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Title

Use of antibiotics and colorectal cancer risk: A primary care nested case-control study in Belgium

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Abstract

Objectives

To examine the association between the use of oral antibiotics and subsequent colorectal cancer risk.

Design

Matched case-control study.

Setting

General practice centres participating in the INTEGO database in Flanders, Belgium.

Participants

In total, 1705 cases of colorectal cancer diagnosed between 01 January 2010 and 31 December 2015 were matched to 6749 controls by age, sex, comorbidity and general practice centre.

Primary outcome measure

The association between the number of prescriptions for oral antibiotics and the incidence of colorectal cancer over a period of 1 – 10 years, estimated by a conditional logistic regression model.

Results

A significantly increased risk of colorectal cancer (OR 1.25, 95 % confidence interval 1.10 to 1.44) was found in subjects with one or more prescriptions compared to those with none after correction for diabetes mellitus. No dose – response relationship was found.

Conclusions

This study resulted in a modestly higher risk of having colorectal cancer diagnosed after antibiotic exposure. The main limitation was missing data on known risk factors, in particular smoking behaviour. This study did not allow us to examine the causality of the relationship, indicating the need of further investigation.

Strengths and limitations of this study

- This case-control study was sufficiently powered to detect an association between antibiotic prescriptions and colorectal cancer.
- The large dataset minimized selection bias.
- This study provided us an insight into the prescription behaviour of the general practitioner.
- Smoking and Obesity, known risk factors for colorectal cancer, were missing in INTEGO database. These potential confounders could not be taken into account in our study.

Funding statement

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Competing interests statement

The authors declare that they have no competing interests.

Title

Use of antibiotics and colorectal cancer risk: A primary care nested case-control study in Belgium

Keywords (6): Antibiotics, colorectal cancer, case-control study, risk factor, drug side effects, primary care

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Data sharing statement

The Intego database is managed at the Department of Public Health and Primary Care of the University of Leuven under the supervision of Prof. Dr. Bert Vaes. The dataset supporting the conclusions of this article is held at the University of Leuven, Belgium, and can be shared upon reasonable request.

Dossier number ethical accordance, KU Leuven, Belgium

The ethical commission of the UZ/ KU Leuven, Belgium approved this study (MP004885).

Introduction

The development of antibiotics during the twentieth century has had a ground breaking impact upon modern medicine.(1) However, their administration is known to be associated with the development of short-term gastro-intestinal symptoms such as diarrhoea. (2) While these conditions are generally self-limiting, both oral and intravenous use of antibiotics have been linked to a more permanent state of distorted colonic balance, one example being a Clostridium difficile infection.(3) However, less is known about long-term side effects, for instance the relationship between antibiotic exposure and development of colorectal cancer (CRC). The development of CRC is typically a multifactorial process with a development time of over 10 years and is dependent on modifiable and unmodifiable factors.(4,5) Having a first degree relative with CRC and having an inflammatory bowel disease are major risk factors (IBD).(5) Minor risk factors are smoking (6), abdominal obesity(7), lack of physical activity, diabetes mellitus (DM), male sex and increased age.(5) Roughly 3-5 percent of CRC is an hereditary form.(5)

Prior epidemiological analyses focusing on the relationship between antibiotic use and CRC predominantly consisted of case-control studies, of which six were found relevant to our subject.(8–13) Five investigated the relationship in a database and one was questionnaire based. Our aim is to evaluate the association between oral antibiotics prescribed in general practice and subsequent diagnosis of colorectal cancer with correction for co-morbidity including DM, an established risk factor for CRC(5).

Methods

Study Design and context

For this nested case-control study we used the Belgium based Integrated Computerized Network (INTEGO). Since its foundation in 1994 general practitioners (GPs) have recorded over 3 million diagnoses and 12 million prescriptions. It covers more than 2 percent of the Flemish population and is representative for the Flemish population in terms of age and gender distribution.(14)

Data

Registered details contain information about the subjects´ age, gender, general practice and date of prescriptions and diagnosis. The latter two were coded using the Anatomic Therapeutic Chemical (ATC) Classification System and the International Classification of Primary Care (ICPC-2), respectively.(14)

Case and control selection

A flow diagram of the patient selection is presented in figure 1. All patients aged above 18 years old registered in the INTEGO database during the period of 01 January 2010 to 31 December 2015 (hereinafter called the selection interval) were eligible for inclusion. Cases were those with a first diagnosis of CRC (ICPC-2 code D75) registered by their GP during the selection interval. The index date used for the CRC cases was equal to the date of their first diagnosis. Everyone with the presence of CRC prior to the selection interval was excluded. Furthermore, subjects with IBD prior to the index date were excluded, since IBD might distort the association between antibiotics and CRC.(5)

Each case was matched to four optimally chosen control subjects matched on age (± 5 years), sex, the number of comorbidities (± 1 disease) and general practice. Controls were assigned the same index date as their case counterpart. Mamouris et al. developed an optimal algorithm to match cases and controls in an optimal, fast, and efficient way. This algorithm is efficient since it accommodates replacement with or without controls, fast since it is executable in seconds even with millions of controls, and optimal, since the closest control is always captured. Specifically, in the scenario that a case has only one control we assured that this control will be matched to this case, thus maximizing the cases to be used in the analysis. For additional information about our applied method we refer to an elaborate paper authored by our statistician Mamouris and co-authored by us.(15)

To clarify the concept of matching on comorbidities, consider a case with 3 chronic diseases the number of comorbidities equals 3. When matching this case to a control, we allow for an absolute difference of one chronic disease, meaning that the controls could have 2, 3 or 4 diseases. A total of 105 chronic diseases were taken into account for the operationalization of comorbidity, of which 51 actually occurred in our study population.(16)

Exposure and covariates

To minimize the potential influence of a protopathic bias, prescriptions one year prior to the index date were not considered. The main exposure was defined as any oral antibiotic therapy during 1 to 10 years prior to the index date, subdivided into the following eight classes (ATC code): Tetracyclines (J01A), amphenicols (J01B), beta lactam antibiotics, penicillines (J01C), other beta-lactam antibacterials (J01D), sulfonamides and trimethoprim (J01E), macrolides, lincosamides and streptogramins (J01F), quinolone antibacterials (J01M) and other antibacterial drugs (J01X). Classes with aminoglycoside (J01G) and combinations of antibacterials (J01R) were not prescribed. These classes consist of chemical subgroups which categorize individual antibiotics on a molecular level.(17)

The cumulative number of prescriptions per drug class prior to the index date was assessed. Multiple prescriptions prescribed during one consultation were added up only if they were of a different molecule. Subjects were categorized based upon total number of prescriptions into the following categories: nonusers, low (1st – 33rd percentile), intermediate (34th- 66th percentile), high (67th-90th percentile), very high (above 90th percentile) and all (1st percentile and above). Multiple categories might allow us to witness a dosage-response relationship. The presence of diabetes mellitus (ICPC-2 code T90) was taken into account as confounder. The main factor for which we lacked registration of sufficient quality and quantity was tobacco use.

Statistical analysis

The proportion of cases and controls using antibiotics, as well as the proportion of subjects using different classes of antibiotics were described. For continuous variables we used Student´s t test and Mann-Whitney U test. The Pearson Chi-square test was used for categorical variables. The primary analysis was a conditional logistic regression analysis to estimate odds ratios (OR) and 95% confidence intervals (CIs) for the association between antibiotic prescriptions for oral use and the subsequent diagnosis of CRC. The multivariate analysis was conditioned on the presence of DM. Based upon literature we decided to consider a minimal odds ratio of 1.20 as a relevant risk factor.(5) The predominance of the J01C class of 45 percent allowed us to estimate odds ratios of those who used one or more prescription of this class.

R studio Team (2019) was used for statistical analysis.(18)

Patient involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Results

Baseline characteristics

The group of 1,705 cases with a first diagnosis of CRC between 01 January 2010 and 31 December 2015 were matched to 6,749 controls. Nineteen cases were excluded due to lack of any controls. As indicated in table 1, there were no statistically significant differences between the case and control groups in the terms used by matching. Due to the near perfect matching in terms of age and gender these factors were not corrected for during the analysis. The top three most prevalent comorbidities, which were similar in controls and cases, by most frequent occurrence were hypertension, hypercholesterolemia and asthma.

Exposure

In total 5,217 antibiotic prescriptions were prescribed for the cases versus 18,263 for the controls, resulting in a total average of respectively 3.06 and 2.71 per patient during the observation period of on average nine years. This correlates to on average 1 prescription for every 3-year period for cases and on average 1 prescription every 3 year and 4 months period for controls. The categorization of cases and controls resulted in a combined group of non-users of 3,590 individuals (42% of total), 2,110 in the low category (25%), 1,056 (12%) in the intermediate category, 1,188 (14%) in the high exposure category and lastly 510 (6%) in the very high group. The percentage non-users in the case group were a bit lower compared the control group (40% versus 43%, $p<0.01$). The highest individual number of prescriptions was 122 prescriptions in total, which appeared a credible number after further investigation.

Figure 2 presents the relative share of the six most prescribed classes of antibiotics per year during the entire observational period. The major share consisted of beta-lactam antibacterials (45% on average), which in turn consisted of 89 percent of amoxicillin. Noteworthy is the gradual decline of other beta-lactam antibacterials, which consisted for over 90 percent of cefuroxime. Lastly there was an apparent increase of the class named other antibacterials, which mainly consisted of nitrofurantoin derivatives (68 percent). Figure 3 presents the time-wise use of prescriptions relative to the index date per case or control. As shown, there is a gradual increase in both the case and the control group. After the first year of measurement it shows a non-crossing pattern, with the cases consistently having a higher number of prescriptions.

Association between antibiotics and risk of colorectal cancer

Table 2 shows the main outcome of our study in terms of crude and adjusted odds ratios. The odds ratio of developing CRC for antibiotic users with one or more prescriptions compared to nonusers was 1.25 (95% CI 1.10 to 1.44). Due to the lack of a clear increase of odds ratios, the confidence intervals of all categories were overlapping with their lesser-exposed category, an evident dose-response relationship could not be shown. The additional comparison of individuals who used one or more prescription of the penicillin and other beta lactam antibacterials (J01C class) to nonusers resulted in a crude odds ratio of 1.13 (95% CI 1.00 to 1.28). Due to this number being low and nearly insignificant we deemed an isolated effect of this subclass unlikely.

Discussion

Statement of the principal findings

Antibiotic prescriptions compared to none were associated with a 25 percent higher chance of developing CRC over a 9-year period, a number that remained significant after correction for DM. All subdivided categories, except for low exposure (1-2 prescriptions), showed a significantly increased odds ratio. A dose-response relationship was not shown.

Strengths and weaknesses

The benefit of the INTEGO database was twofold. First the size of more than a quarter of a million of unique individuals during the selection period has allowed us to investigate a relatively rare disease. Second it has shown its representativeness for the Flemish-Belgian population in terms of the geological spread, age and sex.(14) Exact and recent European statistics comparing outpatient and hospital prescriptions were not found. In the United States the GP, with 24 percent of all antibiotic prescriptions in 2011, accounted for the greatest share of all specialties.(19) Considering the likelihood of the GP being the most frequent prescriber the impact of long-term side effects would be the greatest.

The major limitation of this study is the absence of known risk factors, especially smoking. Furthermore, two studies were found that indicated a lower threshold for doctors to prescribe antibiotics of certain classes for tobacco users.(20,21) Considering the increased relative risk of 1.20 (95% CI 1.10 to 1.30) of developing CRC when comparing smokers to non-smokers found by Kelvin et al.(6) it is doubtful, but not excludable, that our found odds ratio can fully be explained by smoking. One further limitation of the used database is that Belgian patients are free to visit different GPs of their choice, possibly resulting in incomplete patient data regarding prescription of antibiotics and registration of relevant diagnoses.

Comparison to previous studies

Comparing our results to similar studies, differences can be found in terms of investigated population, registration of exposure, diagnosis and method of correction for comorbidities. Kilkkinen et al. (2008) found an increased risk of developing CRC with an odds ratio (OR) of 1.15 (95% CI 1.04 to 1.26) when comparing 6 or more prescriptions versus 0-1 during a follow up period of 3 – 9 years after use.(9) Boursi et al. demonstrated a higher risk of CRC associated with first penicillin usage over 10 years before diagnosis date.(10) Dik et al. established an OR of 1.23 (95% CI 1.08 to 1.40) when comparing eight or more prescriptions versus none during 1-6 years prior to CRC diagnosis.(8) The odds ratio we found when comparing 5 or more prescriptions to non-users was 1.40 (95% CI 1.10 to 1.79). This number is slightly higher, yet comparable to these earlier studies. In accordance with these three studies(8–10) we did not find a significant relationship in our lowest exposure category (1-2 prescriptions) when comparing to non-users. The only study which corrected for smoking behavior was by Armstrong et al. (2020). They found an increased odds ratio of 1.90 (95% CI

1.61 to 2.19) for the overall amount of prescriptions during the entire follow-up duration, which had a median of 6 years and a maximum of 15 years.(13)

Wang et al. investigated the same relationship in a subpopulation of diabetic patients, resulting in an OR of 2.31 (95% CI 2.12 to 2.52).(11) Due to a different population this study was not found comparable. Finally, a study by Cao et al. measured the exposure between the age of 20 and 59 by questionnaire in a group of nurses and compared this to the risk of colorectal adenoma after the age of 60 years. Compared to non-users, women who used antibiotics for ≥ 2 months between age 20 and 39 had a multivariable OR of 1.36 (95% CI 1.03 to 1.79). Women who used ≥2 months of antibiotics between age 40 and 59 had a multivariable OR of 1.69 (95% CI 1.24 to 2.31).(12)

Recommendations for further research and clinical practice

One possible explanation for our results might be the influence of antibiotics on the human gut, which contains a diverse microbial community and has a crucial role in the defence against pathogenic bacteria.(22) Borges-Canha et al. found conclusive evidence of a link between carcinogenesis and microbial dysbiosis. In particular, there are hints that the metabolic environment is involved, which can create a pro-inflammatory state (23). Furthermore, an altered state of colonic microbiota was shown after the use of antibiotics.(24) Human studies with convincing evidence of a direct relationship between antibiotic use and CRC however are lacking.(4,25) Considering the possibility of a permanent altered colonic state after antibiotic exposure, this effect could result in a classical dose-response relationship or according to a threshold like model. The amount of exposure while we measured it seems relatively high in comparison to other countries. A European surveillance study, which investigated outpatient antibiotic use during 1997 – 2009, showed an increase during this time. In addition, Belgium ranked sixth out of thirty-three in terms of packages per 1000 inhabitants per day in 2009.(26) Our results however do support rational use, a trend which seems to become stronger due to the increased presence of antibiotic resistance.(27) Further research with more extensive correction for known risk factors is required to exclude the possibility of a causative correlation. Such presence might indicate further biological study. In our opinion clinical implications of our study are limited due to the inherent limitations of retrospective research.

Conclusion

In this case-control study, prescribed oral antibiotics predicted an increased risk of colorectal cancer without a clear dosage-response relationship. The major limitation was lack of information on known risk factors of cancer, such as smoking. The retrospective observational nature warrants caution interpreting these results as proof of causality. Our inability of disproving the correlation between antibiotic exposure and development of CRC indicates the need of additional investigation. This study further supports the opinion of reserved and prudent usage of a potential lifesaving medicine.

References

1. Zaffiri L, Gardner J, Toledo-Pereyra LH. History of antibiotics. from salvarsan to cephalosporins. J Investig Surg. 2012;25(2):67–77.

2. Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. N Engl J Med [Internet]. 2002 Jan 31;346(5):334–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11821511

3. Song JH, Kim YS. Recurrent Clostridium difficile Infection : Risk Factors , Treatment , and Prevention. 2019;13(1):16–24.

4. Brenner H, Chen C. The colorectal cancer epidemic: challenges and opportunities for primary, secondary and tertiary prevention. Br J Cancer [Internet]. 2018;119(7):785–92. Available from: http://dx.doi.org/10.1038/s41416-018-0264-x

5. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet. 2014;383(9927):1490–502.

6. Tsoi KKF, Pau CYY, Wu WKK, Chan FKL, Griffiths S, Sung JJY. Cigarette Smoking and the Risk of Colorectal Cancer: A Meta-analysis of Prospective Cohort Studies. Clin Gastroenterol Hepatol [Internet]. 2009;7(6):682-688.e5. Available from: http://dx.doi.org/10.1016/j.cgh.2009.02.016

7. Dong Y, Zhou J, Zhu Y, Luo L, He T, Hu H, et al. Abdominal obesity and colorectal cancer risk: systematic review and meta-analysis of prospective studies. Biosci Rep [Internet]. 2017;37(6):1–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29026008%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5725611

8. Dik VK, van Oijen MGH, Smeets HM, Siersema PD. Frequent Use of Antibiotics Is Associated with Colorectal Cancer Risk: Results of a Nested Case–Control Study. Dig Dis Sci [Internet]. 2016;61(1):255–64. Available from: http://link.springer.com/10.1007/s10620-015-3828-0

9. Kilkkinen A, Rissanen H, Klaukka T, Pukkala E, Heliövaara M, Huovinen P, et al. Antibiotic use predicts an increased risk of cancer. Int J Cancer. 2008;123(9):2152–5.

10. Boursi B; Haynes K; Ronac M ;Yu-Xiao Y; Perelman. Impact of antibiotic exposure on the risk of colorectal cancer.

11. Wang J-L, Chang C-H, Lin J-W, Wu L-C, Chuang L-M, Lai M-S. Infection, antibiotic therapy and risk of colorectal cancer: A nationwide nested case-control study in patients with Type 2 diabetes mellitus. Int J Cancer [Internet]. 2014;135(4):956–67. Available from: http://doi.wiley.com/10.1002/ijc.28738

12. Cao Y, Wu K, Mehta R, Drew DA, Song M, Lochhead P, et al. Long-term use of antibiotics and risk of colorectal adenoma. Gut [Internet]. 2017;gutjnl-2016-313413. Available from: http://gut.bmj.com/lookup/doi/10.1136/gutjnl-2016-313413

13. Armstrong D, Dregan A, Ashworth M, White P, McGee C, de Lusignan S. The association between colorectal cancer and prior antibiotic prescriptions: case control study. Br J Cancer [Internet]. 2020;122(6):912–7. Available from: http://dx.doi.org/10.1038/s41416-019-0701-5

14. Truyers C, Goderis G, Dewitte H, Akker M vanden, Buntinx F. The Intego database: background, methods and basic results of a Flemish general practice-based continuous morbidity registration project. BMC Med Inform Decis Mak [Internet]. 2014;14(1):48. Available from:

http://bmcmedinformdecismak.biomedcentral.com/articles/10.1186/1472-6947-14-48

15. Mamouris P, Nassiri V, Molenberghs G, van den Akker M, van der Meer J, Vaes B. Fast and optimal algorithm for case-control matching using registry data: application on the antibiotics use of colorectal cancer patients. BMC Med Res Methodol. 2021;21(1):1–9.

16. Shi X, Nikolic G, Van Pottelbergh G, van den Akker M, Vos R, De Moor B. Development of Multimorbidity Over Time: An Analysis of Belgium Primary Care Data Using Markov Chains and Weighted Association Rule Mining. Journals Gerontol Ser A. 2020;XX(Xx):1–8.

17. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment. 22nd editi. WHO Collaborating Centre for Drug Statistics Methodology, editor. 2019.

18. R Foundation for Statistical Computing, Vienna A. R Core Team (2019). R: A language and environment for statistical computing. [Internet]. 2019. Available from: https://www.r-project.org/

19. Suda KJ, Hicks LA, Roberts RM, Hunkler RJ, Danziger LH. A national evaluation of antibiotic expenditures by healthcare setting in the United States, 2009. J Antimicrob Chemother. 2013;68(3):715–8.

20. Steinberg MB, Akincigil A, Kim EJ, Shallis R, Delnevo CD. Tobacco Smoking as a Risk Factor for Increased Antibiotic Prescription. Am J Prev Med [Internet]. 2016;50(6):692–8. Available from: http://dx.doi.org/10.1016/j.amepre.2015.11.009

21. Blix HS, Hjellvik V, Litleskare I, Rønning M, Tverdal A. Cigarette smoking and risk of subsequent use of antibacterials: A follow-up of 365117 men and women. J Antimicrob Chemother. 2011;66(9):2159–67.

22. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorg 2019, Vol 7, Page 14 [Internet]. 2019;7(1):14. Available from: https://www.mdpi.com/392832

23. Borges-Canha M, Portela-Cidade JP, Dinis-Ribeiro M, Leite-Moreira AF, Pimentel-Nunes P. Role of colonic microbiota in colorectal carcinogenesis: A systematic review. Rev Esp Enfermedades Dig. 2015;107(11):659–71.

24. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci [Internet]. 2011 Mar 15;108(Supplement_1):4554–61. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1000087107

25. Dejea C, Wick E, Sears CL. Bacterial oncogenesis in the colon HHS Public Access. Futur Microbiol [Internet]. 2013;8(4):445–60. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4052711/pdf/nihms513136.pdf

26. Adriaenssens N, Coenen S, Versporten A, Muller A, Minalu G, Faes C, et al. European Surveillance of Antimicrobial Consumption (ESAC): Outpatient antibiotic use in Europe (1997-2009). J Antimicrob Chemother. 2011;66(SUPPL. 6):3–12.

27. ECDC. Surveillance of antimicrobial resistance in Europe (2017). [Internet]. Surveillance of antimicrobial resistance in Europe. 2018. 1–71 p. Available from: https://ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2016

Author Statement

Name	Individual author contribution role (CRediT)
Johannes Van der Meer	Conceptualization, Methodology, Investigation, Writing original draft, Visualization
Pavlos Mamouris	Formal analysis, Data curation, Software
Vahid Nassiri	Formal analysis, Data curation, Software
Bert Vaes	Conceptualization, Methodology, Writing – Review & Editing
Marjan van den Akker	Conceptualization, Methodology, Investigation, Writing – Review & Editing, Supervision

Table 1

Baseline characteristics of cases and controls

	Cases	Controls	p-value*
Absolute number of subjects	1705	6749	
Mean age at index date (+/- standard deviation)	58.85 (13.48)	58.53 (14.11)	0.40
Male gender (percentage)	791 (46.39)	3143 (46.57)	0.92
Prevalence of diabetes mellitus (percentage)	386 (22.64)	877 (12.99)	<0.01
Non-exposed individuals (percentage)	675 (39.59)	2915 (43.19)	<0.01
Average number of co-morbidities per subject	2.35	2.29	0.35

*Pearson Chi-square test was used for categorical variables and Student´s t test for continuous variables

Table 2

Antibiotic prescriptions and odds ratio of developing CRC

Amount of prescriptions compared to none[a]	Odds ratio (95 percent interval lower limit - upper limit)	
	Crude	Adjusted[b]
>0	1.26 (1.10-1.45)	1.25 (1.10-1.44)
1-2	1.11 (0.95-1.31)	1.12 (0.95-1.32)
3-4	1.37 (1.07-1.75)	1.36 (1.06-1.74)
5-122	1.43 (1.12-1.82)	1.40 (1.10-1.79)
5-10	1.31 (1.01-1.71)	1.30 (1.00-1.70)
11-122	2.00 (1.27-3.41)	1.92 (1.17-3.16)

a Category (number of prescriptions): All (>0), low (1-2), intermediate (3-4), high (5-10), very high (11-122)
b Adjusted for presence of DM2

Figure 1

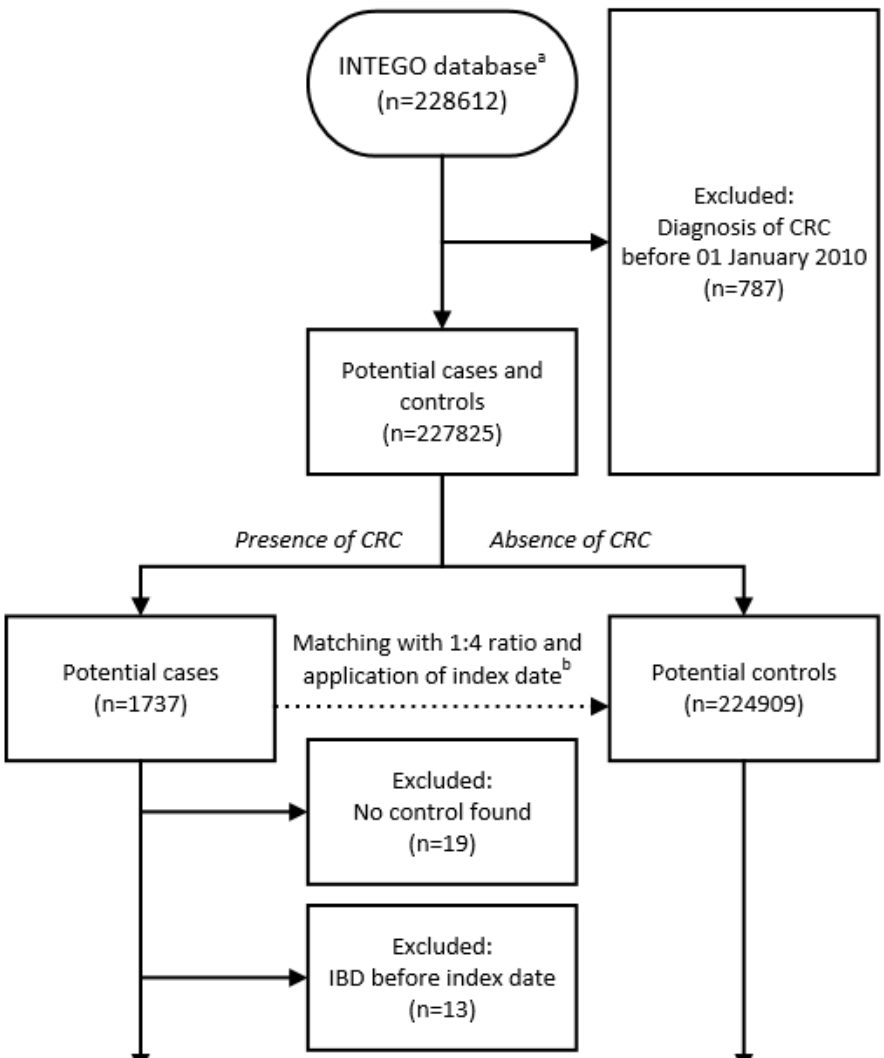
Patient selection flowchart

Figure 2

Relative antibiotic use per class per year (excluding two classes with <2 percent of total prescriptions)

Figure 3

Average cumulative prescriptions per case or control prior to index date



a. Total number of unique eligible subjects in the INTEGO database during 1st January 2010 till 31st December 2015
b. Controls with presence of IBD before the index date were excluded during matching

Figure 1

Patient selection flowchart

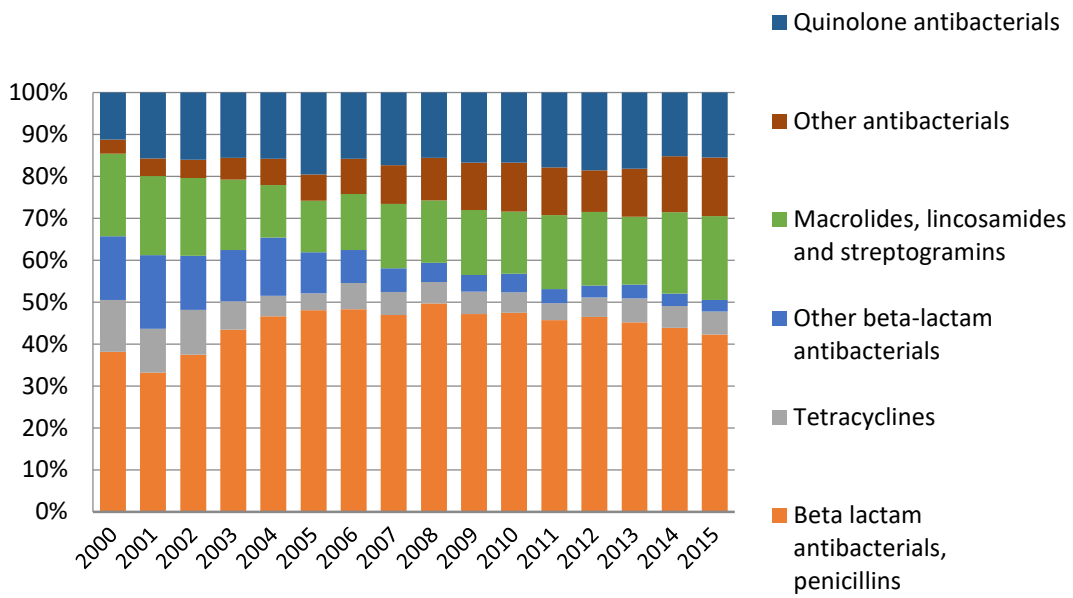
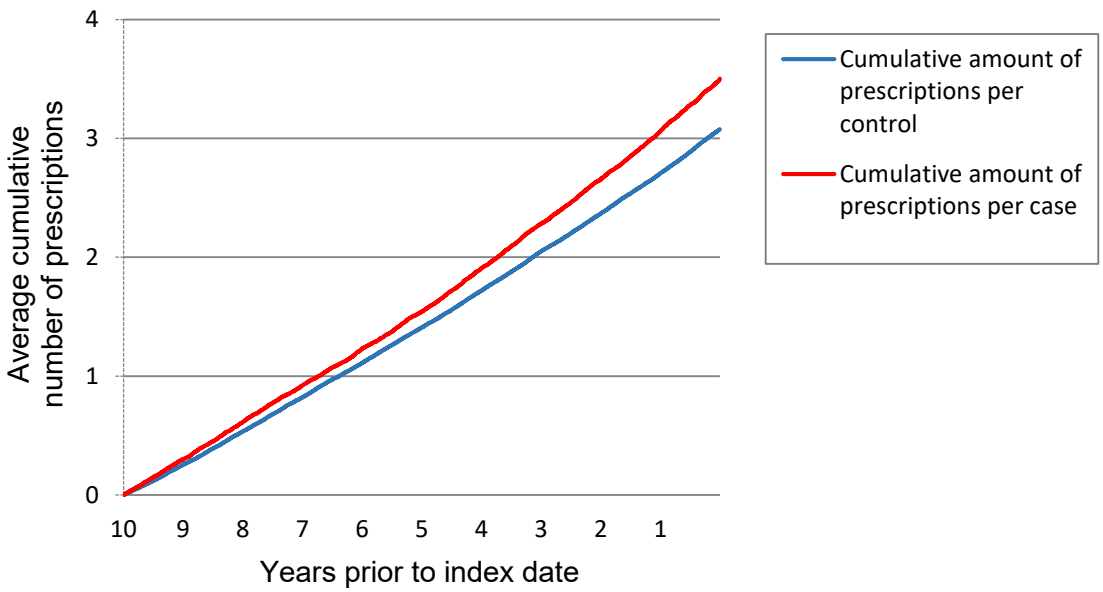


Figure 2

Relative antibiotic use per class per year (excluding two classes with <2 percent of total prescriptions)



*Observation period ranged from 1 – 10 years prior to the index date

Figure 3

Average cumulative prescriptions per case or control prior to index date*

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6,7
		(*d*) If applicable, explain how matching of cases and controls was addressed	5
		(*e*) Describe any sensitivity analyses	6
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1, page 5
		(b) Give reasons for non-participation at each stage	Figure 1, page 5
		(c) Consider use of a flow diagram	Figure 1, page 5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1, page 7
		(b) Indicate number of participants with missing data for each variable of interest	Table 1, page 7
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	7, 8, Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7, 8, Table 2
		(*b*) Report category boundaries when continuous variables were categorized	7, 8, Table 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Use of antibiotics and colorectal cancer risk: A primary care nested case-control study in Belgium

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Title

Use of antibiotics and colorectal cancer risk: A primary care nested case-control study in Belgium

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Abstract

Objectives

To examine the association between the use of oral antibiotics and subsequent colorectal cancer risk.

Design

Matched case-control study.

Setting

General practice centres participating in the INTEGO database in Flanders, Belgium.

Participants

In total, 1705 cases of colorectal cancer diagnosed between 01 January 2010 and 31 December 2015 were matched to 6749 controls by age, sex, comorbidity and general practice centre.

Primary outcome measure

The association between the number of prescriptions for oral antibiotics and the incidence of colorectal cancer over a period of 1 – 10 years, estimated by a conditional logistic regression model.

Results

A significantly increased risk of colorectal cancer (OR 1.25, 95 % confidence interval 1.10 to 1.44) was found in subjects with one or more prescriptions compared to those with none after correction for diabetes mellitus. No dose – response relationship was found.

Conclusions

This study resulted in a modestly higher risk of having colorectal cancer diagnosed after antibiotic exposure. The main limitation was missing data on known risk factors, in particular smoking behaviour. This study did not allow us to examine the causality of the relationship, indicating the need of further investigation.

Strengths and limitations of this study

- This case-control study was sufficiently powered to detect an association between antibiotic prescriptions and colorectal cancer.
- The large dataset minimized selection bias.
- This study provided us an insight into the prescription behaviour of the general practitioner.
- Smoking and Obesity, known risk factors for colorectal cancer, were missing in INTEGO database. These potential confounders could not be taken into account in our study.

Funding statement

Intego is funded on a regular basis by the Flemish Government (Ministry of Health and Welfare).

Competing interests statement

The authors declare that they have no competing interests.

Title

Use of antibiotics and colorectal cancer risk: A primary care nested case-control study in Belgium

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Data sharing statement

The Intego database is managed at the Department of Public Health and Primary Care of the University of Leuven under the supervision of Prof. Dr. Bert Vaes. The dataset supporting the conclusions of this article is held at the University of Leuven, Belgium, and can be shared upon reasonable request.

Dossier number ethical accordance, KU Leuven, Belgium

The ethical commission of the UZ/ KU Leuven, Belgium approved this study (MP004885).

Introduction

The development of antibiotics during the twentieth century has had a ground breaking impact upon modern medicine.(1) However, their administration is known to be associated with the development of short-term gastro-intestinal symptoms such as diarrhoea. (2) While these conditions are generally self-limiting, both oral and intravenous use of antibiotics have been linked to a more permanent state of distorted colonic balance, one example being a Clostridium difficile infection.(3) The human gut contains a diverse microbial community and has a crucial role in the defence against pathogenic bacteria, a balance that can be influenced by numerous factors.(4) Little is known about long-term effects of oral antibiotic exposure, for example in the relationship between antibiotic exposure and development of colorectal cancer (CRC). A systematic review of the link between carcinogenesis and microbial dysbiosis suggests a relationship, yet hard conclusions about causality could not be stated. In particular, there are hints that the metabolic environment is involved, which can create a pro-inflammatory state.(5) In addition a persistent altered microbiotic state after oral antibiotic exposure was shown by Dethlefsen et al. 10 months after initial exposure.(6) Human studies with a clear link between an altered colonic state and colorectal oncogenesis in vivo were not found.

The development of CRC in general is typically a multifactorial process with a development time of over 10 years and is dependent on modifiable and unmodifiable factors.(7,8) Having a first degree relative with CRC and having an inflammatory bowel disease (IBD) are major risk factors.(8) Minor risk factors are smoking (9), abdominal obesity(10), lack of physical activity, diabetes mellitus (DM), male sex and increased age.(8) Roughly 3-5 percent of CRC is a hereditary form.(8) Whether exposure to oral antibiotics might be one of these factors is a complex association to investigate.

The importance of investigating the potential presence of such a relationship can be demonstrated by the current disease burden caused by the development of CRC, which is significant by measurement of incidence and consequence. For instance in Europe it is estimated to account for twelve percent of all cancers and cancer-related deaths annually.(11) Analysis of geographical distribution between 21 European countries during the first two decades of the twenty-first century resulted in great intercountry differences.(12) Outpatient antibiotic use also differs greatly between countries in this region. This was shown by an analysis of 33 European countries resulting in a factor of 3.8 when comparing the highest and lowest national consumption.(13) Considering the heterogenetic geographical distribution of both antibiotic use and CRC incidence an investigation in a country where this has not yet been analysed yet might be of additional value.

Our aim is to evaluate the association between oral antibiotics prescribed in Flemish general practice and subsequent diagnosis of colorectal cancer with correction for co-morbidity including DM, an established risk factor for CRC.(8)

A thorough search with this focus can be summarized by describing the results of three meta-analyses, which combined analysed a total of eleven individual case-control and cohort studies.(14–24) In 2019 Syanolu et al.(25) investigated a total of eight studies and their quantitative synthesis resulted in a significant odds ratio of 1.20 (95% CI 1.10 – 1.32) when cumulatively assessing the number of prescriptions. In their conclusion they consider a weak

association between exposure and outcome but no clear signs for a dose-response relationship. The other two meta-analyses were both published in 2020, were both based upon the same ten studies and resulted in comparable results. Qu et al.(26) analysed a total of over 4,8 million participants, which resulted in an odds ratio of 1.09 (95% CI 1.02-1.17). In particular, the additional analysis of anti-aerobic antibiotics showed an increased risk. Lastly Simin et al.(27) compared ever-users to none users which resulted in an odds ratio of 1.17 (95% CI of 1.05-1.30). Limitations mentioned by these meta-analyses are the high heterogeneity between studies in terms of measurement of exposure, measurement of outcome, differing antibiotic use per country and potential biases such as residual confounding. In addition to the already present studies, ours will add weight in its geographical location since this is the first study in Belgium, with its own prescription behaviour and CRC diagnostic procedures and incidence.

Methods

Study Design and context

For this nested case-control study we used the Belgium based Integrated Computerized Network (INTEGO). Since its foundation in 1994 general practitioners (GPs) have recorded over 3 million diagnoses and 12 million prescriptions. It covers more than 2 percent of the Flemish population and is representative for the Flemish population in terms of age and gender distribution.(28)

Data

Registered details contain information about the subjects´ age, gender, general practice and date of prescriptions and diagnosis. The latter two were coded using the Anatomic Therapeutic Chemical (ATC) Classification System and the International Classification of Primary Care (ICPC-2), respectively.(28)

Case and control selection

A flow diagram of the patient selection is presented in figure 1. All patients aged above 18 years old registered in the INTEGO database during the period of 01 January 2010 to 31 December 2015 (hereinafter called the selection interval) were eligible for inclusion. Cases were those with a first diagnosis of CRC (ICPC-2 code D75) registered by their GP during the selection interval. The index date used for the CRC cases was equal to the date of their first diagnosis. Everyone with the presence of CRC prior to the selection interval was excluded. Furthermore, subjects with IBD prior to the index date were excluded, since IBD might distort the association between antibiotics and CRC.(8)

-Figure 1 here-

Each case was matched to four optimally chosen control subjects matched on age (± 5 years), sex, the number of comorbidities (± 1 disease) and general practice. Controls were assigned the same index date as their case counterpart. Mamouris et al. developed an optimal algorithm to match cases and controls in an optimal, fast, and efficient way. This algorithm is efficient since it accommodates replacement with or without controls, fast since it is executable in seconds even with millions of controls, and optimal, since the closest control is always captured. Specifically, in the scenario that a case has only one control we assured that this control will be matched to this case, thus maximizing the cases to be used in the analysis. For additional information about our applied method we refer to an elaborate paper authored by our statistician Mamouris and co-authored by us.(29)

To clarify the concept of matching on comorbidities, consider a case with 3 chronic diseases the number of comorbidities equals 3. When matching this case to a control, we allow for an absolute difference of one chronic disease, meaning that the controls could have 2, 3 or 4 diseases. A total of 105 chronic diseases were taken into account for the operationalization of comorbidity, of which 51 actually occurred in our study population.(30)

Exposure and covariates

To minimize the potential influence of a protopathic bias, prescriptions one year prior to the index date were not considered. The main exposure was defined as any oral antibiotic therapy during 1 to 10 years prior to the index date, subdivided into the following eight classes (ATC code): Tetracyclines (J01A), amphenicols (J01B), beta lactam antibiotics, penicillines (J01C), other beta-lactam antibacterials (J01D), sulfonamides and trimethoprim (J01E), macrolides, lincosamides and streptogramins (J01F), quinolone antibacterials (J01M) and other antibacterial drugs (J01X). Classes with aminoglycoside (J01G) and combinations of antibacterials (J01R) were not prescribed. These classes consist of chemical subgroups which categorize individual antibiotics on a molecular level.(31)

The cumulative number of prescriptions per drug class prior to the index date was assessed. Multiple prescriptions prescribed during one consultation were added up only if they were of a different molecule. Subjects were categorized based upon total number of prescriptions into the following categories: nonusers, low (1st – 33rd percentile), intermediate (34th- 66th percentile), high (67th-90th percentile), very high (above 90th percentile) and all (1st percentile and above). Multiple categories might allow us to witness a dosage-response relationship. The presence of diabetes mellitus (ICPC-2 code T90) was taken into account as confounder. The main factor for which we lacked registration of sufficient quality and quantity was tobacco use. In addition certain genetic variants, such as Lynch syndrome, increase the chance of development of CRC.(8) Due to missing details in the database about these often hereditary disorders we were not able to correct for these types.

Statistical analysis

The proportion of cases and controls using antibiotics, as well as the proportion of subjects using different classes of antibiotics were described. For continuous variables we used Student´s t test and Mann-Whitney U test. The Pearson Chi-square test was used for categorical variables. The primary analysis was a conditional logistic regression analysis to estimate odds ratios (OR) and 95% confidence intervals (CIs) for the association between

antibiotic prescriptions for oral use and the subsequent diagnosis of CRC. The multivariate analysis was conditioned on the presence of DM. Based upon literature we decided to consider a minimal odds ratio of 1.20 as a relevant risk factor.(8) The predominance of the J01C class of 45 percent allowed us to estimate odds ratios of those who used one or more prescription of this class.

R studio Team (2019) was used for statistical analysis.(32)

Ethics Statement

The Intego procedures were approved by the ethical review board of the KULeuven Faculty of Medicine (no. ML 1723) and by the Belgian Privacy Commission (no. SCSZG/13/079).

Patient and Public Involvement

No patients were involved in the development of the research question, study design or interpretation of the data.

Results

Baseline characteristics

The group of 1,705 cases with a first diagnosis of CRC between 01 January 2010 and 31 December 2015 were matched to 6,749 controls. Nineteen cases were excluded due to lack of any controls. As indicated in table 1, there were no statistically significant differences between the case and control groups in the terms used by matching. Due to the near perfect matching in terms of age and gender these factors were not corrected for during the analysis. The top three most prevalent comorbidities, which were similar in controls and cases, by most frequent occurrence were hypertension, hypercholesterolemia and asthma.

Table 1

Baseline characteristics of cases and controls

	Cases	Controls	p-value*
Absolute number of subjects	1705	6749	
Mean age at index date (+/- standard deviation)	58.85 (13.48)	58.53 (14.11)	0.40
Male gender (percentage)	791 (46.39)	3143 (46.57)	0.92
Prevalence of diabetes mellitus (percentage)	386 (22.64)	877 (12.99)	<0.01
Non-exposed individuals (percentage)	675 (39.59)	2915 (43.19)	<0.01
Average number of co-morbidities per subject	2.35	2.29	0.35

*Pearson Chi-square test was used for categorical variables and Student´s t test for continuous variables

Exposure

In total 5,217 antibiotic prescriptions were prescribed for the cases versus 18,263 for the controls, resulting in a total average of respectively 3.06 and 2.71 per patient during the observation period of on average nine years. This correlates to on average 1 prescription for every 3-year period for cases and on average 1 prescription every 3 year and 4 months period for controls. The categorization of cases and controls resulted in a combined group of non-users of 3,590 individuals (42% of total), 2,110 in the low category (25%), 1,056 (12%) in the intermediate category, 1,188 (14%) in the high exposure category and lastly 510 (6%) in the very high group. The percentage non-users in the case group were a bit lower compared the control group (40% versus 43%, p<0.01). The highest individual number of prescriptions was 122 prescriptions in total, which appeared a credible number after further investigation.

-Figure 2 here-

Figure 2 presents the relative share of the six most prescribed classes of antibiotics per year during the entire observational period. The major share consisted of beta-lactam antibacterials (45% on average), which in turn consisted of 89 percent of amoxicillin. Noteworthy is the gradual decline of other beta-lactam antibacterials, which consisted for over 90 percent of cefuroxime. Lastly there was an apparent increase of the class named other antibacterials, which mainly consisted of nitrofurantoin derivatives (68 percent). Figure 3 presents the time-wise use of prescriptions relative to the index date per case or control. As shown, there is a gradual increase in both the case and the control group. After the first year of measurement it shows a non-crossing pattern, with the cases consistently having a higher number of prescriptions.

-Figure 3 here-

Association between antibiotics and risk of colorectal cancer

Table 2 shows the main outcome of our study in terms of crude and adjusted odds ratios. The odds ratio of developing CRC for antibiotic users with one or more prescriptions compared to nonusers was 1.25 (95% CI 1.10 to 1.44). Due to the lack of a clear increase of odds ratios, the confidence intervals of all categories were overlapping with their lesser-exposed category, an evident dose-response relationship could not be shown. The additional comparison of individuals who used one or more prescription of the penicillin and other beta lactam antibacterials (J01C class) to nonusers resulted in a crude odds ratio of 1.13 (95% CI 1.00 to 1.28). Due to this number being low and nearly insignificant we deemed an isolated effect of this subclass unlikely. Analysis of other classes did not result in significant odds ratios.

Table 2

Antibiotic prescriptions and odds ratio of developing CRC

Amount of prescriptions compared to none[a]	Odds ratio (95 percent interval lower limit - upper limit)	
	Crude	Adjusted[b]
>0	1.26 (1.10-1.45)	1.25 (1.10-1.44)
1-2	1.11 (0.95-1.31)	1.12 (0.95-1.32)
3-4	1.37 (1.07-1.75)	1.36 (1.06-1.74)
5-122	1.43 (1.12-1.82)	1.40 (1.10-1.79)
5-10	1.31 (1.01-1.71)	1.30 (1.00-1.70)
11-122	2.00 (1.27-3.41)	1.92 (1.17-3.16)

a Category (number of prescriptions): All (>0), low (1-2), intermediate (3-4), high (5-10), very high (11-122)
b Adjusted for presence of DM2

Discussion

Statement of the principal findings

Antibiotic prescriptions compared to none were associated with a 25 percent higher chance of developing CRC over a 9-year period, a number that remained significant after correction for DM. All subdivided categories, except for low exposure (1-2 prescriptions), showed a significantly increased odds ratio. A dose-response relationship was not shown.

Strengths and weaknesses

The benefit of the INTEGO database was twofold. First the size of more than a quarter of a million of unique individuals during the selection period has allowed us to investigate a relatively rare disease. Second it has shown its representativeness for the Flemish-Belgian population in terms of the geological spread, age and sex.(28) Exact and recent European statistics comparing outpatient and hospital prescriptions were not found. In the United States the GP, with 24 percent of all antibiotic prescriptions in 2011, accounted for the greatest share of all specialties.(33) Considering the likelihood of the GP being the most frequent prescriber the impact of long-term side effects would be the greatest. Two unique features of our study were the use of an optimal matching procedure (29) which optimized the use of controls and matching based upon general disease burden using the co-morbidity index.

The major limitation of this study is the absence of known risk factors, especially smoking. Furthermore two studies were found that indicated a lower threshold for doctors to prescribe antibiotics of certain classes for tobacco users.(34,35) Considering the increased relative risk of 1.20 (95% CI 1.10 to 1.30) of developing CRC when comparing smokers to non-smokers found by Kelvin et al.(9) it is doubtful, but not excludable, that our found odds ratio can fully be explained by smoking. One further limitation of the used database is that Belgian patients are free to visit different GPs of their choice, possibly resulting in incomplete patient data regarding prescription of antibiotics and registration of relevant diagnoses. Lastly it should be stated that while a case-control study is often suited to test a certain hypothesis about the link between a risk factor and an outcome, its retrospective nature limits the power of investigating causality.

Comparison to previous studies

Comparing our results to similar studies, differences can be found in terms of investigated population, registration of exposure, diagnosis and method of correction for comorbidities. Our odds ratio of any versus none users of 1.25 (95% CI 1.10 to 1.44) is very similar to the pooled results from the meta-analysis of Simin et al.(27) who found an odds ratio of 1.17 (95% CI 1.05 to 1.30). Ten studies lay the foundation for this meta-analysis, of which three were conducted in the United States(15–17), five in Europe (United Kingdom N=3(19,21,22), the Netherlands N=1(14), Finland N=1(20)), one in New Zealand(23) and one in Taiwan(18). In 2009 Belgium ranked 6th out of 33 countries for the amount of antibiotic prescriptions and

was higher than the three aforementioned European countries, suggesting relatively high exposure in our study.(13) Potential explanations for a positive correlation might be a causative relationship or potentially persistent lacking correction for a common confounder. A clear dose – response relationship was generally not considered shown, and lacking in our study. However, considering the described permanent effect of antibiotics upon the microbiome(6) it might behave more like a threshold type of model instead.

For detailed results comparable individual case-control studies are more suitable for comparison. Our odds ratio when comparing our group with high exposure, meaning 5 or more prescriptions, to non-users was 1.40 (95% CI 1.10 to 1.79). Dik et al. established an OR of 1.23 (95% CI 1.08 to 1.40) when comparing eight or more prescriptions versus none during 1-6 years prior to CRC diagnosis.(14) Kilkkinen et al. (2008) found an increased risk of developing CRC with an odds ratio (OR) of 1.15 (95% CI 1.04 to 1.26) when comparing 6 or more prescriptions versus 0-1 during a follow up period of 3 – 9 years after use.(20) Our number is slightly higher, yet in line with these studies. In accordance with these studies(14,20) we did not find a significant relationship in our lowest exposure category (1-2 prescriptions) when comparing to non-users. One study which corrected for smoking behavior was by Armstrong et al. (2020). They found an increased odds ratio of 1.90 (95% CI 1.61 to 2.19) for the overall amount of prescriptions during the entire follow-up duration, which had a median of 6 years and a maximum of 15 years.(21)

Recommendations for further research and clinical practice

One possible explanation for our results might be the influence of antibiotics on the human gut, which contains a diverse microbial community and has a crucial role in the defence against pathogenic bacteria.(4) Human studies with convincing evidence of a direct relationship between antibiotic use and CRC however are lacking.(7,36) In order to study such a relationship one needs further investigation of the drug – microbiome relationship and its long term effects. Considering the possibility of a permanent altered colonic state after antibiotic exposure, it is questionable whether this effect could result in a classical dose-response relationship or would behave as a threshold type model. The amount of exposure while we measured it seems relatively high in comparison to other countries.(13) We consider our study, which was performed in a unique region by a unique matching procedure, has added weight to the hypothesis of an existent correlation between the exposure to oral antibiotics and CRC development. Its results highlight the value of additional research to improve the understanding of the interaction between antibiotics and colorectal cancer. Our results do support rational use, a trend which seems to become stronger due to the increased presence of antibiotic resistance.(37) In our opinion additional clinical implications of our study are limited due to the inherent limitations of retrospective research.

Conclusion

In this case-control study, prescribed oral antibiotics predicted an increased risk of colorectal cancer without a clear dosage-response relationship. The major limitation was lack of information on known risk factors of cancer, such as smoking. The retrospective observational nature warrants caution interpreting these results as proof of causality. Our inability of disproving the correlation between antibiotic exposure and development of CRC indicates the need of additional investigation. This study further supports the opinion of reserved and prudent usage of a potential lifesaving medicine.

References

1. Zaffiri L, Gardner J, Toledo-Pereyra LH. History of antibiotics. from salvarsan to cephalosporins. J Investig Surg. 2012;25(2):67–77.

2. Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. N Engl J Med [Internet]. 2002 Jan 31;346(5):334–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11821511

3. Song JH, Kim YS. Recurrent Clostridium difficile Infection : Risk Factors , Treatment , and Prevention. 2019;13(1):16–24.

4. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorg 2019, Vol 7, Page 14 [Internet]. 2019;7(1):14. Available from: https://www.mdpi.com/392832

5. Borges-Canha M, Portela-Cidade JP, Dinis-Ribeiro M, Leite-Moreira AF, Pimentel-Nunes P. Role of colonic microbiota in colorectal carcinogenesis: A systematic review. Rev Esp Enfermedades Dig. 2015;107(11):659–71.

6. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci [Internet]. 2011 Mar 15;108(Supplement_1):4554–61. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1000087107

7. Brenner H, Chen C. The colorectal cancer epidemic: challenges and opportunities for primary, secondary and tertiary prevention. Br J Cancer [Internet]. 2018;119(7):785–92. Available from: http://dx.doi.org/10.1038/s41416-018-0264-x

8. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet. 2014;383(9927):1490–502.

9. Tsoi KKF, Pau CYY, Wu WKK, Chan FKL, Griffiths S, Sung JJY. Cigarette Smoking and the Risk of Colorectal Cancer: A Meta-analysis of Prospective Cohort Studies. Clin Gastroenterol Hepatol [Internet]. 2009;7(6):682-688.e5. Available from: http://dx.doi.org/10.1016/j.cgh.2009.02.016

10. Dong Y, Zhou J, Zhu Y, Luo L, He T, Hu H, et al. Abdominal obesity and colorectal cancer risk: systematic review and meta-analysis of prospective studies. Biosci Rep [Internet]. 2017;37(6):1–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29026008%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5725611

11. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer [Internet]. 2018 Nov 1;103:356–87. Available from: https://doi.org/10.1016/j.ejca.2018.07.005

12. Cardoso R, Guo F, Heisser T, Hackl M, Ihle P, De Schutter H, et al. Colorectal cancer incidence, mortality, and stage distribution in European countries in the colorectal cancer screening era: an international population-based study. Lancet Oncol. 2021;22(7):1002–13.

13. Adriaenssens N, Coenen S, Versporten A, Muller A, Minalu G, Faes C, et al. European Surveillance of Antimicrobial Consumption (ESAC): Outpatient antibiotic use in Europe (1997-2009). J Antimicrob Chemother. 2011;66(SUPPL. 6):3–12.

14. Dik VK, van Oijen MGH, Smeets HM, Siersema PD. Frequent Use of Antibiotics Is

Associated with Colorectal Cancer Risk: Results of a Nested Case–Control Study. Dig Dis Sci [Internet]. 2016;61(1):255–64. Available from: http://link.springer.com/10.1007/s10620-015-3828-0

15. Falagas ME, Walker AM, Jick H, Ruthazer R, Griffith J, Snydman DR. Late incidence of cancer after metronidazole use: a matched metronidazole user/nonuser study. Clin Infect Dis an Off Publ Infect Dis Soc Am. 1998 Feb;26(2):384–8.

16. Friedman GD, Coates AO, Potter JD, Slattery ML. Drugs and colon cancer. Pharmacoepidemiol Drug Saf. 1998 Mar;7(2):99–106.

17. Friedman GD, Jiang SF, Udaltsova N, Quesenberry CP, Chan J, Habel LA. Epidemiologic evaluation of pharmaceuticals with limited evidence of carcinogenicity. Int J Cancer. 2009;125(9):2173–8.

18. Wang J-L, Chang C-H, Lin J-W, Wu L-C, Chuang L-M, Lai M-S. Infection, antibiotic therapy and risk of colorectal cancer: A nationwide nested case-control study in patients with Type 2 diabetes mellitus. Int J Cancer [Internet]. 2014;135(4):956–67. Available from: http://doi.wiley.com/10.1002/ijc.28738

19. Boursi B; Haynes K; Ronac M ;Yu-Xiao Y; Perelman. Impact of antibiotic exposure on the risk of colorectal cancer.

20. Kilkkinen A, Rissanen H, Klaukka T, Pukkala E, Heliövaara M, Huovinen P, et al. Antibiotic use predicts an increased risk of cancer. Int J Cancer. 2008;123(9):2152–5.

21. Armstrong D, Dregan A, Ashworth M, White P, McGee C, de Lusignan S. The association between colorectal cancer and prior antibiotic prescriptions: case control study. Br J Cancer [Internet]. 2020;122(6):912–7. Available from: http://dx.doi.org/10.1038/s41416-019-0701-5

22. Zhang J, Haines C, Watson AJM, Hart AR, Platt MJ, Pardoll DM, et al. Oral antibiotic use and risk of colorectal cancer in the United Kingdom, 1989-2012: A matched case-control study. Gut. 2019;68(11):1971–8.

23. Didham RC, Reith DM, McConnell DW, Harrison KS. Antibiotic exposure and breast cancer in New Zealand. Breast Cancer Res Treat. 2005;92(2):163–7.

24. Cao Y, Wu K, Mehta R, Drew DA, Song M, Lochhead P, et al. Long-term use of antibiotics and risk of colorectal adenoma. Gut [Internet]. 2017;gutjnl-2016-313413. Available from: http://gut.bmj.com/lookup/doi/10.1136/gutjnl-2016-313413

25. Sanyaolu LN, Oakley NJ, Nurmatov U, Dolwani S, Ahmed H. Antibiotic exposure and the risk of colorectal adenoma and carcinoma: a systematic review and meta-analysis of observational studies. Color Dis. 2020;22(8):858–70.

26. Qu G, Sun C, Sharma M, Uy JP, Song EJ, Bhan C, et al. Is antibiotics use really associated with increased risk of colorectal cancer? An updated systematic review and meta-analysis of observational studies. Int J Colorectal Dis. 2020;35(8):1397–412.

27. Simin J, Fornes R, Liu Q, Olsen RS, Callens S, Engstrand L, et al. Antibiotic use and risk of colorectal cancer: a systematic review and dose–response meta-analysis. Br J Cancer [Internet]. 2020;123(12):1825–32. Available from: http://dx.doi.org/10.1038/s41416-020-01082-2

28. Truyers C, Goderis G, Dewitte H, Akker M vanden, Buntinx F. The Intego database: background, methods and basic results of a Flemish general practice-based continuous morbidity registration project. BMC Med Inform Decis Mak [Internet].

2014;14(1):48. Available from: http://bmcmedinformdecismak.biomedcentral.com/articles/10.1186/1472-6947-14-48

29. Mamouris P, Nassiri V, Molenberghs G, van den Akker M, van der Meer J, Vaes B. Fast and optimal algorithm for case-control matching using registry data: application on the antibiotics use of colorectal cancer patients. BMC Med Res Methodol. 2021;21(1):1–9.

30. Shi X, Nikolic G, Van Pottelbergh G, van den Akker M, Vos R, De Moor B. Development of Multimorbidity Over Time: An Analysis of Belgium Primary Care Data Using Markov Chains and Weighted Association Rule Mining. Journals Gerontol Ser A. 2020;XX(Xx):1–8.

31. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment. 22nd editi. WHO Collaborating Centre for Drug Statistics Methodology, editor. 2019.

32. R Foundation for Statistical Computing, Vienna A. R Core Team (2019). R: A language and environment for statistical computing. [Internet]. 2019. Available from: https://www.r-project.org/

33. Suda KJ, Hicks LA, Roberts RM, Hunkler RJ, Danziger LH. A national evaluation of antibiotic expenditures by healthcare setting in the United States, 2009. J Antimicrob Chemother. 2013;68(3):715–8.

34. Steinberg MB, Akincigil A, Kim EJ, Shallis R, Delnevo CD. Tobacco Smoking as a Risk Factor for Increased Antibiotic Prescription. Am J Prev Med [Internet]. 2016;50(6):692–8. Available from: http://dx.doi.org/10.1016/j.amepre.2015.11.009

35. Blix HS, Hjellvik V, Litleskare I, Rønning M, Tverdal A. Cigarette smoking and risk of subsequent use of antibacterials: A follow-up of 365117 men and women. J Antimicrob Chemother. 2011;66(9):2159–67.

36. Dejea C, Wick E, Sears CL. Bacterial oncogenesis in the colon HHS Public Access. Futur Microbiol [Internet]. 2013;8(4):445–60. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4052711/pdf/nihms513136.pdf

37. ECDC. Surveillance of antimicrobial resistance in Europe (2017). [Internet]. Surveillance of antimicrobial resistance in Europe. 2018. 1–71 p. Available from: https://ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2016

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Johannes Van der Meer: Conceptualization, Methodology, Investigation, Writing original draft, Visualization.
Pavlos Mamouris: Formal analysis, Data curation, Software.
Vahid Nassiri: Formal analysis, Data curation, Software.
Bert Vaes: Conceptualization, Methodology, Writing – Review & Editing.
Marjan van den Akker: Conceptualization, Methodology, Investigation, Writing – Review & Editing, Supervision.

Figure 1

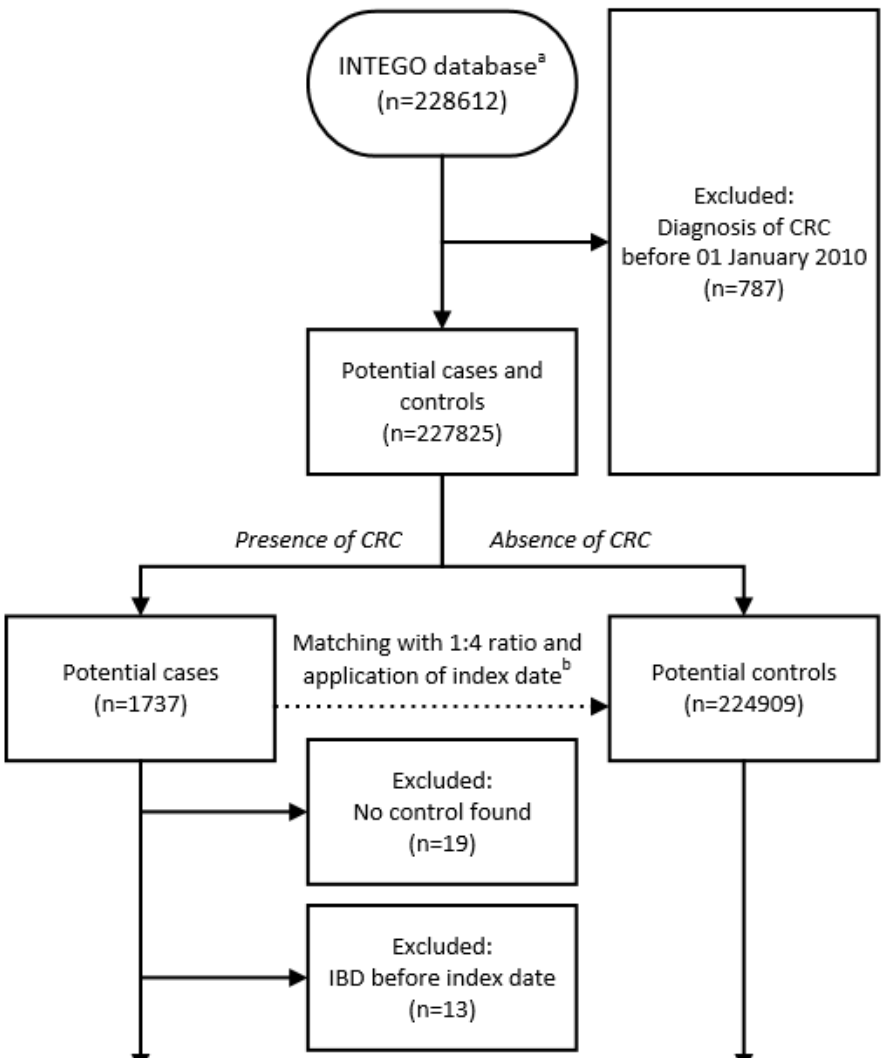
Patient selection flowchart

Figure 2

Relative antibiotic use per class per year (excluding two classes with <2 percent of total prescriptions)

Figure 3

Average cumulative prescriptions per case or control prior to index date



a. Total number of unique eligible subjects in the INTEGO database during 1st January 2010 till 31st December 2015
b. Controls with presence of IBD before the index date were excluded during matching

Figure 1

Patient selection flowchart

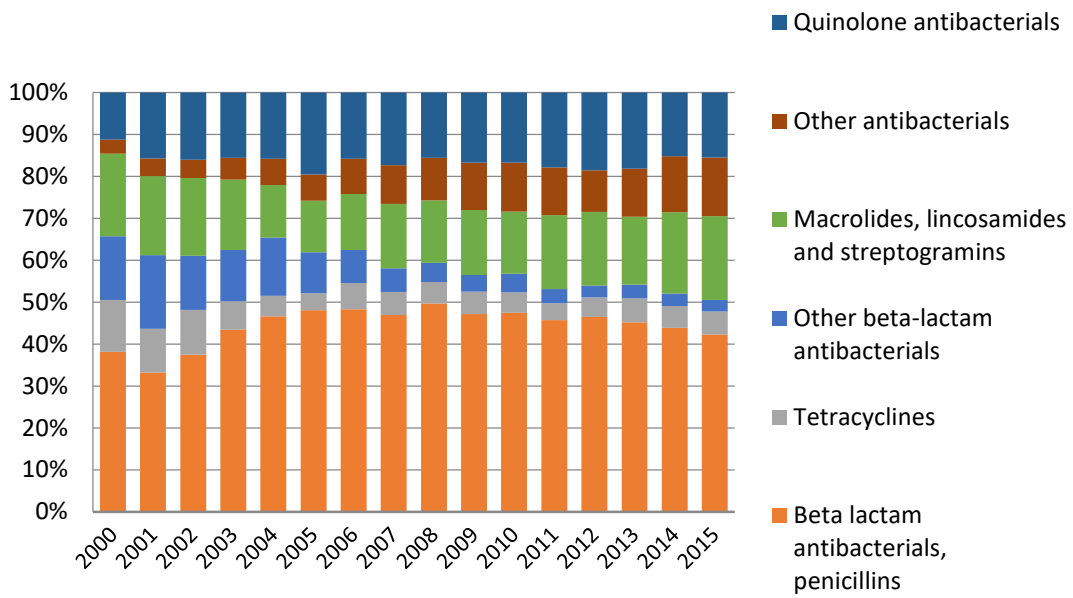
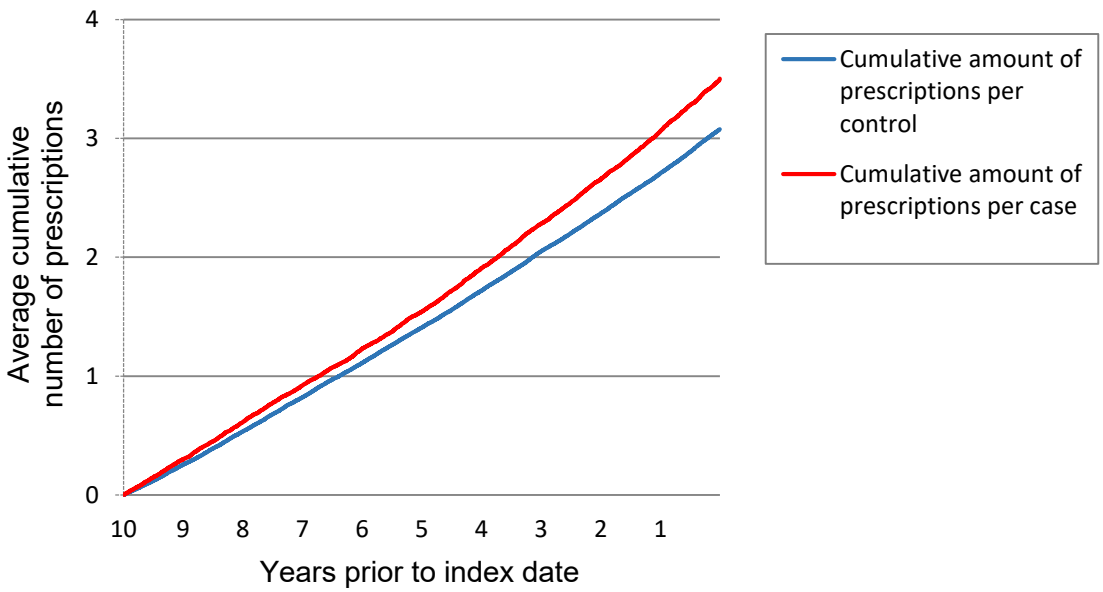


Figure 2

Relative antibiotic use per class per year (excluding two classes with <2 percent of total prescriptions)



*Observation period ranged from 1 – 10 years prior to the index date

Figure 3

Average cumulative prescriptions per case or control prior to index date*

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6,7
		(*d*) If applicable, explain how matching of cases and controls was addressed	5
		(*e*) Describe any sensitivity analyses	6
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1, page 5
		(b) Give reasons for non-participation at each stage	Figure 1, page 5
		(c) Consider use of a flow diagram	Figure 1, page 5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1, page 7
		(b) Indicate number of participants with missing data for each variable of interest	Table 1, page 7
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	7, 8, Table 2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7, 8, Table 2
		(*b*) Report category boundaries when continuous variables were categorized	7, 8, Table 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9,10
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.