Full title: Antibiotic exposure and the risk of colorectal adenoma and carcinoma: systematic review and meta-analysis of observational studies.

Short title: Antibiotic exposure and the risk of colorectal adenoma and carcinoma

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Abstract

Background

Colorectal cancer (CRC) incidence is increasing and evidence suggests that bowel microbiome mala-daptation may be associated with colorectal carcinogenesis. Antibiotic consumption may cause bowel microbiome imbalance but research assessing an association between antibiotic exposure and CRC is inconsistent. The aim of this systematic review and meta-analysis was to appraise and synthesise the available evidence.

Methods

MEDLINE, EMBASE and CINAHL databases were searched for published observational studies. We included eight studies of 3,408,312 patients. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) for the odds of CRC following antibiotic exposure were estimated. Sensitivity analyses were performed according to exposure definition, study design and risk of bias.

Results

A weak association between antibiotic exposure and CRC was demonstrated when exposure was assessed cumulatively by the number of prescriptions (OR 1.204, 95% CI 1.097-1.322, p <0.001) or duration of antibiotic exposure (OR 1.168, 95% CI 1.087-1.256, p <0.001). Antibiotic exposure assessed as a binary variable demonstrated no association with CRC.

Conclusion

The findings suggest a weak association between cumulative antibiotic consumption and risk of CRC but no causal conclusions can be made. Limitations include the heterogeneity and quality of the available research, particularly with regard to measurement of antibiotic exposure.

What does this paper add to the literature?

This systematic review synthesises and appraises the current evidence for a potential association between antibiotic exposure and CRC. Specifically, it highlights limitations in the available research that should be addressed in future research.

1. Introduction

The incidence of colorectal cancer (CRC) is increasing worldwide. In 2012, it was the third most common cancer globally and second most common in Europe (1). Recognized risk factors include family history, body mass index, smoking, diet, and inflammatory bowel disease (IBD) (2). Recent studies suggest that antibiotic exposure may also be a risk factor for CRC, increasing the risk of carcinogenesis through bowel microbiome imbalance (3-13). The bowel microbiome is a diverse composition of approximately 100 trillion micro-organisms that play a role in digestion, the immune system and protection from pathogenic organisms (11, 14-16). Maladaptation of the bowel microbiome may relate to the development of CRC through several mechanisms, including chronic inflammation, altered effects on the local immune system or toxins and carcinogenic metabolites released by the bowel flora (11, 14, 15, 17).

Antibiotic use can affect the bowel microbiome by reducing the diversity of the gut flora within a day of starting antibiotics and this may persist for a prolonged period (18-23). This effect differs between individuals and depends on antibiotic class, route of administration and duration of use (18-21, 24). A causal relationship between antibiotic consumption and CRC would be of considerable concern given the increasing rates of antibiotic use within Europe, especially within the elderly where CRC incidence is higher (22, 23, 25). However, current research investigating an association between antibiotic exposure and colorectal carcinoma has been inconclusive and inconsistent (3-10).

A systematic review and meta-analysis of observational studies was performed to appraise and synthesise published studies investigating the relationship between antibiotic exposure and incident colorectal adenoma and carcinoma. The aim was to assess whether the use of antibiotics is associated with the development of pre-cancerous or cancerous lesions in adults.

2. Methods

This was a systematic review and meta-analysis of observational studies. A protocol was prospectively registered with the International prospective register of systematic reviews (PROSPERO) database (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=79979). The Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines were used for reporting(26).

Search Strategy and Study Eligibility

A comprehensive electronic literature search strategy was constructed using both medical subject heading (MeSH) terms and free text search terms relating to antibiotics and colorectal adenomas or carcinomas. The search strategy was created by three investigators (LS, NO and UN) and key words selected by two clinicians (LS and NO). The search was conducted by LS using Ovid®, Wolters Kluwer online search tool. Three electronic databases were searched: MEDLINE, EMBASE and CINAHL. The search strategy was produced initially in accordance with MEDLINE (see supplementary figure S1), then adapted to the other databases. There were no restrictions in terms of language or date. Hand searching of references of relevant studies was also undertaken. The initial search was undertaken on the 23rd October 2017 and repeated on the 25th June 2018.

Inclusion criteria were published case-control or cohort studies from primary and secondary care that investigated the association between antibiotic exposure and incident colorectal adenoma or carcinoma in adults over 18 years of age. Randomised control trials, case series and case reports were

excluded. The review protocol stated that studies enrolling patients less than 30 years old, patients with IBD or patients with a genetic predisposition to CRC were to be excluded. However it was subsequently decided to include these studies because these individuals only comprised a relatively small proportion of the included population. Sensitivity analyses were conducted to assess the impact these studies had on the overall risk estimates. Potentially eligible studies were extracted and organised in EndNote software, Thompson Reuters. Two investigators (LS and NO) independently reviewed the titles and abstracts. Eligible studies at this stage underwent a full text paper review against our inclusion and exclusion criteria by three authors (LS, NO and HA).

Data extraction

For each eligible observational study, data extraction was performed by two reviewers independently (LS and NO). Data were collected on the primary author, date of publication, type of study, the country and setting of the study and duration of follow-up. Main study data extracted were patient demographics, sample size, number of cases and controls in case-control studies and the number of exposed and unexposed participants in cohort studies, which antibiotic or antibiotics were assessed, and the primary outcome — incident colorectal adenomas or carcinomas. These data were collected for the meta-analysis but also to assist with sensitivity analyses. Data on subject inclusion and exclusion criteria were recorded in order to assess for potential confounding and limiting factors. Extra information was requested from three authors for the meta-analysis, correspondence was received from one author initially and a second after the initial meta analysis was conducted.

Risk of bias and study quality assessment

Studies were assessed for methodological quality and risk of bias using the Effective Public Health Practice Project (EPHPP) quality assessment tool(27). EPHPP assesses quantitative studies based on a number of components resulting in either a weak, moderate or strong rating as well as an overall

global rating of study strength. This assessment was performed independently by two reviewers (LS and NO). Disagreements were discussed and resolved by a third reviewer (HA).

Data analysis and synthesis

Data were pooled statistically and meta-analyses conducted on available outcomes using a random-effects model. All analyses were undertaken and forest plots created using Comprehensive Meta-Analysis software (version 3). Results were expressed as Odds Ratios (OR) with 95% confidence intervals (95% CI) for dichotomous outcomes. In some cases, relative risks (RR) were used in the meta-analyses, as they are interchangeable and a good estimate of OR when the disease or outcome is rare in the population (typically prevalence less than 10%) as is the case in CRC or colorectal adenomas(28, 29).

Mean effect sizes (MES) were estimated for different types of antibiotic exposures from the same studies. This integrative approach is characterised by the inclusion of multiple effect sizes per study and is a novel approach in dealing with effect size multiplicity in systematic reviews and meta-analyses (30).

Higher antibiotic exposure was categorised as more than 6 courses, reflecting definitions in 4 studies. Where antibiotic duration was expressed as days of use, we defined more than 2 months as 'higher use'. The rationale for this was that most antibiotics are prescribed for respiratory tract infections with course durations of 6-10 days equating to about 60 days of use if 6 courses were prescribed as per the previous definition (31, 32).

Sensitivity analyses were performed according to age, inclusion of patients with IBD or diabetes and risk of bias for the key review findings. These were undertaken as patients with CRC under the age of 30 potentially may be more likely to have a genetic predisposition and those with diabetes or IBD are at an increased risk of CRC compared to the general population. We also did a post-hoc sensitivity

analysis excluding the study by Friedman et al (9). The published study methods suggested that this study only included patients more than 20 years old. However, later contact with the author revealed the study did not restrict based on age and included patients <18 years. Therefore, sensitivity analyses were undertaken to assess its impact.

Statistical tests for heterogeneity were performed and assessment undertaken for evidence of publication bias graphically using Funnel plots and statistically using Egger's test (33).

For outcomes for which it was not possible to produce a meta-analysis, data was narratively synthesized. No subgroup analyses were conducted.

3. Results

Study characteristics

Eight studies were included in this systematic review. Three were cohort studies (4, 6, 7) and five were case-control studies (3, 5, 8-10), of which four were nested case-control studies (3, 5, 9, 10) (Figure 1). Detailed study characteristics and results are shown in table 1 and supplementary figure S2 respectively.

PRISMA 2009 Flow Diagram(34)

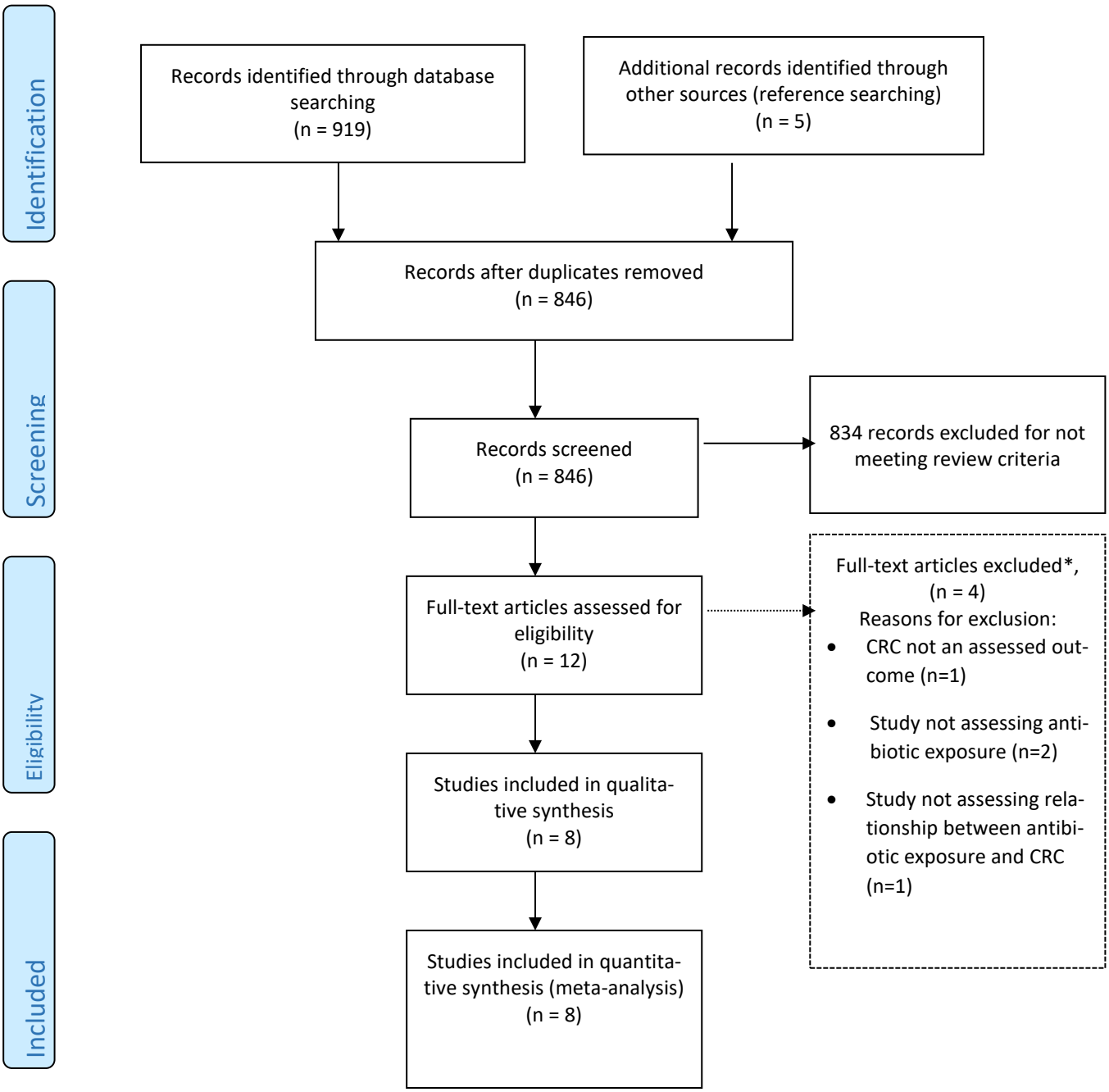

Figure 1. PRISMA flow diagram of results of database literature searching. *Excluded references (35-38).

Reference	Country	Population	Intervention	Comparison	Outcome	Study Design	Sample size	Duration from antibiotic exposure to CRC/adenoma diagnosis	Main indication for antibiotic exposure
Boursi et al, 2015 "Impact of antibiotic exposure on the risk of colorectal cancer"	U.K.	The Health Improvement Network (THIN). Inclusion criteria - aged >40 years. Exclusion criteria - IBD, CRC syndromes and incomplete records. Sex - 55.1% male in cases and controls	Multiple Antibiotic classes *	Four controls matched according to age, sex, GP practice site and duration of follow up	Colorectal cancer	Nested Case control	103,044	not restricted in main study results (Median follow up 6.5 years)	Most common indication was respiratory tract infection
Cao et al, 2017 "Long-term use of antibiotics and risk of colorectal adenoma"	U.S.A.	Nurses' Health Study (NHS) - Female nurses only. Inclusion criteria – aged ≥ 60 in 2004, reported history of antibiotic exposure and at least one colonoscopy between 2004-2010. Exclusion criteria – UC/CRC or polyp before 2004	Antibiotics in general	Control within same 2-year period as cases with a normal colonoscopy	Colorectal adenoma	Cohort	16,642	not restricted	not stated

Dik et al, 2015 "Frequent Use of Antibiotics Is Associated with Colorectal Cancer Risk: Results of a Nested Case-Control Study"	Netherlands	Achmea Health Database. Inclusion criteria – aged >18 years at CRC diagnosis between 2006-11. Exclusion criteria – IBD, < 6 years follow-up. Sex – 47.1% male in cases and controls	Multiple Antibiotic classes **	Four controls matched according to sex and date of birth	Colorectal cancer	Nested Case control	20,017	1-6 years	not stated
Falagas et al, 1998 "Late Incidence of Cancer After Metronidazole Use: A Matched Metronidazole User/Nonuser Study"	U.S.A.	Group Health Cooperative (GHC). Inclusion criteria – metronidazole script issued between 1st Jan 1975 – 31st December 1983 for exposure group. Age >18 years. Exclusion criteria not stated. Sex proportions not stated	Metronidazole	Controls matched for age, gender and year of enrolment in GHC	Colorectal cancer	Cohort	10,444	> 7 years	not stated

Friedman et al, 1998 "Drugs and colon cancer"	U.S.A.	Kaiser Permanente Medical care programme, Utah residents and Minnesota. Inclusion criteria –CRC aged 30-79 between 1st Oct 1991 – 30th Sept 1994. Exclusion criteria – not black/white or Hispanic ethnicity, 'not mental competent' to complete interview, IBD or FAP. Sex proportions not stated	Penicillin (and other drugs)	Controls matched according to sex and 5-year age group	Colorectal cancer	Case control	4403	'about 2 years'	not stated
Friedman et al, 2009 "Epidemiologic evaluation of pharmaceuticals with limited evidence of carcinogenicity"	U.S.A.	Kaiser Permanente Medical care programme. Inclusion criteria – CRC diagnosed between Aug 1994 – June 2006. Age not restricted. Exclusion criteria and age range not stated. Sex proportions not stated	Metronidazole (and other drugs)	Ten controls matched according to sex, year of birth and year of starting drug	Colon cancer	Nested Case control	113,278 [A1]	≥ 2 years	not stated

Kilkkinen et al, 2008 "Antibiotic use predicts an increased risk of cancer"	Finland	Finland Population register, linkage with Finnish Cancer and Drug prescription Registries. Inclusion criteria – aged 30-79 and resident in Finland on 1st Jan 1995. Exclusion criteria – diagnosed with CRC with 1953 and 1997 or died between 1st Jan 1995 and 31st Dec 1997. Sex proportions not stated	Antibiotics in general	Not stated	Colon and rectal cancer	Cohort	3,112,624	not restricted [B1]	not stated

Wang et al, 2014 "Infection, antibiotic therapy and risk of colorectal cancer: A nationwide nested case-control study in patients with Type 2 diabetes mellitus"	Taiwan	Taiwan National Health Insurance (NHI), Diabetic cohort. Inclusion criteria – Type 2 Diabetes between 1st Jan and 31st Dec 2000. Exclusion criteria – aged <30 or >100 years, died before 1st July 2000, potential Type 1 Diabetes, history of IBD or CRC, colon diverticulosis diagnosed 1 year before CRC, <1year between antibiotics and CRC diagnosis. Sex – colon cancer, 54.02% male in cases and controls; rectal cancer 56.34% in cases and controls	Antibiotics in general	Up to four controls, matched according to sex, age (within 5 years) and follow up duration	Colon and rectal cancer	Nested Case control	27,860	> 1 year [C1]	not stated

Table 1. Detailed Study Characteristics

* Antibiotic classes included Penicillin, Cephalosporins, Macrolides, Tetracyclines, Sulphonamides, Quinolones, Nitroimidazoles

** Antibiotic classes included Penicillin, Macrolides, Tetracyclines, Sulphonamides and trimethoprim, Quinolones, Nitrofurantoin derivatives

[A1]Exact number of controls not stated - approximate value based on paper statement that 10 controls were matched to each case.

[B1]Study states that limiting the result to those with at least 5 years follow did not significantly affect the results.

[C1]Mean duration from exposure to CRC was 1,424 days for colon cancer and 1,397 days for rectal cancer

Risk of bias and quality assessment

Six studies scored a moderate rating for quality and risk of bias according to the EPHPP. Two studies achieved a strong global rating (3, 8) (Table 2).

Study	Study Design	Selection bias			Study design			Confounders			Blinding			Data collection method			Withdrawals and dropouts				Global rating		
		S	M	W	S	M	W	S	M	W	S	M	W	S	M	W	S	M	W	NA	S	M	W
Boursi, 2015	Nested Case-control		X			X			X			X		X						X	X		
Cao, 2017	Cohort Study		X			X		X				X			X				X			X	
Dik, 2015	Nested Case-control		X			X				X		X			X					X		X	
Falagas, 1998	Cohort		X			X				X		X			X					X		X	
Friedman, 1998	Case-control		X			X		X				X			X					X	X		
Friedman, 2009	Nested Case-control		X			X				X		X			X					X		X	
Kilkkinen, 2008	Cohort		X			X				X		X			X					X		X	
Wang, 2014	Nested Case-control		X			X				X		X			X		X					X	

Table 2. Methodological quality and risk of bias assessment using the Effective Public Health Practice Project (EPHPP) tool.
Key for study ratings, S – strong, M – moderate and W – weak, NA – not applicable.

Antibiotic exposure and risk of adenoma or CRC

Effect of any antibiotic exposure

FIGURE 2 HERE

Fig 2. Odds Ratios (OR) of developing CRC or adenomas with any antibiotic exposure.
Forest plot of the odds of developing CRC with any antibiotic exposure. **Study relative weighting:** Cao 2018 - 21.32%, Dik 2016 - 22.64%, Falagas 1998 - 4.46%, Friedman 1998 - 13.80%, Friedman 2009 - 17.27%, Wang 2014 - 20.50%**. Key** * mean effect size (MES). See supplementary figures S3-5 for MES plots.

Six studies reported associations between any antibiotic use and incident colorectal adenoma or carcinoma. Any antibiotic use was defined as any prescription for antibiotics during the study period. Meta-analysis of these studies showed that antibiotic exposure defined in this manner was not significantly associated with incident CRC (pooled odds ratio (OR) 1.058, 95% CI 0.913-1.225, p=0.453). Restricting the analysis based on study participants age, study quality (moderate quality, low risk of bias), and exclusion of studies including participants with IBD or type 2 diabetes did not significantly affect our estimates. Removing the study by Friedman et al (9) resulted in an increase in the OR to 1.127 (95% CI 0.992-1.280) but the association remained statistically non-significant (p=0.066).

Effect of higher antibiotic exposure

FIGURE 3 HERE

Fig 3. Odds Ratios (OR) of developing CRC or adenomas in patients with higher antibiotic exposure.
Forest plot of the odds of developing CRC or adenomas with higher antibiotic exposure. **Study relative weighting:** Boursi 2015 - 35.47%, Cao 2018 - 13.45%, Dik 2016 - 23.44%, Kilkkinen 2008 - 27.64%. **Key** - Boursi 2015 = > 10 course of antibiotics, Cao 2018 = > 2 months of antibiotics, Dik 2016 = > 8 courses of antibiotics, Kilkkinen 2008 = > 6 course of antibiotics. * mean effect size (MES). See supplementary figures S6-8 for MES plots.

Four studies reported associations between stratified antibiotic exposure and incident CRC or adenomas with comparable higher antibiotic exposures. Higher antibiotic exposure was defined as more than 6 courses during the study period (range — more than 6 to more than 10 courses). More than 2 months duration of antibiotics was included within this analysis as it was comparable to the course ranges described above. Meta-analysis found that high antibiotic exposure, as described above, was associated with an increased odds of CRC (pooled OR 1.204, 95% CI 1.097-1.322, p=0.000). Restricting the analysis, as described with the previous outcome, did not affect our estimates.

More *Prolonged duration of antibiotic exposure*

FIGURE 4 HERE

Fig 4. Odds Ratios (OR) of developing CRC or adenomas in patients with more prolonged antibiotic exposure.
Forest plot of the odds of developing CRC with more prolonged antibiotic exposure. **Study relative weighting:** Boursi 2015 - 74.82%, Cao 2018 - 1.42%, Dik 2016 - 23.76%. **Key** - Boursi 2015 = > 56 days duration, Cao 2018 = > 2 months of antibiotics, Dik 2016 = > 70 days duration. * Mean effect size (MES). See supplementary figure S9 for MES plots.

Three studies reported cumulative duration of antibiotic exposure and incident colorectal adenoma or cancer, with comparable more prolonged duration categories. These studies assessed cumulative exposure as opposed to courses of potentially variable duration. More prolonged duration was analysed (range — more than 56 days to more than 70 days of antibiotic exposure). Meta-analysis of these

studies demonstrated that more prolonged antibiotic exposure was associated with an almost 17% increased odds of CRC (pooled OR 1.168, 95% CI 1.087-1.256, p=0.000). Again restricting the analysis did not affect our estimates.

Risk of Bias across studies

The funnel plot suggests there is potentially publication bias based on asymmetry (see supplementary figure S10). However the Eggers regression intercept (Intercept =-1.471, p=0.520) suggests no publication bias. This must be interpreted with caution however as its use with less than 10 studies leads to a reduction in its power(39).

GRADE Evaluation of Certainty of Findings

'Summary of findings tables' were created for primary outcomes (see tables 3-5). Quality of evidence was assessed for each outcome using the five GRADE criteria (GRADEpro GDT) (39, 40). Decisions and justifications to down — or upgrade the quality of studies are documented within footnotes.

Based on the GRADE certainty of evidence assessment, for the three exposures studied, the subsequent risk of developing CRC and adenomas has a very low certainty of evidence.

Question: Does any antibiotic exposure increase the risk of developing colorectal cancer and adenomas?
Setting: Inpatient and outpatient
Bibliography: Cao et al, Dik et al, Falagas et al, Friedman et al 1998, Friedman et al 2009 and Wang et al

Certainty assessment							№ of patients		
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Antibiotic exposure	No Antibiotic exposure	
Development of Colorectal Cancer or Adenomas									
6	observational studies	serious [a]	very serious [b]	not serious	serious [c]	publication bias strongly suspected [d]	Unable to calculate due to missing raw data	Unable to calculate due to missing raw data	(0

Table 3. GRADE Assessment of whether antibiotic exposure increases the risk of developing CRC or adenomas. Please note that the risk of b

CI: Confidence interval; **OR:** Odds ratio

Explanations

a. One of the studies (Friedman et al 1998) had a low risk of bias, whereas the remaining five were high risk of bias due confounding factors and withdrawals and follow up. Please see Study Quality assessment for further details of risk of bias for each study.
b. Downgraded on inconsistency due to a high I_2 value of 79% and a large Chi Squared value.
c. Downgraded due to wide confidence intervals, despite large sample size.
d. Funnel plot shows asymmetry suggestive of publication bias.

Question: Does higher antibiotic exposure increase the risk of developing colorectal cancer or adenomas?
Setting: Inpatient and Outpatient
Bibliography: Boursi et al, Cao et al, Dik et al and Kilkkinen et al

Certainty assessment							№ of patients		
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	High antibiotic exposure	No antibiotic exposure	Re (95
Colorectal cancer or adenoma									
4	observational studies	serious [a]	serious [b]	not serious	not serious	publication bias strongly suspected [c]	Unable to calculate due to missing raw data	Unable to calculate due to missing raw data	**OR** (1.087

CI: Confidence interval; **OR:** Odds ratio

Table 4. GRADE Assessment of whether higher antibiotic exposure increases the risk of developing CRC or adenomas. Please note that the risk of bias was asses

Explanations

a. Downgraded because apart from Boursi et al, the other studies had high risk of bias due to confounding factors.
b. Downgraded due to a relatively elevated I_2 of 62% and high Chi Squared value.
c. Unable to formally assess publication bias as too few studies but strongly suspected based on fact it was strongly suspected in the above assessment (see table 3)

Question: Does more prolonged antibiotic exposure increase the risk of developing colorectal cancer or adenomas?
Setting: Inpatient and outpatient
Bibliography: Boursi et al, Cao et al and Dik et al.

Certainty assessment							№ of patients		
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Prolonged Antibiotic Exposure**	**No Antibiotic Exposure**	
Development of Colorectal cancer or adenoma									
3	observational studies	serious [a]	not serious	not serious	not serious	publication bias strongly suspected [b]	Unable to calculate due to missing raw data	Unable to calculate due to missing raw data	(1

Table 5. GRADE Assessment of whether more prolonged antibiotic exposure increases the risk of developing CRC or adenomas. Please note

CI: Confidence interval; **OR:** Odds ratio

Explanations

a. Downgraded as Cao et al is at high risk of bias due to withdrawal and drop-out rates whereas Dik et al is at high risk of bias due to confounding factor bias.
b. Unable to formally assess publication bias as too few studies but strongly suspected based on fact it was strongly suspected in the above assessment (see table 3)

4. Discussion

Summary of findings

This systematic review found that antibiotic exposure, assessed as a binary variable, had no significant association with colorectal adenoma or carcinoma. However, when antibiotic exposure was categorised using cumulative measures, such as individuals exposed to more than 6 courses of antibiotics or more than 2 months duration of treatment, they had a relatively small increased odds of developing colorectal adenoma or carcinoma. However, the observed association should be interpreted with caution due to the small effect size and potential for bias and confounding.

Of the studies included in this systematic review, four found an association between antibiotic exposure and CRC (3-6). Studies by Boursi et al and Dik et al categorised antibiotics according to class and demonstrated a dose dependent increase in CRC risk with penicillin whereas no linear relationship was demonstrated in Cao et al and Kilkkinen et al where antibiotics were not categorised according to class (3-6). The four remaining studies gave opposing results. Three studies focussed on specific antibiotic groups (penicillin and metronidazole). No association was found in two of these studies and the study by Friedman 09 et al showed reduced odds of developing CRC with metronidazole (7-9). Finally, Wang et al analysed the effect of general antibiotic exposure on CRC risk in a diabetic cohort and again found no association. However, further analysis demonstrated that anaerobic antibiotic exposure was associated with an increased CRC risk (10).

Association does not necessarily imply causation and the Bradford-Hill criteria provides a useful framework for appraising an association for possible causation (40). Firstly, current knowledge suggests that a potential association between antibiotic exposure and CRC is biologically plausible. It is known that the microbiome differs between individuals with CRC and 'healthy' people but also differs within the same individual between cancer tissue and unaffected bowel (18, 41-44). There is also experimental evidence from animal studies where mice without an established microbiome (germ-free) living in a germ free environment develop less CRC (14, 45, 46). It has been hypothesized that gut microbiome

imbalance could result in CRC formation by creating a pro-inflammatory environment via a number of mechanisms (47-50). Research has also resulted in different microbe populations being isolated leading to two proposed models, the 'bacterial driver-passenger model' and the 'alpha bugs' model (51, 52). Antibiotics are well known to alter the gut microbiome and it is via this mechanism it is speculated they might contribute to CRC development (18-21, 53).

However, in spite of the above, the evidence against a causal relationship remains substantial. Uncertainty about the temporality of an association is significant as it is not clear whether dysbiosis precedes CRC development or occurs as a result. In addition, there is experimental evidence supporting a link between microbiome dysbiosis and CRC but not with regard to antibiotics and CRC. Also the results of the included studies are mixed and do not consistently demonstrate an association. Finally, this review amalgamated all the observational research in this area and only demonstrates a weak association. This could be explained by bias within the studies, discussed below, but also by confounding factors,. One of which could include reduced immune system function resulting in an increased risk of cancer and infection necessitating antibiotic use. Another plausible confounder relates to health seeking behaviour, where those more likely to seek antibiotics maybe more likely to present with symptoms relating to CRC or attend screening.

Strengths and limitations

To the best of our knowledge, this is the first systematic review and meta-analysis of observational studies that quantify the association between antibiotic exposure and risk of CRC. All included studies were of at least moderate quality according to the EPHPP. Furthermore, the included studies had adequate statistical power, with the meta-analysis sample sizes ranging from nearly 140,000 patients to more than 3 million.

However, this review also has a number of significant limitations. Firstly, included studies were heterogeneous in how antibiotic exposure was characterized from general exposure to focusing on specific

groups. The route and setting of antibiotic exposure differed between studies, with some focusing on an outpatient population whereas others included hospital inpatients and potentially intravenous administration. These differences may impact the concentrations of antibiotic exposure but also classes of antibiotics used, leading to differing effects upon the individuals gut flora. A further area of heterogeneity was with regard to how antibiotic exposure was stratified which led to some studies not being included in the quantitative synthesis. It is also important to note that the method of capturing data on antibiotic exposure differed between studies, from interviews and questionnaires to interrogation of healthcare databases.

A number of studies did not stratify or consider all known confounding risk factors for CRC, thus potentially confounding the study results. Of those studies that did include patients with IBD or potentially familial CRC syndromes, the proportions were not stated. A further major limiting factor is the relatively short time between antibiotic exposure and the development of CRC in the majority of studies (table 1). It is generally hypothesised that colorectal carcinogenesis is a stepwise process that takes 8-15 years to develop (3, 10), therefore follow up needs to be long enough to identify any causative links. In addition, antibiotic prescribing tends to be highest in children and the elderly within primary care (23). It is not clear if any included studies analysed antibiotic exposure during childhood, a potentially crucial time period of dysbiosis. Also, none of the included studies tried to differentiate microbiome associated events between initiation of CRC as polyp prevalence and progression through more advanced stages.

Epidemiological approaches to bacterial driven mechanisms of CRC are limited by differences in composition and comparability between mucosal and stool samples, right and left colon, adenomas and carcinomas and the different molecular subtypes and clinical categories within CRC (54-56). This makes causality assessment for bacterial populations challenging. Available evidence of adaptation and evolution in the commensal microbiome also suggests that comparability between studies is challenging and makes a further likely contribution to heterogeneity (57). Another potential limitation is

the inclusion of adenomas within our analysis as it has been suggested the microbiome may differ along the adenoma-colorectal cancer continuum (56). However, currently there is a paucity of good quality evidence to support changes in the microbiome at different stages leading up to CRC.

Only one study analysed patients according to lesion characteristics (Cao et al) and whether participants were symptomatic therefore limiting further analyses. This study demonstrated longer antibiotic exposure at age 40-59 was more strongly associated with proximal adenomatous lesions (4). They also demonstrated that between 15-25% of participants had symptoms at the time of endoscopy (4). Finally, the indication for antibiotics was only analysed in one study (Cao et al). This raises the concern that antibiotics could have been given for gastrointestinal infection, which is acting as a confounding factor. Another plausible explanation is that patients presenting with symptoms suggestive of gastrointestinal infection had already developed CRC.

Conclusions

Our findings suggest there may be an association between antibiotic consumption and the risk of incident CRC. However, published literature is heterogeneous and inconsistent with a number of potentially significant confounding factors and therefore no causal conclusions can be made. Further large cohort studies with clearly defined antibiotic exposure, adjustment for confounding factors and long-term follow-up are needed to allow more conclusive understanding of whether there is a causal relationship.

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