Antibiotics Use and Subsequent Risk of Colorectal Cancer: A Swedish Nation-wide Population-based Study

Supplementary Materials

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Methods

Descriptions of the Swedish national registries

The Swedish Colorectal Cancer Register: A national register in which all diagnosed cases of rectal cancer (from 1995 onwards) and colon cancer (from 2007 onwards) are recorded. Individual data on tumor characteristics, treatments and several other clinical variables are reported to the register. The coverage is 99.3% for colon cancer and 98.5% for rectal cancer, and the evaluation of key variables, including TNM staging, have shown that the register has good validity [1-3].

The Swedish Cancer Register: The register was founded in 1958 and covers the whole Swedish population. All new diagnosis of cancer and some selected premalignant lesions are reported to the register and approximately 60 000 malignant cases are registered every year. Reporting of the data is obligatory by law for every health care providers and the completeness is around 96% [4]. The register contains individual patient data, comprehensive medical data such as ICD code, diagnosis date, date of death and histopathology. Since 2004, TNM staging data have been included in the register.

The Total Population Register: The Total Population Register is the foundation of official population and household statistics. The register is maintained by the government agency Statistics Sweden (SCB) and it is the largest population register in Sweden with complete socio-demographic data such as age, sex and marital status since 1968. Through the personal identity number, assigned to all residents staying at least 1 year in Sweden, data from the register can be used for medical research purposes, and the register also allows to identify controls from the general population [5].

The Swedish Prescribed Drug Register: A nation-wide pharmaco-epidemiological register started in July 2005, which includes data on all prescribed drugs dispensed at pharmacies in the entire country as well as information on dispensed medical devices and consumables. Drugs administered at hospital settings, vaccines and over-the-counter medicines are not included in the register. The register includes detailed information, such as ATC codes, drug names, dosage and package, as well as the dates of prescription and dispensing. The register provides the valuable and reliable data on drug exposure and is useful for researches [6].

The Longitudinal Integration Database for Health Insurance and Labor Market Studies (LISA by Swedish acronym): The LISA database started in 1990 is one of the largest databases in Sweden, and socioeconomic information such as country of birth and level of education is available for all residents of Sweden from 16 years of age, registered on December 31 each year. The participation in the register is compulsory and the quality of the database is generally high [7].

The Swedish Inpatient Register: The register, established in 1964, is the largest register regarding public health in Sweden and a principle source of data for numerous research projects. It is compulsory for all physicians, private and publicly funded, to deliver data to the register (except for visits in primary care). The inpatient register has complete national coverage since 1987, and the validity of the register is generally high [8].

Selection of cases and controls

All primary CRC cases identified between January 1st, 2010 and December 31st, 2016 were selected from the Swedish Colorectal Cancer Register. The starting date was selected to ensure a minimum of 4.5 years follow-up with complete exposure data. For each CRC case, five controls were selected at random from all individuals present in the Total Population Register (RTB), as of 2018-06-19. Controls had to be born the same year, be of the same sex and be living in the same county as their index case at the time of diagnosis. We used incidence density sampling to minimize bias when using cases and controls with different follow-up times [9], cases were therefore eligible to be used as controls prior to their own CRC diagnosis date. All controls were selected without replacement. Prior to control selection, the Swedish Cancer Register was used to remove individuals with a previous cancer diagnosis (except non-melanoma skin cancer) from the selection pool as they are at increased risk of secondary malignancies. Twenty-nine cases lacked eligible matched controls and were therefore excluded, and three cases had less than five eligible controls. The final study population included 40 545 CRC cases and 202 720 controls (**Figure 1**).

Classification of antibiotics

Antibiotics were classified into nine groups based on their mechanisms of action: narrow-spectrum penicillins, broad-spectrum beta lactams, tetracyclines, quinolones, nitrofurantoins, sulfonamides/trimethoprims, macrolides/lincosamides, metronidazoles/tinidazoles, and other antibacterial agents. Antibiotics were also categorized into two groups based on whether or not they inhibit anaerobic bacteria, which is the dominant form of gut microbiota [10, 11]. Antibiotics with effect on both anaerobic and aerobic bacteria, and metrodinazoles/tinidazoles, which only affect anaerobic bacteria, were categorized as anti-anaerobic antibiotics, and antibiotics with primarily or exclusive effect on aerobic bacteria were categorized as anti-aerobic antibiotics.

Covariates

Socioeconomic information regarding level of education (primary school up to 9 years, secondary school and postsecondary school), country of birth (Sweden, the rest of Europe and non-European countries) and marital status (married, widower/widow, unmarried and divorced) were retrieved by linking the study population to the LISA database.

To account for health care use during the study period we extracted data on number of specialist visits and hospitalizations (in continuous variable) from the Swedish Inpatient

Register, a nation-wide register of inpatient and specialist outpatient care. In order to limit the effect of reverse confounding, we included visits and hospitalizations occurring two or more years prior to CRC diagnosis for the cases, and two or more years prior to the diagnosis of the index case for the controls.

Rationale for sub-group analyses and sensitivity analyses

The study was based on the hypothesis that antibiotics use would be associated with increased risk of CRC by altering the gut microbiome. Our pre-specified primary analyses included full cohort analysis (using all antibiotics use prior to diagnosis as categorical variables), tumor-site-specific analyses and analyses of antibiotics classes (including anti-anaerobic/anti-aerobic antibiotics and using binary antibiotics exposure for simplicity). We also calculated risk estimates for methenamine hippurate (a urinary tract antiseptic which is used prophylactically in patients with recurrent urinary tract infections and has no known effect on gut microbiota) in order to address the microbiome hypothesis (that antibiotics use increases CRC risk by altering the gut microbiota). To account for possible reverse causation, pre-specified sensitivity analyses were performed by excluding antibiotics use during the two years preceding the CRC diagnosis of cases (and for the controls, the two years before diagnosis of their index case) in all primary analyses. Based on the results of the sensitivity analyses, a two-year washout period was included in all subsequent analyses. After completion of the primary and sensitivity analyses, we followed up with pre-specified secondary analyses, stratifying by sex, age and tumor stage.

Thereafter, we conducted post-hoc analyses based on our initial findings. In addition to exclusion of antibiotics use two years prior to CRC diagnosis in all analyses after the primary analyses, the post-hoc analyses included more detailed investigation of the different antibiotic classes (stratified by site and sex), follow up time from exposure to diagnosis, and total number of antibiotic prescriptions during the study period.

References

1. Jörgren F, Johansson R, Damber L*, et al.* Validity of the Swedish Rectal Cancer Registry for patients treated with major abdominal surgery between 1995 and 1997. Acta oncologica 2013;52(8):1707-1714.

2. Moberger P, Sköldberg F, Birgisson H. Evaluation of the Swedish Colorectal Cancer Registry: an overview of completeness, timeliness, comparability and validity. Acta oncologica (Stockholm, Sweden) 2018;57(12):1611.

3. Kodeda K, Nathanaelsson L, Jung B*, et al.* Population-based data from the Swedish Colon Cancer Registry. British Journal of Surgery 2013;100(8):1100-1107.

4. Barlow L, Westergren K, Holmberg L*, et al.* The completeness of the Swedish Cancer Register – a sample survey for year 1998. Acta Oncologica 2009;48(1):27-33.

5. Ludvigsson JF, Almqvist C, Bonamy A-KE*, et al.* Registers of the Swedish total population and their use in medical research. European journal of epidemiology 2016;31(2):125-136.

6. Wettermark B, Hammar N, Michaelfored C*, et al.* The new Swedish Prescribed Drug Register—Opportunities for pharmacoepidemiological research and experience from the first six months. Pharmacoepidemiology and Drug Safety 2007;16(7):726-735.

7. Ludvigsson J, Svedberg P, Olén O*, et al.* The longitudinal integrated database for health insurance and labour market studies (LISA) and its use in medical research. European Journal of Epidemiology 2019;34(4):423-437.

8. Ludvigsson JF, Andersson E, Ekbom A*, et al.* External review and validation of the Swedish national inpatient register. BMC public health 2011;11(1):450-450.

9. Lubin JH, Gail MH. Biased selection of controls for case-control analyses of cohort studies. Biometrics 1984;40(1):63-75.

10. Neish AS. Microbes in Gastrointestinal Health and Disease. Gastroenterology 2009;136(1):65-80.

11. Brook I. Antimicrobial treatment of anaerobic infections. Expert Opinion on Pharmacotherapy 2011;12(11):1691-1707.

Supplementary Table 1: Classification of antibiotics types

Antibiotics types	ATC codes[a]
Narrow-spectrum penicillins	J01CE01, J01CE02, J01CE08, J01CF01, J01CF02, J01CF05
Broad-spectrum beta lactams	J01CA01, J01CA02, J01CA04, J01CA08, J01CA12, J01CR02, J01CR05, J01DB01, J01DB05, J01DC02, J01DC08, J01DD01, J01DD02, J01DD04, J01DD13, J01DD14, J01DD54, J01DH02, J01DH03, J01DH51
Tetracyclines	J01AA02, J01AA04, J01AA06, J01AA07
Qinolones	J01MA01, J01MA02, J01MA06, J01MA12, J01MA14
Nitrofurantoins	J01XE01
Sulfonamides/trimethoprims	J01EA01, J01EE01, J04AB02
Macrolides/Linkosamides	J01FA01, J01FA06, J01FA09, J01FA10, J01FA15, J01FF01
Metronidazoles/Tinidazoles	J01XD01, P01AB01, P01AB02
Other antibacterial agents	A07AA06, A07AA09, A07AA12, J01GB, J01GB03, J01GB06, J01XA01, J01XA02, J01XC01, J01XX, J01XX01, J01XX08, J01XX09, J01XX11, J04AB04, J04AM02, J04AM05, J04AM06
Methenamine hippurate	J01XX05
Anti-anaerobic antibiotics[b]	A07AA09, A07AA12, J01CA01, J01CA02, J01CA04, J01CA12, J01CE01, J01CE02, J01CE08, J01CR02, J01CR05, J01DH02, J01DH03, J01DH51, J01FF01, J01XA01, J01XA02, J01XD01, J04AB02, J04AB04, J04AM02, J04AM05, J04AM06, P01AB01, P01AB02
Anti-aerobic antibiotics[c]	A07AA06, J01AA02, J01AA04, J01AA06, J01AA07, J01CA08, J01CF01, J01CF02, J01CF05, J01DB01, J01DB05, J01DC02, J01DC08, J01DD01, J01DD02, J01DD04, J01DD13, J01DD14, J01DD54, J01EA01, J01EE01, J01FA01, J01FA06, J01FA09, J01FA10, J01FA15, J01GB, J01GB03, J01GB06, J01MA01, J01MA02, J01MA06, J01MA12, J01MA14, J01XC01, J01XE01, J01XX, J01XX01, J01XX08, J01XX09, J01XX11, J04AC01, J04AK01, J04AK02, J04BA02

[a]The Anatomical Therapeutic Chemical Classification System by WHO Collaboration Center for Drug Statistic Methodology (https://www.whocc.no/atc_ddd_index/)
[b]Antibiotics with effect on both anaerobic and aerobic bacteria, and metrodinazoles/tinidazoles (which only affect anaerobic bacteria) were categorized as anti-anaerobic antibiotics.
[c]Antibiotics that primarily or only affect aerobic bacteria were categorized as anti-aerobic antibiotics.

Supplementary Table 2: Characteristics of colorectal cancer cases

Tumor characteristics	Cases (n=40 545)
Age at diagnosis in years, mean (SD)	71.9 (11.7)
Age at diagnosis, n (%)	
<50 year	1 862 (4.6)
50-59 year	3 725 (9.2)
60-69 year	9 977 (24.6)
70-79 year	13 748 (33.9)
≥80 year	11 233 (27.7)
Tumor location, n (%)	
Proximal colon	14 765 (36.4)
Distal colon	11 864 (29.3)
Rectum	13 371 (33.0)
Unknown[a]	545 (1.3)
Tumor stage, n (%)	
Stage I-II	16 585 (40.9)
Stage III-IV	16 485 (40.7)
Unknown[b]	7 475 (18.4)
Follow-up time[c], years	
Mean (SD)	7.6 (2.1)
Median (IQR)	8 (6-9)

[a]Colon cancer with no additional information on location (proximal and distal). Abbreviations: SD, Standard Deviation; IQR, Interquartile Range.
[b]Tumor stage data not registered.
[c]Follow-up time from study start (July 1, 2005) to date of diagnosis.

Supplementary Table 3: Associations between antibiotics use and risk of colorectal cancer by sex

Tumor site	Antibiotics use[a]	Men		Women	
		Case(n) / Control(n)	Adjusted OR[b] (95% CI)	Case(n) / Control(n)	Adjusted OR[b] (95% CI)
Colorectum	No use	8 036 / 41 334	1 (Reference)	5 678 / 28 802	1 (Reference)
	Low	2 357 / 11 598	1.03 (0.98-1.09)	2 388 / 11 870	1.00 (0.95-1.05)
	Moderate	8 420 / 41 638	1.02 (0.99-1.06)	8 116 / 40 276	0.99 (0.95-1.03)
	High	2 094 / 10 128	1.03 (0.97-1.09)	2 320 / 11 854	0.94 (0.88-0.99)
	Very high	551 / 2 587	1.03 (0.93-1.14)	585 / 2 633	1.00 (0.91-1.11)
	P_{trend}[c]		0.18		0.16
Proximal colon	No use	2 255 / 12 225	1 (Reference)	2 237 / 12 332	1 (Reference)
	Low	710 / 3 561	1.07 (0.98-1.18)	997 / 5 077	1.07 (0.98-1.16)
	Moderate	2 596 / 12 771	1.08 (1.01-1.15)	3 622 / 17 420	1.11 (1.04-1.18)
	High	751 / 3 199	1.19 (1.08-1.31)	1 089 / 5 253	1.04 (0.95-1.13)
	Very high	200 / 804	1.18 (1.00-1.41)	308 / 1 183	1.16 (1.00-1.33)
	P_{trend}[c]		<.001		0.01
Distal colon	No use	2 495 / 12 835	1 (Reference)	1 556 / 7 847	1 (Reference)
	Low	724 / 3 591	1.02 (0.93-1.11)	661 / 3 311	0.98 (0.89-1.09)
	Moderate	2 686 / 12 938	1.04 (0.98-1.11)	2 239 / 10 875	1.00 (0.93-1.08)
	High	618 / 3 234	0.96 (0.86-1.06)	594 / 3 231	0.89 (0.80-0.99)
	Very high	154 / 787	0.95 (0.79-1.15)	137 / 671	0.97 (0.79-1.18)
	P_{trend}[c]		0.87		0.27
Rectum	No use	3 189 / 15 778	1 (Reference)	1 786 / 8 212	1 (Reference)
	Low	905 / 4 298	1.04 (0.96-1.13)	702 / 3 296	0.95 (0.86-1.05)
	Moderate	3 030 / 15 450	0.97 (0.91-1.02)	2 150 / 11 356	0.86 (0.80-0.92)
	High	699 / 3 570	0.95 (0.87-1.05)	587 / 3 182	0.84 (0.76-0.94)
	Very high	192 / 974	0.97 (0.82-1.15)	131 / 734	0.85 (0.70-1.05)
	P_{trend}[c]		0.18		<.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. Antibiotics use during the two years preceding CRC diagnosis was excluded to account for possible reverse causation.

[b]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 two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). CI = confidence interval.

[c]The P_{trend} represents a trend test in which the five categories of antibiotics use were included in the model as a continuous variable.

Supplementary Table 4: Associations between antibiotics classes and proximal colon cancer risk

Antibiotics classes	Antibiotics use[a]					P_{trend}[c]
	No use	Low	Moderate	High	Very high	
Narrow-spectrum penicillins						
Case (n) / Control (n)	8 387 / 43 003	1 115 / 5 540	4 757 / 23 051	445 / 1 960	61 / 271	
Adjusted OR[b] (95% CI)	1 (Reference)	1.01 (0.94-1.08)	1.02 (0.98-1.07)	1.02 (0.91-1.14)	0.92 (0.69-1.23)	0.32
Broad-spectrum beta lactams						
Case (n) / Control (n)	10 636 / 54 257	1 515 / 7 590	2 397 / 11 151	185 / 718	32 / 109	
Adjusted OR[b] (95% CI)	1 (Reference)	0.99 (0.93-1.05)	1.04 (0.98-1.09)	1.12 (0.94-1.33)	1.18 (0.78-1.77)	0.11
Tetracyclines						
Case (n) / Control (n)	11 354 / 57 844	1 431 / 6 937	1 645 / 7 718	267 / 1 033	68 / 293	
Adjusted OR[b] (95% CI)	1 (Reference)	1.02 (0.96-1.09)	1.04 (0.98-1.10)	1.22 (1.06-1.40)	1.06 (0.81-1.39)	0.01
Qinolones						
Case (n) / Control (n)	12 265 / 63 098	1 391 / 6 057	1 001 / 4 230	98 / 379	10 / 61	
Adjusted OR[b] (95% CI)	1 (Reference)	1.13 (1.06-1.20)	1.12 (1.04-1.21)	1.11 (0.88-1.39)	0.51 (0.24-1.07)	<.001
Nitrofurantoins						
Case (n) / Control (n)	13 550 / 68 102	842 / 4 094	342 / 1 463	26 / 128	5 / 38	
Adjusted OR[b] (95% CI)	1 (Reference)	0.99 (0.92-1.07)	1.07 (0.95-1.21)	0.82 (0.53-1.26)	0.59 (0.23-1.53)	0.84
Sulfonamides/trimethoprims						
Case (n) / Control (n)	12 991 / 66 270	1 012 / 4 504	672 / 2 748	68 / 212	22 / 91	
Adjusted OR[b] (95% CI)	1 (Reference)	1.10 (1.02-1.18)	1.16 (1.06-1.27)	1.39 (1.05-1.84)	1.01 (0.63-1.62)	<.001
Macrolides/Linkosamides						
Case (n) / Control (n)	13 190 / 66 818	1 125 / 5 072	400 / 1 724	34 / 163	16 / 48	
Adjusted OR[b] (95% CI)	1 (Reference)	1.06 (0.99-1.13)	1.05 (0.94-1.18)	0.85 (0.57-1.25)	1.34 (0.74-2.40)	0.16
Metronidazoles/Tinidazoles						
Case (n) / Control (n)	13 969 / 69 939	648 / 3 240	143 / 621	4 / 21	1 / 4	
Adjusted OR[b] (95% CI)	1 (Reference)	0.97 (0.89-1.06)	1.02 (0.85-1.23)	0.64 (0.20-2.06)	1.55 (0.16-15.06)	0.58
Other antibacterial agents						
Case (n) / Control (n)	14 681 / 73 520	34 / 109	26 / 60	12 / 75	12 / 61	
Adjusted OR[b] (95% CI)	1 (Reference)	1.39 (0.94-2.05)	1.59 (0.98-2.60)	0.65 (0.35-1.21)	0.81 (0.44-1.52)	0.89

[a]Antibiotics use was categorized as no use (no prescriptions of specific antibiotics class 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. Antibiotics use during the two years preceding CRC diagnosis was excluded to account for possible reverse causation.

[b]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 two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). CI = confidence interval.

[c]The P_{trend} represents a trend test in which the five categories of antibiotics use were included in the model as a continuous variable.

Supplementary Table 5: Associations between antibiotics use and risk of colorectal cancer by age at diagnosis

Age at diagnosis	Antibiotics use[a]	Proximal Colon		Distal Colon		Rectum	
		Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)
Age < 50 years (early onset) (n= 1 905)	No use	205 / 1 028	1 (Reference)	259 / 1 259	1 (Reference)	309 / 1 547	1 (Reference)
	Low	58 / 327	0.87 (0.62-1.21)	70 / 368	0.86 (0.64-1.17)	92 / 427	0.98 (0.75-1.29)
	Moderate	219 / 1 039	0.99 (0.79-1.25)	236 / 1 203	0.90 (0.72-1.11)	268 / 1 417	0.89 (0.74-1.08)
	High	37 / 245	0.60 (0.39-0.92)	47 / 254	0.76 (0.52-1.10)	61 / 276	1.00 (0.71-1.39)
	Very high	16 / 36	1.53 (0.77-3.07)	12 / 36	1.38 (0.66-2.92)	16 / 63	1.15 (0.62-2.15)
	P_{trend}[c]		0.51		0.27		0.48
Age ≥ 50 years (n= 38 095)	No use	4 287 / 23 529	1 (Reference)	3 792 / 19 423	1 (Reference)	4 666 / 22 443	1 (Reference)
	Low	1 649 / 8 311	1.08 (1.01-1.15)	1 315 / 6 534	1.01 (0.94-1.08)	1 515 / 7 167	1.01 (0.95-1.08)
	Moderate	5 999 / 29 152	1.10 (1.05-1.15)	4 689 / 22 610	1.03 (0.98-1.08)	4 912 / 25 389	0.92 (0.88-0.97)
	High	1 803 / 8 207	1.11 (1.04-1.19)	1 165 / 6 211	0.93 (0.86-1.01)	1 225 / 6 476	0.90 (0.84-0.97)
	Very high	492 / 1 951	1.16 (1.04-1.30)	279 / 1 422	0.95 (0.83-1.10)	307 / 1 645	0.91 (0.79-1.03)
	P_{trend}[c]		<.001		0.70		<.001
Age < 72 years (mean age) (n= 18 590)	No use	1 845 / 9 684	1 (Reference)	2 201 / 10 918	1 (Reference)	2 815 / 13 485	1 (Reference)
	Low	607 / 3 092	1.02 (0.92-1.13)	681 / 3 317	0.99 (0.90-1.09)	842 / 4 056	0.97 (0.89-1.05)
	Moderate	2 252 / 11 073	1.04 (0.97-1.12)	2 413 / 11 905	0.97 (0.91-1.04)	2 657 / 13 944	0.89 (0.84-0.95)
	High	589 / 2 951	0.98 (0.88-1.10)	530 / 3 002	0.83 (0.74-0.92)	662 / 3 323	0.92 (0.83-1.01)
	Very high	204 / 685	1.32 (1.11-1.58)	136 / 663	0.91 (0.75-1.12)	156 / 852	0.84 (0.70-1.01)
	P_{trend}[c]		0.12		0.02		<.001
Age ≥ 72 years (mean age) (n= 21 410)	No use	2 647 / 14 873	1 (Reference)	1 850 / 9 764	1 (Reference)	2 160 / 10 505	1 (Reference)
	Low	1 100 / 5 546	1.10 (1.02-1.19)	704 / 3 585	1.02 (0.93-1.13)	765 / 3 538	1.06 (0.96-1.16)
	Moderate	3 966 / 19 118	1.13 (1.07-1.19)	2 512 / 11 908	1.09 (1.02-1.17)	2 523 / 12 862	0.97 (0.91-1.03)
	High	1 251 / 5 501	1.16 (1.07-1.26)	682 / 3 463	1.03 (0.93-1.15)	624 / 3 429	0.91 (0.82-1.01)
	Very high	304 / 1 302	1.11 (0.96-1.27)	155 / 795	1.02 (0.85-1.23)	167 / 856	1.01 (0.84-1.21)
	P_{trend}[c]		<.001		0.08		0.15

[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. Antibiotics use during the two years preceding CRC diagnosis was excluded to account for possible reverse causation.
[b]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 two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). CI = confidence interval.
[c]The P_{trend} represents a trend test in which the five categories of antibiotics use were included in the model as a continuous variable.

Supplementary Table 6: Associations between antibiotics use and risk of colorectal cancer by age at diagnosis (Men)

Age at diagnosis	Antibiotics use[a]	Proximal Colon		Distal Colon		Rectum	
		Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)
Age < 50 years (early onset) (n= 1 047)	No use	129 / 656	1 (Reference)	158 / 756	1 (Reference)	194 / 998	1 (Reference)
	Low	22 / 184	0.56 (0.34-0.93)	36 / 186	0.93 (0.61-1.41)	48 / 236	1.01 (0.70-1.44)
	Moderate	116 / 522	1.05 (0.78-1.42)	106 / 569	0.84 (0.62-1.13)	152 / 769	0.95 (0.74-1.21)
	High	16 / 103	0.65 (0.35-1.21)	18 / 93	0.84 (0.48-1.49)	25 / 107	0.98 (0.59-1.62)
	Very high	12 / 10	3.98 (1.51-10.51)	6 / 16	1.37 (0.42-4.45)	9 / 30	1.21 (0.52-2.79)
	P_{trend}[c]		0.51		0.35		0.76
Age ≥ 50 years (n= 20 157)	No use	2 126 / 11 569	1 (Reference)	2 337 / 12 079	1 (Reference)	2 995 / 14 780	1 (Reference)
	Low	688 / 3 377	1.10 (1.00-1.21)	688 / 3 405	1.02 (0.93-1.12)	857 / 4 062	1.04 (0.96-1.13)
	Moderate	2 480 / 12 249	1.08 (1.01-1.15)	2 580 / 12 369	1.05 (0.98-1.12)	2 878 / 14 681	0.97 (0.91-1.03)
	High	735 / 3 096	1.21 (1.09-1.33)	600 / 3 141	0.96 (0.87-1.07)	674 / 3 463	0.95 (0.87-1.05)
	Very high	188 / 794	1.14 (0.96-1.36)	148 / 771	0.94 (0.78-1.14)	183 / 944	0.97 (0.81-1.14)
	P_{trend}[c]		<.001		0.75		0.20
Age < 72 years (mean age) (n= 10 500)	No use	1 057 / 5 474	1 (Reference)	1 428 / 7 115	1 (Reference)	1 889 / 9 227	1 (Reference)
	Low	276 / 1 420	1.00 (0.86-1.16)	367 / 1 810	0.99 (0.87-1.12)	474 / 2 381	0.96 (0.86-1.07)
	Moderate	1 018 / 5 013	1.03 (0.93-1.13)	1 332 / 6 476	1.00 (0.92-1.09)	1 588 / 8 128	0.94 (0.87-1.01)
	High	233 / 1 145	1.00 (0.85-1.18)	245 / 1 458	0.79 (0.68-0.93)	349 / 1 731	0.95 (0.83-1.08)
	Very high	87 / 303	1.30 (1.00-1.68)	67 / 336	0.88 (0.66-1.17)	90 / 483	0.87 (0.68-1.10)
	P_{trend}[c]		0.27		0.08		0.06
Age ≥ 72 years (mean age) (n= 10 704)	No use	1 198 / 6 751	1 (Reference)	1 067 / 5 720	1 (Reference)	1 300 / 6 551	1 (Reference)
	Low	434 / 2 141	1.13 (1.00-1.28)	357 / 1 781	1.05 (0.92-1.21)	431 / 1 917	1.15 (1.01-1.30)
	Moderate	1 578 / 7 758	1.11 (1.02-1.21)	1 354 / 6 462	1.10 (1.00-1.20)	1 442 / 7 322	1.01 (0.93-1.11)
	High	518 / 2 054	1.30 (1.15-1.47)	373 / 1 776	1.13 (0.98-1.29)	350 / 1 839	0.98 (0.85-1.12)
	Very high	113 / 501	1.11 (0.88-1.40)	87 / 451	1.04 (0.81-1.34)	102 / 491	1.12 (0.88-1.41)
	P_{trend}[c]		<.001		0.04		0.81

[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. Antibiotics use during the two years preceding CRC diagnosis was excluded to account for possible reverse causation.
[b]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 two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). CI = confidence interval.
[c]The P_{trend} represents a trend test in which the five categories of antibiotics use were included in the model as a continuous variable.

Supplementary Table 7: Associations between antibiotics use and risk of colorectal cancer by age at diagnosis (Women)

Age at diagnosis	Antibiotics use[a]	Proximal Colon		Distal Colon		Rectum	
		Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)
Age < 50 years (early onset) (n= 858)	No use	76 / 372	1 (Reference)	101 / 503	1 (Reference)	115 / 549	1 (Reference)
	Low	36 / 143	1.32 (0.83-2.11)	34 / 182	0.80 (0.51-1.25)	44 / 191	0.89 (0.58-1.35)
	Moderate	103 / 517	0.93 (0.64-1.34)	130 / 634	0.93 (0.68-1.27)	116 / 648	0.77 (0.56-1.05)
	High	21 / 142	0.54 (0.30-0.98)	29 / 161	0.70 (0.42-1.15)	36 / 169	0.89 (0.56-1.41)
	Very high	4 / 26	0.50 (0.15-1.68)	6 / 20	1.35 (0.51-3.55)	7 / 33	1.01 (0.40-2.54)
	P_{trend}[c]		0.08		0.49		0.27
Age ≥ 50 years (n= 17 938)	No use	2 161 / 11 960	1 (Reference)	1 455 / 7 344	1 (Reference)	1 671 / 7 663	1 (Reference)
	Low	961 / 4 934	1.06 (0.97-1.15)	627 / 3 129	1.00 (0.90-1.11)	658 / 3 105	0.96 (0.86-1.06)
	Moderate	3 519 / 16 903	1.11 (1.05-1.18)	2 109 / 10 241	1.01 (0.93-1.09)	2 034 / 10 708	0.86 (0.80-0.93)
	High	1 068 / 5 111	1.05 (0.97-1.15)	565 / 3 070	0.90 (0.80-1.01)	551 / 3 013	0.84 (0.75-0.94)
	Very high	304 / 1 157	1.17 (1.01-1.36)	131 / 651	0.96 (0.78-1.18)	124 / 701	0.85 (0.69-1.04)
	P_{trend}[c]		0.004		0.33		<.001
Age < 72 years (mean age) (n= 8 090)	No use	788 / 4 210	1 (Reference)	773 / 3 803	1 (Reference)	926 / 4 258	1 (Reference)
	Low	331 / 1 672	1.05 (0.91-1.21)	314 / 1 507	0.99 (0.85-1.14)	368 / 1 675	0.97 (0.84-1.11)
	Moderate	1 234 / 6 060	1.05 (0.95-1.16)	1 081 / 5 429	0.94 (0.85-1.05)	1 069 / 5 816	0.82 (0.74-0.90)
	High	356 / 1 806	0.95 (0.82-1.10)	285 / 1 544	0.85 (0.73-1.00)	313 / 1 592	0.87 (0.75-1.01)
	Very high	117 / 382	1.27 (1.00-1.62)	69 / 327	0.95 (0.71-1.25)	66 / 369	0.80 (0.60-1.07)
	P_{trend}[c]		0.46		0.09		<.001
Age ≥ 72 years (mean age) (n= 10 706)	No use	1 449 / 8 122	1 (Reference)	783 / 4 044	1 (Reference)	860 / 3 954	1 (Reference)
	Low	666 / 3 405	1.08 (0.97-1.19)	347 / 1 804	0.99 (0.86-1.14)	334 / 1 621	0.94 (0.82-1.09)
	Moderate	2 388 / 11 360	1.14 (1.06-1.22)	1 158 / 5 446	1.07 (0.97-1.19)	1 081 / 5 540	0.90 (0.81-1.00)
	High	733 / 3 447	1.08 (0.98-1.20)	309 / 1 687	0.93 (0.80-1.09)	274 / 1 590	0.82 (0.70-0.97)
	Very high	191 / 801	1.10 (0.92-1.32)	68 / 344	1.01 (0.75-1.34)	65 / 365	0.91 (0.68-1.22)
	P_{trend}[c]		0.009		0.83		0.02

[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. Antibiotics use during the two years preceding CRC diagnosis was excluded to account for possible reverse causation.
[b]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 two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). CI = confidence interval.
[c]The P_{trend} represents a trend test in which the five categories of antibiotics use were included in the model as a continuous variable.

Supplementary Table 8: Associations between antibiotics use and risk of colorectal cancer by tumor stage

Tumor site	Antibiotics use[a]	Stage I-II		Stage III-IV	
		Case(n) / Control(n)	Adjusted OR[b] (95% CI)	Case(n) / Control(n)	Adjusted OR[b] (95% CI)
Colorectum	No use	5 312 / 28 643	1 (Reference)	5 977 / 29 453	1 (Reference)
	Low	1 966 / 9 634	1.08 (1.02-1.15)	1 940 / 9 649	0.98 (0.92-1.04)
	Moderate	6 854 / 33 505	1.08 (1.03-1.12)	6 596 / 32 810	0.97 (0.93-1.01)
	High	1 964 / 9 016	1.13 (1.06-1.20)	1 571 / 8 488	0.88 (0.83-0.94)
	Very high	489 / 2 127	1.14 (1.02-1.27)	401 / 2 025	0.93 (0.82-1.04)
	P_{trend}[c]		<.001		0.002
Proximal colon	No use	1 791 / 10 633	1 (Reference)	2 149 / 11 019	1 (Reference)
	Low	754 / 3 775	1.17 (1.06-1.28)	735 / 3 826	0.98 (0.89-1.08)
	Moderate	2 778 / 13 168	1.22 (1.14-1.31)	2 651 / 13 037	1.02 (0.95-1.09)
	High	878 / 3 748	1.28 (1.16-1.41)	721 / 3 542	0.99 (0.89-1.09)
	Very high	236 / 861	1.32 (1.12-1.56)	198 / 846	1.08 (0.91-1.28)
	P_{trend}[c]		<.001		0.58
Distal colon	No use	1 518 / 8 133	1 (Reference)	1 847 / 9 058	1 (Reference)
	Low	545 / 2 730	1.05 (0.94-1.17)	604 / 2 961	0.98 (0.89-1.09)
	Moderate	1 956 / 9 289	1.10 (1.02-1.19)	2 053 / 10 110	0.97 (0.90-1.04)
	High	515 / 2 560	1.07 (0.95-1.20)	451 / 2 594	0.83 (0.74-0.93)
	Very high	123 / 573	1.16 (0.94-1.43)	109 / 597	0.84 (0.67-1.05)
	P_{trend}[c]		0.02		0.01
Rectum	No use	1 947 / 9 575	1 (Reference)	1 907 / 9 035	1 (Reference)
	Low	651 / 3 021	1.05 (0.95-1.16)	581 / 2 735	0.99 (0.89-1.10)
	Moderate	2 044 / 10 673	0.93 (0.87-1.00)	1 819 / 9 309	0.92 (0.85-0.99)
	High	543 / 2 602	1.02 (0.92-1.14)	382 / 2 264	0.80 (0.70-0.91)
	Very high	124 / 674	0.92 (0.75-1.13)	92 / 562	0.80 (0.63-1.02)
	P_{trend}[c]		0.24		<.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. Antibiotics use during the two years preceding CRC diagnosis was excluded to account for possible reverse causation.
[b]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 two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). CI = confidence interval.
[c]The P_{trend} represents a trend test in which the five categories of antibiotics use were included in the model as a continuous variable.

Supplementary Table 9: Associations between single prescription of antibiotics and risk of colorectal cancer by follow-up time from exposure to diagnosis[a]

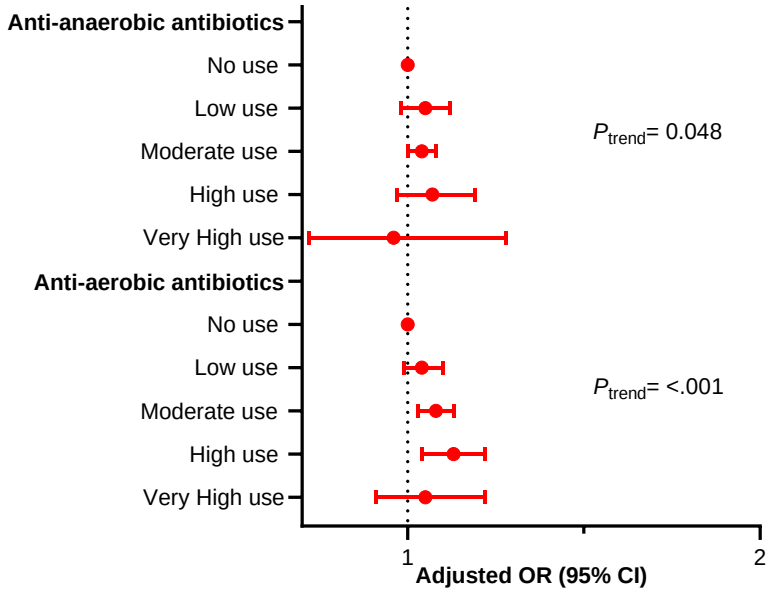
Antibiotics use	0-2 years follow-up		2-5 years follow-up		5-11 years follow-up	
	Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)
Colorectum						
No use	9 705 / 54 489	1 (Reference)	9 705 / 54 489	1 (Reference)	9 705 / 54 489	1 (Reference)
Single prescription	2 193 / 10 109	1.21 (1.15-1.27)	2 752 / 15 295	0.99 (0.94-1.03)	2 477 / 13 410	1.01 (0.96-1.06)
Colon						
No use	6 001 / 35 737	1 (Reference)	6 001 / 35 737	1 (Reference)	6 001 / 35 737	1 (Reference)
Single prescription	1 428 / 6 688	1.26 (1.18-1.35)	1 752 / 10 142	1.01 (0.95-1.07)	1 555 / 8 869	1.01 (0.95-1.08)
Proximal colon						
No use	3 115 / 18 915	1 (Reference)	3 115 / 18 915	1 (Reference)	3 115 / 18 915	1 (Reference)
Single prescription	789 / 3 561	1.34 (1.22-1.46)	913 / 5 388	1.02 (0.94-1.10)	828 / 4 709	1.04 (0.95-1.13)
Distal colon						
No use	2 767 / 16 093	1 (Reference)	2 767 / 16 093	1 (Reference)	2 767 / 16 093	1 (Reference)
Single prescription	618 / 3 002	1.19 (1.08-1.31)	808 / 4 561	1.00 (0.92-1.09)	704 / 3 960	1.00 (0.91-1.09)
Rectum						
No use	3 704 / 18 752	1 (Reference)	3 704 / 18 752	1 (Reference)	3 704 / 18 752	1 (Reference)
Single prescription	765 / 3 421	1.12 (1.03-1.23)	1 000 / 5 153	0.95 (0.88-1.03)	922 / 4 541	1.02 (0.94-1.10)

[a] This is a sensitivity analysis based on follow-up time from antibiotics exposure to diagnosis. Only one antibiotic prescription was included, to allow stratification and to eliminate potential confounding by frequent antibiotics prescriptions.
[b]Odds ratios (OR), adjusted for matching factors (age, sex, county), socioeconomic factors (level of education, country of birth, marital status) and health care utilizations prior the two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). CI = confidence interval.

Supplementary Table 10: Associations between total number of antibiotics prescriptions and risk of colorectal cancer[a]

Antibiotics use	Proximal colon		Distal colon		Rectum	
	Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)	Case (n) / Control (n)	Adjusted OR[b] (95% CI)
No use	4 481 / 24 492	1 (Reference)	4 047 / 20 650	1 (Reference)	4 972 / 23 963	1 (Reference)
Single prescription	2 888 / 14 821	1.04 (0.99-1.10)	2 520 / 12 409	1.02 (0.96-1.07)	2 887 / 13 841	0.99 (0.94-1.05)
2 prescriptions	1 992 / 9 919	1.06 (1.00-1.12)	1 672 / 7 860	1.06 (0.99-1.13)	1 781 / 8 942	0.95 (0.90-1.01)
3 prescriptions	1 370 / 6 556	1.09 (1.02-1.17)	1 013 / 5 164	0.97 (0.90-1.05)	1 059 / 5 807	0.88 (0.81-0.94)
4 prescriptions	1 006 / 4 472	1.17 (1.08-1.26)	703 / 3 395	1.03 (0.94-1.13)	741 / 3 801	0.94 (0.87-1.03)
5 prescriptions	626 / 3 085	1.03 (0.94-1.13)	459 / 2 349	0.96 (0.86-1.07)	454 / 2 483	0.87 (0.78-0.97)
> 5 prescriptions	2 402 / 10 480	1.11 (1.05-1.18)	1 450 / 7 493	0.95 (0.88-1.02)	1 477 / 8 013	0.88 (0.83-0.95)
P_{trend}[c]		<.001		0.13		<.001

[a] This analysis was done to assess the robustness of the associations when antibiotics exposures were defined based on total number of prescriptions rather than defined daily doses.
[b]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 two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). CI = confidence interval.
[c]The P_{trend} represents a trend test in which the categories of antibiotics use were included in the model as a continuous variable.



	Case(n) / Control(n)	Adjusted OR (95% CI)
Anti-anaerobic antibiotics		
No use	7 982 / 41 452	1 (Reference)
Low use	1 182 / 5 687	1.05 (0.98-1.12)
Moderate use	4 997 / 24 083	1.04 (1.00-1.08)
High use	542 / 2 326	1.07 (0.97-1.19)
Very High use	62 / 277	0.96 (0.72-1.28)
Anti-aerobic antibiotics		
No use	6 802 / 36 146	1 (Reference)
Low use	2 384 / 12 048	1.04 (0.99-1.10)
Moderate use	4 368 / 20 505	1.08 (1.03-1.13)
High use	962 / 4 093	1.13 (1.04-1.22)
Very High use	249 / 1 033	1.05 (0.91-1.22)

Supplementary Figure 1: Associations between antibiotics use and risk of proximal colon cancer by antibiotics types (anti-anaerobic vs anti-aerobic). 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 two years preceding colorectal cancer (CRC) diagnosis (number of specialist visits and hospitalizations). 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. Antibiotics with effect on both anaerobic and aerobic bacteria, and metrodinazoles/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. The P_{trend} represents a trend test in which the five categories of antibiotics use were included in the model as a continuous variable. CI = confidence interval.