diagnosis[d]	1.1 (1.7)	0.5 (1.4)	<.001
Hospitalizations more than 2 years before case diagnosis[d]	1.2 (2.5)	1.1 (2.4)	<.001
Antibiotics exposure, No. (%)			
No use	7 568 (18.7)	45 427 (22.4)	
Low (1-10 days)	5 847 (14.4)	28 106 (13.9)	
Moderate (11-60 days)	18 695 (46.1)	90 005 (44.4)	
High (61-180 days)	6 685 (16.5)	31 269 (15.4)	
Very high (>180 days)	1 750 (4.3)	7 913 (3.9)	<.001

[a]P value for Pearson χ^2 test (categorical variables) in which missing categories were excluded or 2-sample *t* test (continuous variables). All tests were 2-sided.
[b]Matching variables.
[c]All other counties in Sweden.
[d]For the matched controls, the diagnosis date of the index case was used.

use of antibiotics, revealed a risk gradient along the colorectal continuum (Figure 2), with the strongest positive association in the ascending colon and an inverse association in the rectum.

In analyses of all antibiotics use, further stratified by sex (Supplementary Table 3, available online), an inverse association for rectal cancer was observed in women only, with an odds ratio of 0.86 (95% CI = 0.80 to 0.92) for moderate use and 0.84 (95% CI = 0.76 to 0.94) for high use vs no use ($P_{trend} < .001$). A statistically significant interaction was found between antibiotics use and sex for rectal cancer ($P_{interaction} = .002$) but not for proximal or distal colon cancer ($P_{interaction} = .81$ and .33, respectively). Similarly, tests for heterogeneity showed statistically significant differences between men and women for rectal cancer ($P_{het} = .004$) but not for proximal or distal colon cancer ($P_{het} = 1.00$ and .35, respectively).

We further examined the association between antibiotics use and risk of CRC separately in 9 classes of antibiotics (Supplementary Table 1, available online). In these analyses, quinolones and sulfonamides and/or trimethoprims were associated with increased risk of proximal colon cancer (Figure 3; Supplementary Table 4, available online), whereas nitrofurantoins, macrolides and/or lincosamides, and notably, metronidazoles and/or tinidazoles (which exclusively inhibit anaerobic bacteria) were inversely associated with rectal cancer (Figure 3). Antibiotics grouped according to effect on anaerobic bacteria or primarily effecting aerobic bacteria showed roughly similar

Table 2. Associations between antibiotics use and risk of colorectal cancer by tumor site

Tumor site and antibiotics use[a]	Including all antibiotics use before diagnosis		Excluding antibiotics use during the 2 years preceding CRC diagnosis	
	No. of cases/controls	Adjusted OR (95% CI)[b]	No. of cases/controls	Adjusted OR (95% CI)[b]
Colorectum				
No use	9 728/54 641	1 (Referent)	13 714/70 136	1 (Referent)
Low	4 209/21 044	1.11 (1.07 to 1.15)	4 745/23 468	1.02 (0.98 to 1.06)
Moderate	18 316/88 401	1.15 (1.12 to 1.18)	16 536/81 914	1.01 (0.98 to 1.04)
High	6 554/30 774	1.17 (1.13 to 1.21)	4 414/21 982	0.98 (0.94 to 1.02)
Very high	1 738/7 860	1.17 (1.10 to 1.24)	1 136/5 220	1.02 (0.95 to 1.09)
P_{trend}[c]		<.001		.97
Colon				
No use	6 019/35 848	1 (Referent)	8 739/46 146	1 (Referent)
Low	2 727/14 144	1.13 (1.08 to 1.19)	3 138/15 874	1.03 (0.98 to 1.07)
Moderate	12 459/59 432	1.23 (1.19 to 1.27)	11 356/55 108	1.06 (1.02 to 1.09)
High	4 721/21 130	1.28 (1.23 to 1.34)	3 128/15 230	1.02 (0.97 to 1.07)
Very high	1 248/5 316	1.27 (1.19 to 1.37)	813/3 512	1.07 (0.98 to 1.17)
P_{trend}[c]		<.001		.009
Proximal colon				
No use	3 129/18 991	1 (Referent)	4 492/24 557	1 (Referent)
Low	1 520/7 707	1.18 (1.10 to 1.26)	1 707/8 638	1.07 (1.00 to 1.14)
Moderate	6 685/32 436	1.23 (1.17 to 1.29)	6 218/30 191	1.09 (1.05 to 1.14)
High	2 663/11 725	1.30 (1.22 to 1.38)	1 840/8 452	1.10 (1.03 to 1.17)
Very high	768/2 966	1.35 (1.23 to 1.49)	508/1 987	1.17 (1.05 to 1.31)
P_{trend}[c]		<.001		<.001
Distal colon				
No use	2 771/16 128	1 (Referent)	4 051/20 682	1 (Referent)
Low	1 174/6 153	1.10 (1.02 to 1.19)	1 385/6 902	1.00 (0.94 to 1.07)
Moderate	5 526/25 810	1.23 (1.17 to 1.29)	4 925/23 813	1.03 (0.98 to 1.08)
High	1 941/8 974	1.25 (1.17 to 1.34)	1 212/6 465	0.93 (0.86 to 1.00)
Very high	452/2 255	1.16 (1.03 to 1.30)	291/1 458	0.96 (0.84 to 1.10)
P_{trend}[c]		<.001		.56
Rectum				
No use	3 709/18 793	1 (Referent)	4 975/23 990	1 (Referent)
Low	1 482/6 900	1.08 (1.01 to 1.15)	1 607/7 594	1.01 (0.94 to 1.07)
Moderate	5 857/28 969	1.02 (0.97 to 1.07)	5 180/26 806	0.92 (0.88 to 0.96)
High	1 833/9 644	0.96 (0.90 to 1.03)	1 286/6 752	0.91 (0.84 to 0.97)
Very high	490/2 544	0.98 (0.88 to 1.09)	323/1 708	0.91 (0.80 to 1.04)
P_{trend}[c]		.44		<.001

[a]Antibiotics use was categorized as no use (no prescriptions during the study period), low (1-10 days), moderate (11-60 days), high (61-180 days), and very high (>180 days) use, using defined daily doses. CI = confidence interval; CRC = colorectal cancer; OR = odds ratio.
[b]Odds ratios, conditioned on matching factors (age, sex, county) and adjusted for socioeconomic factors (level of education, country of birth, marital status) and health-care utilizations prior the 2 years preceding colorectal cancer diagnosis (number of specialist visits and hospitalizations).
[c]The P_{trend} represents a trend test in which the 5 categories of antibiotics use were included in the model as a continuous variable.

associations (Figure 3; Supplementary Figure 1, available online). Associations for rectal cancer in women were consistently inverse across antibiotics classes.

For methenamine hippurate, a urinary tract antiseptic with no known effects on gut microbiota, the associations with overall risk of CRC were null (OR for very high use vs no use = 0.93, 95% CI = 0.81 to 1.08; P_{trend} = .13) (Figure 4). Methenamine hippurate use was related to women, higher age, and higher antibiotics use (respective $P < .001$) (data not shown).

Results stratified by age at diagnosis are presented in Supplementary Table 5 (available online), and further analyses stratified by both age and sex are in Supplementary Tables 6 and 7 (available online). The association between antibiotics use and risk of proximal colon cancer was clearer among participants aged 50 years and older at the time of diagnosis, compared with participants aged younger than 50 years.

In analyses stratified by tumor stage, the positive association between antibiotics use and risk of proximal colon cancer was more pronounced in stage I-II cancer compared with stage III-IV cancer (Supplementary Table 8, available online). In contrast, the inverse association in rectal cancer was limited to stage III-IV.

We also performed analyses based on follow-up time from exposure to diagnosis (Supplementary Table 9, available online). Only individuals with a single antibiotic prescription were included to eliminate potential confounding by frequent prescriptions. The analysis confirmed the presence of reverse confounding. Analyses based on the total number of antibiotics prescriptions showed similar patterns of association as for defined daily doses (Supplementary Table 10, available online).

Discussion

In this nationwide analysis of more than 40 000 CRC cases, we found a robust association between antibiotics use and higher risk of proximal colon cancer, consistent with previous

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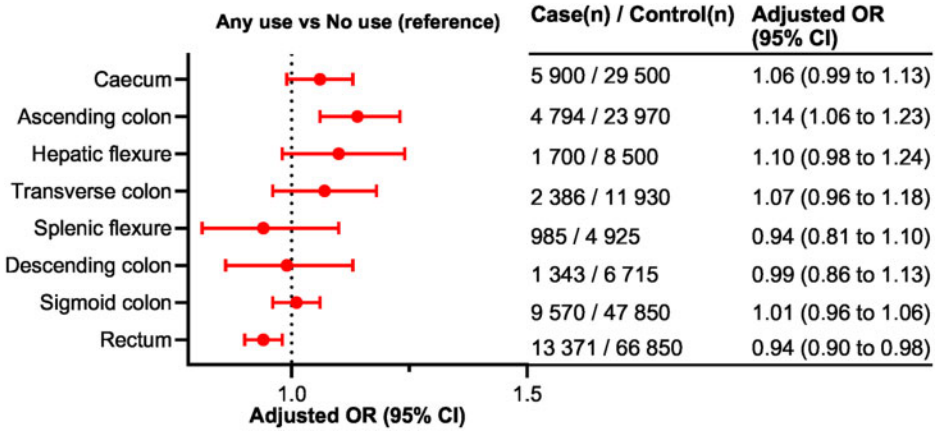

Figure 2. Associations between antibiotics use and risk of colorectal cancer (CRC) by tumor subsites. Odds ratios (OR), conditioned on matching factors (age, sex, county) and adjusted for socioeconomic factors (level of education, country of birth, marital status) and health-care utilizations prior the 2 years preceding CRC diagnosis (number of specialist visits and hospitalizations). Antibiotics use during the 2 years preceding CRC diagnosis was excluded to account for possible reverse causation. CI = confidence interval.

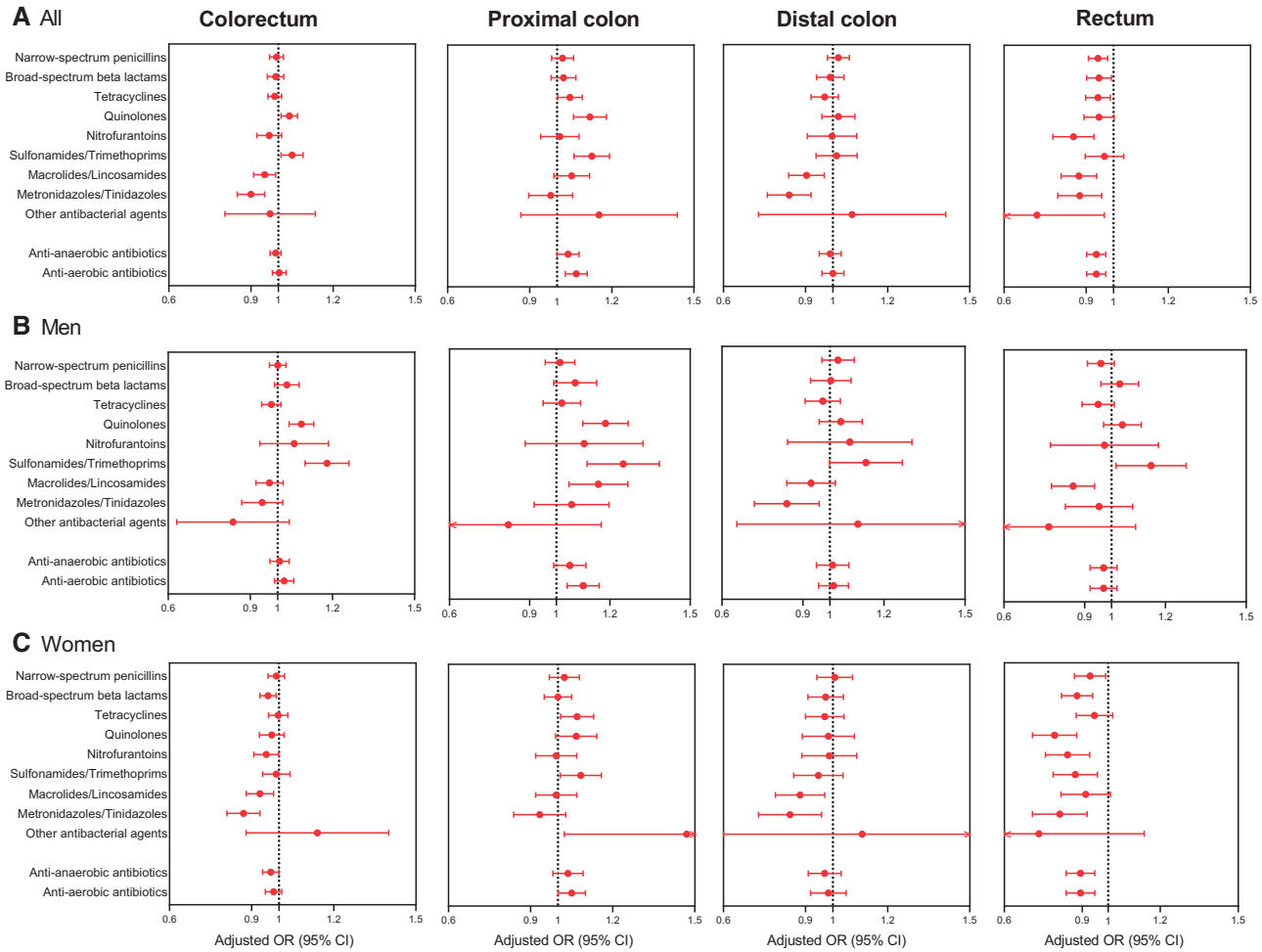

Figure 3. Associations between antibiotics classes and risk of site-specific colorectal cancer, stratified by sex. Odds ratios (OR), conditioned on matching factors (age, sex, county) and adjusted for socioeconomic factors (level of education, country of birth, marital status) and health-care utilizations prior the 2 years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). Antibiotics use during the 2 years preceding CRC diagnosis was excluded to account for possible reverse causation. Antibiotics with effect on both anaerobic and aerobic bacteria, and metrodinazoles and/or tinidazoles (which only affect anaerobic bacteria) were categorized as anti-anaerobic antibiotics. Antibiotics that primarily or only affect aerobic bacteria were categorized as anti-aerobic antibiotics. Any use of specific antibiotics class was compared with no use of the specific antibiotics class (reference category) during the study period. Results for all participants **(A)**, men **(B)**, and women **(C)** are shown. CI = confidence interval.

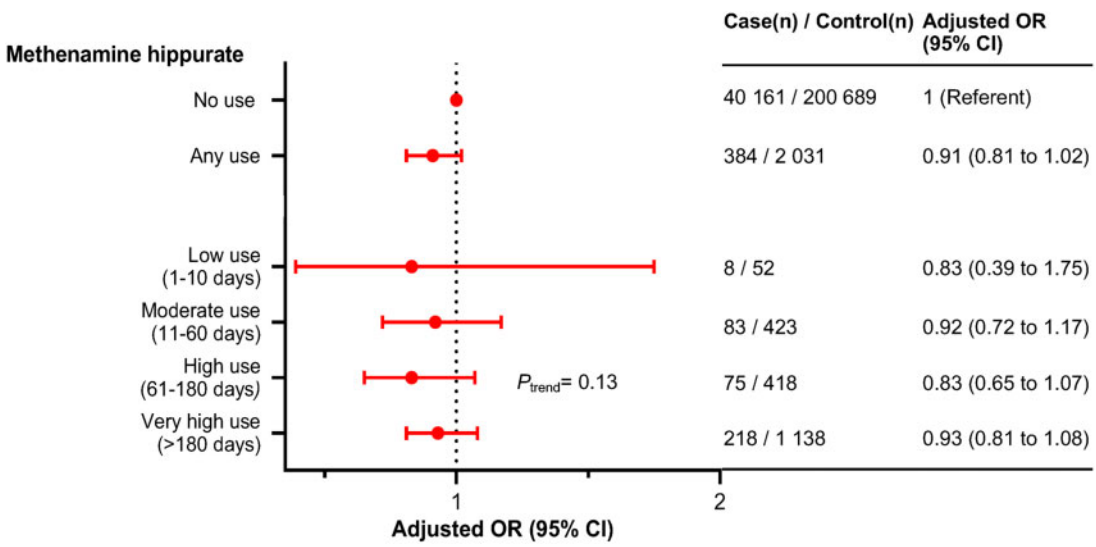

Figure 4. Associations between methenamine hippurate use and risk of colorectal cancer (CRC). Odds ratios (OR), conditioned on matching factors (age, sex, county) and adjusted for socioeconomic factors (level of education, country of birth, marital status) and health-care utilizations prior the 2 years preceding CRC diagnosis (number of specialist visits and hospitalizations). Methenamine hippurate use during the 2 years preceding CRC diagnosis was excluded to account for possible reverse causation. The P_{trend} represents a trend test in which the 5 categories of antibiotics use were included in the model as a continuous variable. CI = confidence interval.

investigations (18–23,29). We also provide important and timely confirmation of the inverse association between antibiotics use and rectal cancer risk recently reported (24). By adding stratification on sex, we show that this inverse association occurs primarily in women. Furthermore, we report results based on fine anatomic detailing of the colorectum, using stratification by follow-up time and stage at diagnosis to explore the role of timing of antibiotics exposure in the carcinogenic process. Finally, a null association between use of methenamine hippurate, a urinary antiseptic acting locally in the urinary bladder, and CRC risk provides indirect support for dysbiosis of the gut flora as a mechanism behind the association between antibiotics use and colonic carcinogenesis.

In our study, analyses stratified by specific anatomical tumor subsites demonstrated that the association was most pronounced in the ascending colon, after which it diminished, supporting the concept of the colon as a continuum (30). The observed risk gradient along the colorectal continuum is consistent with a high microbial impact in the proximal colon and a decreasing concentration of short-chain fatty acids along the colon, resulting in higher bacterial activity, biofilm formation, and fermentation in the proximal compared with the distal colon and rectum (31–34). Moreover, the positive associations between antibiotics use and proximal colon cancer began at the lowest level of antibiotics use, providing a potential justification for reducing antibiotics prescriptions in clinical practice.

The weak inverse association between antibiotics use and rectal cancer risk has been previously reported, with sexually transmitted infection suggested as a possible explanation (24). In our study, sex-stratified analyses revealed a clear sex difference with an inverse association with rectal cancer, mostly pronounced in women. Interaction and heterogeneity tests also supported the potential effect modification by sex for rectal cancer. Though speculative, sexually transmitted infections may be a possible explanation. Infection of the rectum such as *Chlamydia* infection occurs frequently in women as a secondary infection because of the closer proximity to the primary infection site (vagina vs male urethra) (35). *Chlamydia* infections have malignant potential (36) and can persist, triggering inflammation and reducing apoptosis in infected cells (37).

In the analyses stratified by antibiotics classes, quinolones and sulfonamides and/or trimethoprims were associated with increased risk of proximal cancer in both sexes, possibly reflecting the effect of these antibiotics on bacterial diversity (14). The limited effect of these antibiotics on anaerobic bacteria would favor anaerobic bacteria such as Fusobacteria species and Bacteroidetes species, which may have a role in CRC development (8–11,38). On the other hand, nitrofurantoins, macrolides and/or lincosamides, and metronidazoles and/or tinidazoles were inversely associated with rectal cancer. The potent effect of metronidazoles and/or tinidazoles and lincosamides on anaerobic bacteria could reduce Fusobacteria and Bacteroidetes. This is in line with the hypothesis that a gut flora with more abundant Fusobacteria and Bacteroidetes may contribute to CRC development. Our findings support the existence of heterogeneity in antibiotics effects along the colorectum, as concluded by Zhang et al. (24).

Use of methenamine hippurate was associated with higher antibiotics use. This was expected because methenamine hippurate is used for recurrent urinary tract infections, which previously have been treated with antibiotics. Yet, the association between methenamine hippurate use and CRC risk was null, strengthening the interpretation of an etiological role for antibiotics in CRC mediated through the gut microbiome. However, we acknowledge that even though this supports the idea that antibiotics use is causally related indirectly to an increase in the risk of proximal colon cancer, any interpretation of causality should be done with caution.

To understand at what phase of the carcinogenic process the putative role of antibiotics occurs, we conducted subgroup analyses by disease stage and follow-up time from exposure to CRC diagnosis. For proximal colon cancer, antibiotics use was associated with higher risk of stage I-II, but not III-IV disease, possibly suggesting an early role for antibiotics. However, compared with other studies (20,23,24), the follow-up time in our dataset is relatively short, between 2.5 and 9.5 (a median of 6) years

after excluding antibiotics prescribed within 2 years prior to diagnosis. Therefore, we were unable to address the role of antibiotics prescribed more than 10 years prior to diagnosis, probably the most critical time period for CRC initiation, which generally takes more than 10 years to develop. No previous study has investigated associations between antibiotics use and CRC risk stratified by tumor stage, and datasets with longer follow-up times will be required to confirm our findings. Furthermore, the inverse association for rectal cancer was strongest for stage III-IV disease, suggesting that antibiotics might play a role in slowing tumor progression in the rectum. A caveat with respect to follow-up time is the possibility of reverse causation, if symptomatic undiagnosed CRC leads to increased antibiotics use in the late prediagnostic phase, but excluding exposures in the 2 years before diagnosis should minimize this issue.

A major limitation in our study is the possibility of unmeasured confounding such as diet, anthropometric measurements, medical comorbidities such as diabetes or inflammatory bowel disease, and use of other medications. To account for confounding, we adjusted our final model for socioeconomic factors, number of specialist visits, and number of hospitalizations. These factors tend to correlate with CRC risk factors such as diet, lifestyle, and body size (39,40), and the variables for specialist visits and hospitalizations should capture relevant comorbidities to a substantial degree (41–44). These adjustments also account for potential confounding by a health-care–seeking behavior (ie, extensive use of health-care services) resulting in a higher likelihood of being prescribed antibiotics, as well as being referred to colonoscopy for gastrointestinal symptoms. Previous studies that accounted for numerous dietary and lifestyle factors (20) and specific comorbidities such as inflammatory bowel disease (24) reported similar risk estimates to ours, with no material attenuations. Furthermore, confounding by unmeasured CRC risk factors would be expected to yield false-positive associations between antibiotics use and a higher risk of CRC. However, for rectal cancer, an inverse association was observed. For these reasons, although we acknowledge the lack of data on some potential confounders, including specific comorbidities, as a limitation of this study, we do not believe that confounding due to specific comorbidities can explain our findings.

Many countries have implemented CRC screening programs. This might bias results in studies of CRC risk, as some individuals are more likely to comply with the screening program. However, at the time of our study, there was no national CRC screening in Sweden. On the local level, organized screening programs have been in place in Stockholm and Gotland counties since 2008 (45). In the current study, cases and controls are matched on county of residence, and any influence of population-based CRC screening on the observed associations is likely to be minimal.

Other limitations included lack of data on antibiotics administered during inpatient care and data on antibiotics use before 2005 (the start of the pharmaceutical register) resulting in less than 10 years follow-up time. Despite this, the size of our dataset allowed us to identify clear associations, which, in light of previous findings (18–24,29,46), seem likely to be strengthened in the future when more time has passed. We could not account for patient compliance. However, compliance is generally considered high for antibiotics (47), and the register data in our study are for antibiotics not just prescribed but actually dispensed from the pharmacy (and includes primarily oral antibiotics). Finally, the extensive analyses in our study resulted in 32 statistical comparisons, raising the issue of chance findings because of multiple testing. Applying a Bonferroni-corrected P value threshold of .002 (α value divided by the number of hypotheses = 0.05/32), our main findings remain statistically significant.

The main strength of this investigation was the use of high-quality, nationwide, registry-based data, allowing us to conduct the largest and most comprehensive original research study to date on antibiotics and CRC risk. More than 98% of all diagnosed CRC cases have been reported to the Swedish Colorectal Cancer Register, making it a reliable register for research (48,49). The Swedish Prescribed Drug Register, one of the largest pharmacoepidemiological databases in the world, provides complete national data on dispensed drugs in the Swedish population (50). The LISA database and the Swedish Inpatient Register also have high validity (51,52). Our results are, hence, generalizable and comparable with other northern European countries. The large sample size further allowed us to conduct well-powered and detailed subgroup analyses with great precision.

In conclusion, we observed a consistent association between antibiotics use and higher subsequent risk of proximal colon cancer and an inverse association for rectal cancer in women. Our findings strengthen prior evidence and provide new insights into site-specific carcinogenesis as well as indirect support for the role of gut microbiota.

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Data Availability

The data that support the findings of this study may be requested from the included Swedish registers. Ethical and legal restrictions may apply to the availability of these data, and study-specific ethical permissions are required.

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