Oral antibiotic use and risk of colorectal cancer in the United Kingdom, 1989-2012: a matched case-control study

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Summary

Background

Microbiome dysbiosis predisposes to colorectal cancer (CRC), but a population-based study of antibiotic exposure and risk patterns is lacking. In this study, oral antibiotic use on CRC incidence was assessed.

Methods

A matched case-control study (incident CRC cases and up to 5 matched controls) was performed using the Clinical Practice Research Datalink (CPRD; January 1, 1989 to December 31, 2012). Conditional logistic regression was used to assess CRC association with oral antibiotic use, adjusting for potential confounders. Antibiotic exposure in categorical and continuous terms (spline) was investigated for pattern of risk, stratified by specific tumor location.

Findings

28,980 CRC cases and 137,077 controls were identified. Oral antibiotic use was associated with CRC risk, but effects differed by anatomic location. Antibiotic use was found to be associated with excess risk of colon cancer in a dose-dependent fashion (P_{trend}<0·0001). The risk was observed after minimal antibiotic use, and was greatest in the proximal colon and with antibiotics with anti-anaerobic activity. In contrast, an inverse association was detected between antibiotic use and rectal cancers (P_{trend} =0·003), particularly with length of antibiotic exposure >60 days (Adjusted Odds Ratio [AOR], 0·85, 95% CI 0·79–0·93) as compared with no antibiotic exposure. Penicillins were associated with increased risk of colon cancer (AOR,1·09, [1·05-1·13]) whereas tetracyclines were associated with risk reduction for rectal cancer (AOR, 0·90, [0·84-0·97]). Significant

interactions were detected between antibiotic use and tumor location (colon vs rectum, $P_{interaction}$ <0·0001; proximal colon vs distal colon, $P_{interaction}$ =0·0194). The antibiotic-cancer association was found for antibiotic exposure occurring >ten years before diagnosis (AOR, 1·17, [1·06-1·31]).

Interpretation

Oral antibiotic use is associated with an increased risk of colon cancer risk but a reduced risk for rectal cancer. This effect heterogeneity may suggest differences in gut microbiota and carcinogenesis mechanisms along the lower intestine tract.

Funding
This project was funded by Johns Hopkins Fisher Center Discovery Program and Bloomberg~Kimmel Institute for Cancer Immunotherapy.

Word count:

Abstract: 297

Main text: 3545

Introduction

In 2010, the estimated global consumption of antibiotics was 70 billion individual doses, which equates to ten doses per person, and the annual rates continue to grow steadily. [1, 2] Given this widespread use of antibiotics, elucidating the effects of antibiotics on gut microbiota and links with health outcomes has substantial implications for public health. Use of antibiotics, even narrow spectrum antibiotics, exerts strong, persistent effects on the structure of the gut microbiota and impairs the integrity of intestinal barrier.[3, 4] Antibiotics allow for colonization of pathogenic microbes, [5, 6] and thus, may enable colonization with carcinogenic bacteria that induce local inflammation and tumor formation. Consistent with this hypothesis, recent data suggest differential pathogenic influences of the gut microbiota on neoplastic and immune cells along the colorectal continuum.[7]

Several epidemiological studies have suggested an antibiotic-cancer association. A Finnish cohort study reported increased cancer risk for several cancers, including prostate, breast, lung and colon. [8] In a diabetic Asian population, a positive association between colorectal cancer (CRC) and use of anti-anaerobic agents, but not anti-aerobic agents was observed. [9] Yet, a study from the Netherlands found both anti-anaerobic and anti-aerobic agents contributed to increased CRC risk.[10] The Harvard Nurses' Health Study revealed an association between long-term antibiotic use in early-to-middle adulthood (at age 20-39 and 40-59) and excess risk of subsequent colorectal adenomas,

with a stronger association observed for the proximal colon adenomas, and weaker or no association with distal colon or rectal tumors. [11]

However, there are several limitations to the current evidence: information about known CRC risk factors, such as body mass index (BMI), smoking, and alcohol use, was not consistently measured or adjusted for across studies; a lack of sufficient power to test associations by tumor anatomical location, or results combined colon with rectal tumors; effect modification between known risk factors for CRC and antibiotics on cancer risk was not examined; studies with participant self-reported antibiotic use were susceptible to recall bias and limited in information regarding distinct antimicrobial classes and effects on anaerobes/aerobes; and finally, non-linear associations between antibiotics and cancer risk rarely have not been studied.

Our aim was to investigate the associations between antibiotic use and site-specific colorectal cancer risk in the world's largest primary care database. We hypothesized that antibiotic use, which targets the gut microbiota, was associated with colorectal cancer initiation and progression. We explored whether these effects differed by anatomic location.

Methods

Study design and setting

We conducted a matched case-control study using data obtained from the Clinical Research Practice Datalink (CPRD) in the United Kingdom. The CPRD is one of the world's largest electronic medical record (EMR) databases of anonymized clinical records

with population-based data collected prospectively.[12] At the time of this study, CRPD incorporated longitudinal medical records of 11.3 million patients from 674 practices in the United Kingdom (UK), representing 6.9% of the UK population.[12] The details of each drug prescription, including dosage, instructions, and quantity, are automatically recorded in the computer and can be used to determine dose and duration of drug exposure. Large validation studies have suggested that data are of high quality for use in research.[13, 14]

Participants, exposures, and outcomes

The study population was drawn from all up-to-standard[1] practices in the CPRD cohort from January 1, 1989 to December 31, 2012 with a minimum of two years of follow-up. Study entry was the start of observation, defined as patient registration date of the up-to-standard follow-up. The date of observation end was defined as event date (CRC diagnosis date) both for cases and controls.

CRC cases were identified from the clinical or referral record using a set of previously validated READ codes (appendix table 1) which have a sensitivity of 92%, specificity of 99% and a positive predictive value of 98% for CRC.[15] Controls were defined as patients without a diagnosis of CRC recorded at any time in CPRD. Up to five controls were randomly selected and matched to each case on: year of birth (+/-3 years), gender, general practitioner (GP) practice site, and year of registration in the CPRD (+/- 1 year).

Our analysis was restricted to sporadic CRC. Thus, we excluded patients with conditions that predispose to CRC (inflammatory bowel disease [IBD], Peutz-Jeghers, Familial Adenomatous Polyposis [FAP] and Hereditary Nonpolyposis Colorectal Cancer [HNCC]).

[1] Up-to-standard follow-up is the period of good quality data from the practice.

We also excluded patients with immunosuppressive states, including human immunodeficiency virus (HIV) infection, organ transplant and chemotherapy/immunosuppressive drug use because they may impact the risk of colon carcinogenesis. We excluded patients with anal cancers because they most commonly are of squamous cell origin rather than epithelial cell origin of CRC. Age was restricted from 40 to 90 years. We only included oral antibiotic use since the impact of intravenous antibiotics on the gut microbiota is largely unknown. We applied the same exclusion criteria to the controls. For those patients who had an identifiable tumor location, tumors originating from the caecum, ascending colon, hepatic flexure, and transverse colon were classified as those of the proximal colon, whereas splenic flexure, descending, or sigmoid colon tumors were classified as those of the distal colon. Tumors in the rectum or at the rectosigmoid junction were classified as rectal location (appendix table 1).

We quantified antibiotic exposure by calculating the cumulative number of days they were prescribed, as well as the total number of prescriptions written during research-standard CPRD follow-up (from registration date to one year before CRC diagnosis). Antibiotics were categorized based on their action effects on aerobes/aerobes, as well as by drug class (cephalosporins, macrolides, penicillins, quinolones, sulpha/trimethoprim, tetracyclines, and others, appendix table 2). Timing preceding CRC was categorized as exposure occurring in the biologically plausible thresholds 1-10 years and >10 years.

Statistical analysis

Conditional logistic regression was used to estimate ORs and 95% CIs for the risk of incident CRC in relation to antibiotic use. In the main analysis, antibiotic exposure was

evaluated by number of days and by known therapeutic anaerobic effect (yes/no), and class of antibiotics across anatomic site (colon vs rectum; proximal vs distal colon), controlling for BMI, smoking status, alcohol use, history of diabetes diagnosis, and number of colonoscopies received. For time-varying covariates (BMI, smoking status and alcohol use), values were assigned using previously described methods [16] (i.e., use of the earliest records available during research-standard CPRD follow-up, or the most recent previous records if individuals lacked records at the beginning of research-standard follow-up; older values were dropped in a subsequent sensitivity analysis). We initially characterized antibiotic use in days as categorical terms (0 days, 1-15 days, 16-30 days, 31-60 days and >60 days) to quantify the average effect of each duration increase on CRC risk. To test for trend in the risk of CRC across different categories of length of prescription, we included the antibiotic categories as a continuous variable in the adjusted model. We then fitted fully adjusted models with a restricted cubic spline by taking the number of days of antibiotic use as a continuous term to assess possible non-linearity in any detected antibiotic-cancer association overall and by tumor location. Effect modification was evaluated by introducing interaction terms (one at a time) between antibiotic use and location (colon vs rectum, and proximal colon vs distal colon), as well as known risk factors for CRC including BMI, smoking status, alcohol use, and diabetes history to the full model. In order to examine the temporal relationship between CRC and antibiotic exposure, we analyzed antibiotic use with time preceding diagnosis of 1-10 years and >10 years among patients with at least 15 years of follow-up. For missing data on BMI, smoking status, and alcohol use, we employed multiple imputation to impute the missing data by using chained equations with ten imputed datasets assuming that BMI,

alcohol, and smoking were missing at random,[17] as well as by including missing values as a category in each variable in the fully adjusted model.[18]

In sensitivity analyses, we excluded CRC cases occurring within one year after registration to address concerns of possible prevalent cases at study entry, included only lifestyle factors recorded during follow-up period, included only antibiotic prescriptions more than 3 years prior to CRC diagnosis to guard against reverse causality (i.e., antibiotic use being affected by undiagnosed CRC), and assigned transverse colon cases to distal colon instead of proximal colon. In addition, we assessed how different categorization of antibiotic anti-anaerobic and anti-aerobic effects impact CRC risk. The categorizations assessed were (appendix table 3): 1) a restricted list of agents with predominant anti-anaerobic or anti-aerobic effects, 2) exclusion of vancomycin from the anaerobic antibiotic list, 3) categorization of cephalosporins as anti-anaerobic antibiotics, 4) categorization of ampicillin/amoxicillin as anti-aerobic antibiotics, 5) exclusion of cephalosporins from the anti-aerobic antibiotic list, and 6) assessment of amoxicillin, the most commonly used oral antibiotic, as an anti-aerobic antibiotic agent, to test if the impact of amoxicillin alone on the results.

This study was approved by the Institutional Review Board of the Johns Hopkins University. Statistical analyses were performed using Stata/MP 15. 1 (StataCorp, College Station, TX). All tests of significance used two-sided p-values at the $p<0.05$ level.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

In the CPRD, 28,980 eligible CRC cases and 137,077 matched controls were identified using the selection algorithm (Figure 1). Participants with CRC were more likely to be overweight (35·2% vs 33·8%) and obese (18·6 vs 16·4%), to have history of smoking (49·9% vs 46·9%), have moderate-heavy alcohol use (13·8% vs 11·4%) , have diabetes history (8·8% vs 7·7%), and to undergo colonoscopy (3·5% vs 2·9%), and less likely to have chronic NSAID use (7·2% vs 9·0%) compared to the control group. As compared to participants with rectal cancer, those with colon cancer were more likely to be female (46·9% vs 39·2%), overweight-obese (44·9% vs 41·5%), smokers (49·3% vs 46·9%), alcohol users (74·0% vs 72·6%), have a diabetes history (9·1 % vs 8·2%), and to undergo colonoscopy (3·9% vs 2·6%) compared to those with rectal cancer (Table 1 and Appendix Table 4).

Participants had a median follow-up of 8·1 years (Interquartile range [IQR], 4·9-12·3 years), Antibiotics had been prescribed to 20,278 (70·0%) CRC cases and 93,862 (68·5%) controls (P<0·001). Participants who subsequently developed colon cancers were more likely to be exposed to antibiotics as compared with controls (71·3% vs 69·1%, P<0·001), whereas participants with rectal cancers had comparable exposure to antibiotics (67·1% vs 67·2%, P=0·96). Both anti-anaerobic antibiotics and anti-aerobic

antibiotics were more likely to be prescribed in cases who subsequently developed colon cancer cases relative to controls, yet rectal cancer cases had less anti-aerobic antibiotic exposure than controls (Table 2). Of those with known CRC location, participants with proximal colon cancers were more likely to have antibiotic exposure, particularly those with anti-anaerobic effects, whereas participants with distal colon cancers had similar antibiotic exposure compared to controls, regardless of antibiotic spectrum (appendix Table 5). The most common prescriptions for antibiotics were penicillins (80·7%), macrolides (30·4%), sulpha and trimethoprim (28·9%), cephalosporins (25·1%), tetracyclines (20·3%) and quinolones (14·3%). Most participants (59·5%) were exposed to more than one antibiotic class. By antibiotic class (any vs. none), participants with colon cancers had increased use of cephalosporins and quinolones, whereas those with rectal cancers received fewer prescriptions for tetracyclines and macrolides (Table 2).

Use of any antibiotics was associated with CRC risk compared with non-use, but the effect size and pattern across antibiotic exposure category differed by tumour location (Figure 2 and Figure 3). In the colon, excess risk was observed with increased use of antibiotics in a dose-dependent fashion, with no use as the reference (1-15 days/16-30 days/31-60 days/>60 days vs non-users, AORs: 1·08 [1·04–1·13]/1·14[1·08–1·20]/1·15[1·09–1·22]/1·17[1·10-1·23]; P_{trend}<0·0001), adjusted for BMI, smoking, alcohol use, diabetes status, chronic NSAID/aspirin use, and number of colonoscopies. The effect of antibiotic use on cancer risk was more marked in the proximal colon (1-15 days/16-30 days/31-60 days/>60 days vs non-users, AORs: 1·14 [1·02–1·28]/1·15[1·01–1·32]/1·32[1·15–1·51]/1·09[0·94-1·25]; P_{trend}= 0·046), whereas no association was

observed for each of the exposure categories on distal colon cancer risk (all P>0·10, P_{trend}= 0·40). In the rectum, use of antibiotics showed an inverse association with CRC, with a maximum risk reduction of 15% for antibiotic use exceeding 60 days, as compared with non-use (AOR, 0·85, 95% CI 0·79–0·93, P_{trend}=0·003, Figure 2).

Antibiotics with anti-anaerobic action properties were associated with an increased colon cancer risk (P_{trend}<0·001), particularly in the proximal colon (Figure 4, appendix Figure 1, and appendix Figure 2). An inverse association with antibiotic exposure was observed for rectal cancer, regardless of effects on anaerobes (agents with anti-anaerobic activity, P_{trend} =0.015, agents with anti-aerobic activity, P_{trend} =0.005). By antibiotic class, use of penicillins was associated with an increased colon cancer risk (AOR 1·09, [1·05–1·13]), particularly in the proximal colon, but not cephalosporins, quinolones, macrolides, or sulpha/trimethoprim (appendix Figure 1). In contrast, tetracycline use decreased rectal cancer risk (AOR, 0·90, [0·84-0·97], appendix Figure 1).

Figure 5 shows the adjusted non-linear pattern of antibiotic-cancer association by tumor location. For colon cancers, exposure to antibiotics was associated with substantially increased risk, with the effect increased after minimal antibiotic use and plateaued after 60 days of cumulative exposure. Antibiotics were associated with an increased proximal colon cancer risk at minimum exposure levels but antibiotics were not associated with risk of distal colon cancer. For rectal cancer, there was a reduced risk of cancer associated with cumulative exposure to any antibiotic; however this effect was not observed until after 30 days of cumulative exposure and plateaued after 90 days (Figure 5). Significant

interaction was found between antibiotic use and tumor location (colon vs rectum) ($P_{interaction}$<0·0001), as well as between antibiotic use and location in the colon (proximal colon vs distal colon) ($P_{interaction}$ =0·0194). No interactions were observed between the use of antibiotics and known risk factors for CRC (BMI, smoking status, alcohol use, and diabetes history, all P>0·10) at each anatomical location.

Limiting the analysis to participants with at least 15 years of follow-up, use of antibiotics for more than 10 years before the cancer diagnosis was associated with an increased colon cancer risk (AOR, 1·17, [1·06-1·31]) as compared to non-antibiotic users, whereas antibiotic use 1-10 years before cancer diagnosis was not associated with colon cancer risk (AOR, 1·00, [0·89-1·10]); no association was found between use of antibiotics and rectal cancer risk by time window (>10 year users vs non users, AOR: 0·98, [0·84-1·13]; 1-10 year user vs non users, AOR:0·93, [0·91-1·09]).

Our main findings were robust to a range of sensitivity analyses (appendix Tables 6-9). When ampicillin/amoxicillin was considered as a primarily anti-aerobic antibiotic, the effects from anti-anaerobic and anti-aerobic agent exposure on colon cancer risk inverted, (appendix Figure 2-3) suggesting this class of antibiotics was the dominant contributor to the outcomes presented.

Discussion

In this largest analysis of antibiotic-CRC association to date, we demonstrated that use of oral antibiotics associated with CRC risk, but the effect size and pattern of risk varied

by anatomical location in the colorectum. A dose-dependent increase in colon cancer risk was observed for any antibiotic use. This positive association was driven by anti-anaerobic antibiotics and was limited to the proximal colon, with risk increased after minimal antibiotic use. However, a reduced cancer risk from antibiotic exposure was shown in the rectum. Penicillin exposure was strongly associated with increased colon cancer risk, whereas an inverse association was found with rectal cancer for tetracyclines. The association between antibiotic exposure and colon cancer was seen in participants with required antibiotic exposure more than ten years prior to CRC detection.

Several studies suggest an increased risk for CRC or colorectal adenoma from antibiotic use.[8, 10, 11, 19] Our results add to this evidence base by systematically investigating the dose-response relationships by tumor location, antibiotic action against anaerobes/aerobes, and by antibiotic classes. These analyses, which examined linear and non-linear effects along with evaluation of effect modification by known risk factors for CRC, enhance the understanding of the antibiotic-cancer association.

Results from this study were broadly consistent with previous reports (appendix table 10). We extended previously reported antibiotic-colon cancer associations by observing that the dose-response pattern is non-linear and can increase with even a single antibiotic course but plateaus after 60 cumulative days. Consistent with previous studies, penicillins were associated with significantly increased colon cancer risk.[10, 19] We additionally demonstrated that a penicillin-cancer association was only detected in the proximal colon.

We uniquely showed a protective role of antibiotics for rectal cancer, especially tetracyclines. This contrasts with those reports of a null or positive association, which could have been due to limited sample size and combined analyses of rectal cancers with colon cancers.[9, 11] Several studies have reported the anti-inflammatory effects of tetracyclines and their potential anti-neoplastic role. [20, 21] However, this association needs to be confirmed in other large cohorts and through mechanistic studies for additional validation.

In our study, the effect on CRC risk was most significant after oral exposure to anti-anaerobic agents, which markedly disrupt the microbiota organization and structure in the colon since the gut microbiota is predominately composed of anaerobes. It is possible that the disrupted microbiota enables acquisition or development of a carcinogenic colon microbiota. Previous data have suggested that not only select bacteria but a select consortium may contribute to colon carcinogenesis.[7, 22-24]

While the exact mechanism of differential antibiotic-cancer association by anatomic location is unknown, it is possible that putative carcinogenic bacteria, such as *Fusobacterium, Porphyromonas, Enterococcaceae*, and *Bacteroides–Prevotella*, as well as toxin-producing species including some *B. fragilis* and *E. coli*, may be differentially distributed along the colorectal tract or that colon epithelial cells display differing regional sensitivity to the microbiota. For example, *Fusobacterium nucleatum*, that is associated with a subset of CRC, was reported in one study to decrease in a gradient fashion from cecum to rectum, with proximal colon cancers harboring the highest *F. nucleatum* levels.[7]

A recent meta-analysis using high-resolution bacterial 16S rRNA gene profiling did not confirm this pattern, but did identify an association between a small consortium of bacteria (a symbiont with capacity for tumorigenesis [*Bacteroides fragilis*], and oral pathogens including *Fusobacterium nucleatum*, *Parvimonas micra*, *Gemella morbillorum*) and CRC risk.[24] CRC tissues were enriched for polymicrobial invasive biofilms, particularly on right-sided tumors.[24] Thus, the greater antibiotic impact on the proximal colon may reflect disruption of biofilm formation that have been linked with procarcinogenesis.[23, 25] Additionally, the emergence of data showing differential frequencies of tumor molecular characteristics, subsite-specific clinicopathological differences, and response to treatment implies that the development of proximal colon, distal colon and rectal cancers differ mechanistically. [26]

There are several strengths to a study of this breadth. This is the first use of the CRPD to facilitate compiling the largest CRC cohort with matched cases and controls and available high-quality data on the use of antibiotics, lifestyle factors (BMI, smoking, alcohol use), comorbidity as well as other medications. We were able to investigate the relationships between antibiotics and CRC with greater precision and power than has been previously described, controlling for potential confounders. Second, in the UK, registration with a primary care GP is essentially universal (> 98%), enabling prospective and unbiased collection of longitudinal clinical data in CPRD, with almost all (99·7%) prescriptions recorded.[27] Data from CPRD are largely representative of the UK population and results are thought to be generalizable to the UK population and to comparable countries. Third, we systematically examined the possible non-linearity and effect modification of an antibiotic-cancer association and provide additional evidence for microbiota causality in

CRC development. Further, availability of information regarding the cancer location, dose and classes of antibiotics provided insights to the heterogeneity of the antibiotic-cancer association and implied differences in microbiota mechanisms along the GI tract.

There are several limitations for our study. First, potential residual confounding may still exist despite adjustment for known risk factors associated with CRC. Second, missing data for life-style factors ranged from 10~20%. However, we employed two approaches to deal with missingness, and obtained consistent results. Third, data not captured in CPRD include prescriptions in secondary care, prescriptions filled, and treatment adherence. Certain patient groups are missing from primary care records, such as prisoners, private patients, some residential homes and the homeless, and we excluded individuals with immune-deficient conditions; thus interpretation with respect to those populations is limited. In addition, CPRD does not have information on food intake, physical activity and family history that may influence CRC risk. Fourth, the number of patients with recorded Read codes specifying whether the colon cancer was located in the proximal vs distal colon was limited; thus, analyses in these anatomical groups may be underpowered and results should be interpreted with caution. Lastly, misclassification of antibiotic effect on anaerobes and by antibiotic class may exist. To test these associations, we employed several sensitivity analyses comparing therapeutic anti-anaerobic and anti-aerobic antibiotics and analyzed how specific drugs may change/drive the effect size and pattern and results showed consistency with our main analysis.

In conclusion, our results provide evidence supporting a potential causal relationship between antibiotic-induced microbiome dysbiosis, which may influence subclinical

mucosal inflammation, and colon tumor formation. Substantial heterogeneity in the magnitude and pattern of antibiotic effects exist anatomically in the colon consistent with different microbial-driven mechanisms of cancer pathogenesis occurring along the GI tract. More clinical and translational studies are warranted to test the interplay of antibiotics of different activities and class on the colonic anaerobic and aerobic microbiota and mechanisms of carcinogenesis.

Contributors

All authors were involved in the study design. JZ and CH did the literature search and conducted the statistical analysis. JZ, CH, SC, KG and CS contributed to interpretation of data. JZ, CH and CS wrote the first draft. All authors contributed to further drafts and approved the final manuscript.

Declaration of Interests (COI)

To be added. All other authors have nothing to declare.

Acknowledgements

The authors thank Dr. Krishnan Bhaskaran for his suggestions on characterization of life style factors in the CRPD and for generously sharing codes facilitating our non-linearity modeling analyses. AW is supported by CRUK Pioneer Award Grant 22645.

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