The effect of past antibiotic exposure on diabetes risk

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Abstract

Objective—Gut microbiota influence metabolic pathways relevant to the pathogenesis of obesity, insulin-resistance and diabetes. Antibiotic therapy can alter the microbiota and is commonly used in western countries. We sought to evaluate whether past antibiotic exposure increases diabetes risk.

Research design and methods—We conducted a nested case-control study using a large population-based database from the United Kingdom (UK). Cases were defined as those with incident diagnosis of diabetes. For every case, 4 eligible controls matched on age, sex, practice-site, and duration of follow-up before index-date were selected using incidence-density sampling. Exposure of interest was antibiotic therapy >1 year before index-date. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using conditional logistic regression. The risk was adjusted for body mass index (BMI), smoking, last glucose level and number of infections before index-date, as well as past medical history of coronary artery disease and hyperlipidemia.

Results—The study included 208,002 diabetic cases and 815,576 matched controls. Exposure to a single antibiotic prescription was not associated with higher adjusted diabetes risk. Treatment with 2–5 antibiotic courses was associated with increase in diabetic risk for penicillin, cephalosporins, macrolides and quinolones with adjusted OR ranging from 1.08 (95%CI 1.05–1.11) for penicillin to 1.15 (95%CI 1.08–1.23) for quinolones. The risk increased with the number of antibiotic courses and reached 1.37 (95%CI 1.19–1.58) for >5 courses of quinolones. There was no association between exposure to anti-virals and anti-fungals and diabetes risk.

Conclusions—Exposure to certain antibiotic groups increases diabetes risk.

Keywords

Antibiotic; penicillin; type 2 diabetes; type 1 diabetes; risk factor

Introduction

The composition and diversity of the gut microbiota affect pathways related to metabolism, inflammation and immunity and are highly associated with human health and disease. Specific changes in the microbiota were described in a wide range of diseases from inflammatory bowel disease (IBD) to atherosclerosis and cancer (1–4).

In the past decades obesity, insulin resistance and type 2 diabetes have become a worldwide epidemic (5). Recent studies emphasize the role of human gut microbiota in the pathogenesis of those metabolic conditions, showing increased prevalence among individuals with altered microbiota and low bacterial diversity (6,7). Additional studies demonstrated different microbiota composition in children with type 1 diabetes. Those works suggested difference in immune reactivity against certain bacterial strains in the pathogenesis of the disease as well as other auto-immune diseases (8,9).

Diet may alter the microbiota composition, influencing the body's ability to utilize energy from food. Changes in the relative abundance of Bacteroidetes and Firmicutes, the two dominant bacterial phyla in the human gut, were described in obese subjects (6). Diets high in animal protein and animal fat were also associated with Bacteroides abundance while Prevotella was associated with diets high in carbohidrates (7,10,11). Similarly, in the ob/ob mouse model there was reduced abundance of Bacteroidetes and increase in Firmicutes compared to lean mice. Those changes in microbiota composition were shown to increase the capacity to harvest energy from diet (12). Germ-free mice remain lean despite consuming a high-fat, sugar-rich diet (13) and compensate for the reduced energy harvest due to lack of microbiota by higher dietary intake (14). Moreover, the obese trait was shown to be transmissible by colonization of germ-free mice with obese mice microbiota (15).

Prolonged inflammation was also associated with insulin resistance and diabetes. Several works suggest that gut micobiota is involved in chronic intestinal inflammation (5), increased gastrointestinal permeability and metabolic endotoxemia, contributing to the development of insulin resistance (16). Increase in the level of bacterial 16S rDNA in blood was associated with higher diabetes risk in humans (17). Additionally, mice deficient in TLR5 or in components of the inflammasome were prone to metabolic syndrome and type 2 diabetes (18, 19).

Moreover, microbial products, such as short chain fatty acids (SCFA), affect metabolism (2). Those small molecules were also shown to regulate intestinal gluconeogenesis, thus influencing diabetes risk (20, 21).

Two recent studies demonstrated change in gut microbiota in patients with type 2 diabetes. In those large metagenome wide association studies from China and Europe, there was an

increase in the gram negative Bacteroides species and certain Lactobacillus species among diabetic patients as well as increased expression of pro-inflammatory genes (3, 4)

Two additional clinical observations support the association between gut microbiota and diabetes risk. Following fecal transplantation from lean donors, insulin sensitivity of recipients with metabolic syndrome increased along with levels of butyrate-producing intestinal microbiota (22). In post-gastric-bypass individuals diabetes can resolve even before any change in weight, possibly due to shift in bacterial population with reduction in firmicutes and increase in bacteroides species. Those changes may be caused by the surgical procedure itself or be the consequence of change in diet (23,24).

Antibiotic therapy can alter the gut microbiota. In mice model, low dose penicillin disrupt the microbiota, alter metabolic genes and induce adiposity (25,26). In both ob/ob and diet-induced obese mice antibiotic therapy maximally suppressing the numbers of aerobic and anaerobic bacteria improved glycemic control independent of food intake and changed the hormonal, inflammatory, and metabolic status of the host (27). Recent works in humans showed that antibiotic exposure among infants during the first year of life was associated with an increased risk of overweight and central adiposity later in life (28). However, it's unclear whether change in the microbiota is the cause or a consequence of insulin resistance and diabetes.

Since antibiotics are commonly used in western countries, the aim of the current study is to evaluate whether past antibiotic exposure is associated to diabetes risk.

Subjects and methods

Study Design

We conducted a nested case-control study with incidence density sampling using The Health Improvement Network (THIN), a large population-based database from the UK. This design is computationally more efficient than a cohort study, and produces odds ratios (ORs) that are unbiased estimates of incidence-rate ratios (29). The study was approved by the Institutional Review Board at the University of Pennsylvania and by the Scientific Review Committee of THIN.

Data source

THIN is a large population-based electronic medical records database from the UK that contains comprehensive medical records on approximately 10 million patients under the care of general practitioners in 570 practices. THIN includes information on patient demographics, socioeconomic status, medical diagnoses, lab results and drug prescriptions. Under the National Health Services, 98% of the UK population receives all forms of health care through their general practitioners, thus the demographic and geographic distributions of the THIN population are broadly representative of those of the general UK. Registration date is defined as the date when patients were first registered with a practice in THIN and Vision date is the date that a practice began using in-practice Vision software which collects information for the THIN database (30). Data quality is monitored through routine analysis

of the entered data (31,32). The database has been previously used for pharmaco-epidemiology studies, showing excellent quality of information (33,34).

Study cohort

All people receiving medical care from 1995–2013 from a THIN practitioner were eligible for inclusion. 1,804,170 (15.34%) patients without acceptable medical records (i.e., patients with incomplete documentation or out of sequence date of birth, registration date, date of death, or date of exit from the database) were excluded. Follow-up started at the later of either the Vision date or 183 days after the date at which the patient registered with the general practitioner (35), and ended on the diabetes diagnosis date for those diagnosed with diabetes during the follow-up and on the earliest of date of death, transferring out of the database, or reach the end date of the database for controls.

Case selection

Cases were defined as all individuals in the cohort with at least one Read code (the standard clinical terminology system used in general practices in the UK) for diabetes mellitus during the follow-up period. Subjects who were diagnosed with the diabetes within the first 183 days after initiation of follow-up were excluded in order to avoid prevalent cases (35).

Selection of controls

Selection of the control group was based on incidence-density sampling (29). The eligible control pool for each case consisted of all individuals without a diagnosis of diabetes at the date the case was diagnosed. For each case, up to four eligible controls were matched on age at index, sex, practice site, duration and calendar period of follow-up.

Exposures and Covariates

The primary exposure of interest was any exposure more than one year prior to the index date to one of seven antibiotic groups commonly used in the outpatient setting: penicillins, cephalosporins, macrolides, tetracyclines, sulphonamides, quinolones, and imidazole. The analysis was performed for each antibiotic class separately. We also assessed anti-viral and anti-fungal medications as potential negative controls. For each of the antibiotic classes we evaluated the effect of number of antibiotic courses (0, 1, 2–5 and >5 courses). As potential confounders, we examined a comprehensive list of variables including obesity (BMI>30), smoking history (ever or never); medical co-morbidities including coronary artery disease, and hyperlipidemia treated with statins; number of previous urinary, skin and respiratory infections; and last glucose measure before or at diagnosis date. All covariates were measured prior to the index date.

Statistical Analysis

The baseline characteristics of cases and controls were compared using chi-square test for categorical variables and t-test for continuous variables. The primary analysis was a conditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CIs) for the association between number of antibiotic courses for each antibiotic class and diabetes risk. The reference exposure group consisted of individuals without documented

therapy with the specific antibiotic. The analysis was adjusted for all potential confounders. Bonferroni correction was performed to account for multiple comparisons due to assessment of 9 treatment groups (7 antimicrobial groups as well as anti-viral and anti-fungal). The analysis was repeated only among individuals without urinary or skin infections before index date to minimize indication bias. For penicillins, due to the large number of prescriptions and high statistical power, we were able to repeat the analysis among individuals with last antibiotic prescription more than 5 years before diagnosis date. In this analysis we didn't adjust to last glucose value and number of infections before index date since they occurred after exposure. In an additional sensitivity analysis we evaluated the association between antibiotic exposure and auto-immune type 1 diabetes compared to type 2 disease that is associated with insulin resistance. The definition for type 1 diabetes included individuals with specific Read codes for the disease, individuals who initiated insulin therapy within 90 days of diagnosis and individuals below the age of 30 at the time of diagnosis. Furthermore, since glucose level might be part of the causal pathway between insulin resistance and diabetes, infection and antibiotic use we repeated the analysis without adjustment to this variable.

All analyses were performed using STATA 13 (Stata Corp., College Station, Tx, USA).

Results

The study population included 208,002 diabetic cases and 815,576 matched controls (Table 1). As expected, diabetic patients had elevated risk for high BMI, hyperlipidemia and coronary artery disease, as part of the metabolic syndrome. Additionally, cases had higher rate of infections before diagnosis date compared to controls, especially of the urinary tract (19.3% vs. 15.1%) and skin (17.8% vs. 12.4%). The most commonly prescribed antibiotic among the study population was penicillin with 426,159 subjects (41.6%) receiving at least one prescription during follow-up period, followed by macrolides (150,996 subjects, 14.8%) and TMP/SMX (120,494 subjects, 11.8%).

The univariate analysis demonstrated increased diabetes risk with all antibiotic groups, reaching to an OR of 2.12 (95% CI 2.08–2.17) for exposure to more than 5 antibiotic courses of penicillins (Table 2). In the multivariate analysis, a single antibiotic prescription more than one year before diagnosis date was not associated with higher adjusted diabetes risk in all antibiotic groups after applying the Bonferroni correction. The only exception was treatment with cephalosporins that was associated with a modest increase in diabetes risk (adjusted OR 1.09, 95% CI 1.05–1.13). Treatment with 2–5 antibiotic courses was associated with increase in diabetes risk for penicillin, cephalosporins, macrolides and quinolones with adjusted OR ranging from 1.08 (95% CI 1.05–1.11) for penicillin to 1.15 (95% CI 1.08–1.23) for quinolones. The risk increased with the number of antibiotic courses and reached 1.37 (95% CI 1.19–1.58) for more than 5 courses of quinolones. For 2–5 antibiotic courses there was a modest increase in diabetes risk with TMP/SMX use (adjusted OR 1.1, 95% CI 1.05–1.15). For more than 5 antibiotic courses there was also an elevated risk with tetracyclines (adjusted OR 1.21, 95% CI 1.09–1.33). There was no association between treatment with imidazole, anti-virals and anti-fungals and diabetes risk independent of number of treatment courses (Table 2).

In a sensitivity analysis performed only among subjects without urinary tract, or skin infections before index date there was no change in results apart from lack of association between any number of TMP/SMX courses and diabetes risk (Table 3). Additionally, among subjects with last penicillin prescription more than 5 years before diagnosis with diabetes the unadjusted ORs were 1.18 (95%CI 1.15–1.21), 1.57 (95%CI 1.43–1.74) and 1.87 (95%CI 1.39–2.52) for 1, 2–5 and more than 5 antibiotic courses, respectively, all with p<0.0001. The adjusted ORs were 1.05 (95%CI 1.02–1.08, p=0.002), 1.23 (95%CI 1.1–1.37, p<0.0001), 1.33 (95%CI 0.95–1.86, p=0.09), respectively.

When only subjects with type 1 diabetes were analyzed, there was an association only between exposure to more than 5 courses of penicillin or 2–5 courses of cephalosporin and disease risk (ORs 1.41 95%CI 1.11–1.78 and 1.63 95%CI 1.26–2.11, respectively). No association was observed between other antibiotic groups or number of antibiotic courses prescribed and diabetes risk (Table 4).

In an additional sensitivity analysis not adjusted to last glucose level before or at diagnosis, there was an even higher increase in diabetes risk. In this analysis the risk was demonstrated with all antibiotic groups and with both type 1 and type 2 diabetes (data not shown). The risk for type 1 diabetes increased with the number of antibiotic courses from 1.09 (95%CI 1.03–1.16) for a single course of penicillin to 1.67 (95%CI 1.53–1.83) for more than 5 courses.

Discussion

In this large population based study we showed an association between past antibiotic exposure and diabetes risk. This association was more pronounced with increased number of antibiotic courses prescribed. While the univariate analysis demonstrated a higher risk with exposure to all antibiotic groups and for both type 1 and type 2 diabetes, the adjusted analysis revealed an increase in risk only after exposure to more than one course of penicillin, cephalosporins, macrolides and quinolones and showed almost no change in risk for participants with type 1 diabetes. The OR reached 1.15 (95% CI 1.08–1.23, p<0.0001) for 2–5 courses and 1.37 (95%CI 1.19–1.58, p<0.0001) for more than 5 courses of quinolones. There was no increase in risk with exposure to anti-viral or anti-fungal medications. There was no change in association when a stratified analysis, only among subjects without previous urinary, or skin infections before index date was performed.

Those results are consistent with previous studies in humans, showing microbial changes in patients with type 2 diabetes (3,4) and increased risk of overweight among infants who were exposed to antibiotics during the first year of life (28). They are also consistent with mice models showing disruption of the microbiota, altered metabolic gene expression and induced adiposity following low dose antibiotic (25,26). However, in models of obese mice antibiotic administration with maximal suppression of the microbiota improved glycemic control (27). One possible explanation is that certain bacteria are necessary in order to cause the phenotype of obesity and diabetes and low dose antibiotics might increase the "necessary pro-diabetes microbiota". The fact that germ free mice remain lean even after consuming western diet supports the role of a "pro-diabetes microbiota" (13).

The current study had more than 1 million subjects, of them 208,002 diabetic patients, with follow-up of up to 18 years. Only individuals that were diagnosed more than 6 months after initiation of follow-up were considered as cases in order to avoid prevalent cases. Over 40% of participants had at least one antibiotic prescription during the study duration. By matching cases and controls on duration and calendar period of follow-up as well as practice site we avoided time-window bias and minimized confounding secondary to different clinical judgment regarding antibiotic prescription and type of antibiotic used.

In order to avoid possible protopathic bias (i.e., infections in the period before diagnosis could be the results of infections associated with undiagnosed diabetes) we analyzed only antibiotic prescriptions given more than one year before diagnosis date. Adjustment to the number of previous urinary, skin and respiratory tract infections, as well as the sensitivity analysis among subjects without previous urinary and skin infections, known to be higher in diabetic patients, limited the possibility of confounding by indication. For penicillins, we were able to analyze the effect of antibiotic exposure more than 5 years before diabetes was diagnosed, allowing us to evaluate possible reverse causation in which the pre-diabetes state of insulin resistance increased infection risk and antibiotic use. The results for this sensitivity analysis were similar to the overall analysis pointing against possible overuse of antibiotics due to pre-diabetes state.

We further adjusted to obesity, hyperlipidemia treated with statins, last glucose level before or at diagnosis and coronary artery disease. All those variables, together with diabetes, are part of the metabolic syndrome. Those parameters are associated with diabetes as well as with change in the microbiota and thus serve as possible confounders.

The current work had several limitations. Information regarding BMI was missing for approximately 30% of the study population, since unlike prescription or medical diagnosis information in THIN, recording of BMI data was not mandated by the general practices. We analyzed subjects with missing BMI information as a separate group and additionally performed complete-subject analysis which showed no change in results. Of note, obesity is the known risk factor for diabetes and metabolic syndrome, the percent of obese (BMI>30) participants in the current study was approximately 22% compared to the reported 26% of the UK population in 2010 (HSCIC: Statistics on Obesity, Physical Activity and Diet - England, 2012).

Cases were defined according to at least one Read code for diabetes mellitus. Since many codes did not specify the type of diabetes that was diagnosed we included, in addition to specific type 1 diabetes codes, age less than 30 and initial insulin prescription within 90 days of diagnosis as additional definitions for the disease. The overall analysis was performed in patients with either type 1 or type 2 diabetes.

Our observational study didn't include information regarding the microbiota composition of cases and controls and thus cannot infer causality. However, since change in the microbiota was previously demonstrated in humans in the entire spectrum of pathologies from obesity to insulin resistance and diabetes and since earlier studies showed antibiotic exposure as a risk factor for obesity (3,4,28), our results may support a possible hypothesis that change in

the gut microbiota secondary to antibiotic exposure increase diabetes risk. The need for recurrent antibiotic exposures in addition to the lack of known direct harmful effect of antibiotics on the beta cells of the pancreas are in concordance with such a hypothesis.

We had no information regarding whether the antibiotics prescribed were actually taken, however we expect that if compliance was actually lower it would have change our results toward the null hypothesis. People may also use more than one type of antibiotic during follow-up, making it difficult to differentiate between the effects of different antibiotic groups. Our study results, showing differential effect between groups as well as compared to anti-viral and most anti-fungal medications point against possible surveillance bias (i.e people with infections are more likely to be diagnosed with diabetes). Since we performed multiple comparison for exposure to various pre-defined antibiotic groups we used the conservative Bonferonni correction in the final statistical analysis.

Importantly, although both unadjusted and adjusted analysis showed an association between antibiotic exposure and diabetes risk, the results differ in the magnitude of risk, the antibiotic groups associated with the risk and the effect on type 1 diabetes (increased risk in the univariate analysis vs. almost no risk in the adjusted analysis). While the lack of association in type 1 diabetes might be explained by the different auto-immune pathogenesis of the disease, possibly viral induced (36,37), we couldn't rule out over adjustment, decreasing the risk toward the null hypothesis, as an alternate explanation for our results. For this reason we conducted a sensitivity analysis without adjustment for last glucose levels that might serve as part of a causal pathway between antibiotic exposure and diabetes risk. In this analysis antibiotic exposure was associated with type 1 diabetes. Those contrasting results prevent us from clear conclusions regarding type 1 diabetes and further studies are needed.

In summary, we demonstrated a higher adjusted risk for type 2 diabetes among individuals with recurrent exposures to penicillin, cephalosporins, macrolides and quinolones. There was no increase in adjusted risk for exposure to anti-viral or anti-fungal medications. Despite the observational design and possibility of unmeasured confounding, the results are consistent with previous works that described altered microbiota in obese individuals as well as in subjects with insulin resistance and increase in obesity among individuals exposed to antibiotics during the first year of life. It is also consistent with animal models showing that the obese phenotype can be transformed by transplanting "obesity related bacteria" or induced by low dose antibiotic. Future studies in humans should focus on the changes in microbiota that are caused by repeated antibiotic exposure; the effect of antibiotic exposure in different age groups; the genetic pathways that are affected by the microbiota; and whether antibiotic administration in diabetic patients might improve glycemic control.

Acknowledgments

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Table 1

Characteristics of cases and controls

	Cases (N=208,002)	Controls (N=815,576)	Crude OR
Age (yr, SD)	60.5 (16.0)	60.2 (16.0)	NA
Sex (% Male, N)	53.3% (110,831)	53.3% (434,574)	NA
Follow-up duration (yr, SD)	5.5 (3.9)	5.5 (3.9)	NA
Obesity (BMI>30) (%, N)	40.6% (84,415)	15.1% (123,300)	4.49 (4.44–4.55)
Ever smoking (%, N)	46.6% (96,977)	36.4% (296,843)	1.65 (1.63–1.67)
Coronary artery disease (%, N)	13.3% (27,694)	7.0% (56,895)	2.18 (2.14–2.21)
Hyperlipidemia Rx Statins (%, N)	22.1% (45,905)	13.5% (109,683)	2.1 (2.07–2.13)
Urinary tract infection (%, N)	19.3% (40,051)	15.1% (123,329)	1.05 (1.04–1.05)
Skin infection (%, N)	17.8% (37,082)	12.4% (101,027)	1.11 (1.11–1.12)
Pneumonia (%, N)	3.8% (7,954)	2.7% (21,727)	1.28 (1.26–1.31)
Upper respiratory tract infection(%, N)	16.9% (35,225)	14.1% (114,990)	1.11 (1.1–1.11)
Last glucose before or at diagnosis date (mmol/L, SD)	9.1 (3.8)	5.3 (1.0)	2.76 (2.73–2.79)

Table 2

Diabetes risk as a function of the number of specific antibiotic courses

Antibiotic type	Number of antibiotic courses	Cases (N=208,002)	Controls (N=815,576)	Crude OR	Adjusted OR *,1
Penicillins	0	110,539 (53.14%)	486,880 (59.7%)	Ref.	Ref.
	1	34,344 (16.51%)	133,063 (16.32%)	1.24 (1.22–1.25) P<0.0001	1.02 (0.99–1.06) P=0.18
	2–5	45,356 (21.81%)	150,883 (18.5%)	1.51 (1.49–1.53) P<0.0001	1.08 (1.05–1.11) P<0.0001
	>5	17,763 (8.54%)	44,750 (5.49%)	2.12 (2.08–2.17) P<0.0001	1.23 (1.18–1.28) P<0.0001
Cephalosporines	0	181,218 (87.12%)	734,860 (90.1%)	Ref.	Ref.
	1	15,497 (7.45%)	49,092 (6.02%)	1.34 (1.31–1.37) P<0.0001	1.09 (1.05–1.13) P<0.0001
	2–5	9,266 (4.45%)	26,889 (3.3%)	1.49 (1.46–1.53) P<0.0001	1.11 (1.06–1.17) P<0.0001
	>5	2,021 (0.97%)	4,735 (0.58%)	1.89 (1.79–2.0) P<0.0001	1.32 (1.19–1.46) P<0.0001
Macrolides	0	170,682 (82.06%)	701,900 (86.06%)	Ref.	Ref.
	1	20,535 (9.87%)	67,448 (8.27%)	1.3 (1.28–1.32) P<0.0001	1.05 (1.01–1.08) P=0.01
	2–5	13,654 (6.56%)	38,824 (4.76%)	1.52 (1.49–1.55) P<0.0001	1.11 (1.07–1.16) P<0.0001
	>5	3,131 (1.51%)	7,404 (0.91%)	1.86 (1.78–1.94) P<0.0001	1.32 (1.22–1.43) P<0.0001
Quinolones	0	192,616 (92.6%)	770,205 (94.44%)	Ref.	Ref.
	1	9,803 (4.71%)	30,392 (3.73%)	1.31 (1.28–1.35) P<0.0001	1.05 (1.0–1.1) P=0.05
	2–5	4,675 (2.25%)	12,866 (1.58%)	1.49 (1.44–1.54) P<0.0001	1.15 (1.08–1.23) P<0.0001
	>5	908 (0.44%)	2,113 (0.26%)	1.78 (1.65–1.93) P<0.0001	1.37 (1.19–1.58) P<0.0001
TMP/SMX	0	180,047 (86.56%)	723,037 (88.65%)	Ref.	Ref.
	1	16,375 (7.87%)	56,353 (6.91%)	1.19 (1.17–1.22) P<0.0001	0.98 (0.94–1.02) P=0.31
	2–5	9,652 (4.64%)	30,700 (3.76%)	1.31 (1.28–1.34) P<0.0001	1.1 (1.05–1.15) P<0.0001

Antibiotic type	Number of antibiotic courses	Cases (N=208,002)	Controls (N=815,576)	Crude OR	Adjusted OR*,[1]
	>5	1,928 (0.93%)	5,486 (0.67%)	1.48 (1.41–1.56) P<0.001	1.08 (0.98–1.19) P=0.14
Tetracyclines	0	186,719 (89.77%)	745,298 (91.38%)	Ref.	Ref.
	1	12,902 (6.2%)	42,940 (5.26%)	1.22 (1.2–1.255) P<0.0001	1.06 (1.01–1.11) P=0.008
	2–5	6,527 (3.14%)	21,068 (2.58%)	1.27 (1.23–1.31) P<0.0001	1.05 (0.99–1.12) P=0.07
	>5	1,854 (0.89%)	6,270 (0.77%)	1.21 (1.15–1.28) P<0.0001	1.21 (1.09–1.33) P=0.001
Imidazole	0	198,482 (95.42%)	785,703 (96.34%)	Ref.	Ref.
	1	7,129 (3.43%)	23,240 (2.85%)	1.23 (1.2–1.27) P<0.0001	1.06 (1.0–1.12) P=0.03
	2–5	2,202 (1.06%)	6,245 (0.77%)	1.43 (1.36–1.51) P<0.0001	1.06 (0.97–1.17) P=0.21
	>5	189 (0.09%)	388 (0.05%)	1.97 (1.66–2.35) P<0.0001	1.26 (0.9–1.76) P=0.18
Anti-fungals	0	195,344 (93.91%)	773,351 (94.82%)	Ref.	Ref.
	1	6,833 (3.29%)	22,275 (2.73%)	1.23 (1.19–1.26) P<0.0001	1.04 (0.98–1.09) P=0.22
	2–5	4,683 (2.25%)	16,216 (1.99%)	1.16 (1.12–1.2) P<0.0001	1.05 (0.99–1.12) P=0.1
	>5	1,142 (0.55%)	3,734 (0.46%)	1.23 (1.15–1.31) P<0.0001	1.01 (0.9–1.15) P=0.82
Anti-virals	0	203,035 (97.61%)	797,785 (97.82%)	Ref.	Ref.
	1	4,351 (2.09%)	15,443 (1.89%)	1.11 (1.07–1.15) P<0.0001	0.99 (0.93–1.06) P=0.76
	2–5	465 (0.22%)	1,756 (0.22%)	1.05 (0.95–1.16) P=0.37	0.92 (0.77–1.11) P=0.4
	>5	151 (0.07%)	592 (0.07%)	1.01 (0.84–1.21) P=0.91	0.95 (0.69–1.31) P=0.75

*
Adjusted for BMI, smoking, coronary artery disease, hyperlipidemia treated with statins, last glucose level before index date, number of urinary tract, skin and respiratory infections before index date.

[1]
After the Bonferroni correction, the p-value needed to show statistical significance was 0.006.

Table 3

Diabetes risk among subjects without previous urinary or skin infections, as a function of the number of specific antibiotic courses

Antibiotic type	Number of antibiotic courses	Cases (N=139,732)	Controls (N=440,587)	Crude OR	Adjusted OR*,1
Penicillins	0	80,349 (57.5%)	283,591 (64.37%)	Ref.	Ref.
	1	23,702 (16.96%)	70,488 (16.0%)	1.25 (1.23–1.28) P<0.0001	1.03 (0.99–1.08) P=0.13
	2–5	27,607 (19.76%)	70,447 (15.99%)	1.52 (1.49–1.54) P<0.0001	1.1 (1.06–1.15) P<0.0001
	>5	8,074 (5.78%)	16,061 (3.65%)	2.03 (1.96–2.09) P<0.0001	1.26 (1.18–1.34) P<0.0001
Cephalosporines	0	127,493 (91.24%)	412,250 (93.57%)	Ref.	Ref.
	1	8,112 (5.81%)	19,448 (4.41%)	1.35 (1.31–1.38) P<0.0001	1.13 (1.06–1.2) P<0.0001
	2–5	3,663 (2.62%)	7,985 (1.81%)	1.49 (1.43–1.55) P<0.0001	1.19 (1.1–1.3) P<0.0001
	>5	464 (0.33%)	904 (0.21%)	1.68 (1.49–1.88) P<0.0001	1.23 (0.98–1.55) P=0.08
Macrolides	0	118,791 (85.01%)	390,337 (88.59%)	Ref.	Ref.
	1	12,300 (8.8%)	31,353 (7.12%)	1.3 (1.27–1.33) P<0.0001	1.05 (1.0–1.1) P=0.08
	2–5	7,274 (5.21%)	16,314 (3.7%)	1.48 (1.44–1.53) P<0.0001	1.11 (1.04–1.18) P=0.001
	>5	1,367 (0.98%)	2,583 (0.59%)	1.75 (1.64–1.87) P<0.0001	1.45 (1.27–1.67) P<0.0001
Quinolones	0	132,504 (94.83%)	423,175 (96.05%)	Ref.	Ref.
	1	4,963 (3.55%)	12,447 (2.83%)	1.27 (1.23–1.32) P<0.0001	1.1 (1.03–1.19) P=0.007
	2–5	1,968 (1.41%)	4,447 (1.01%)	1.42 (1.34–1.5) P<0.0001	1.24 (1.12–1.38) P<0.0001
	>5	297 (0.21%)	518 (0.12%)	1.85 (1.6–2.14) P<0.0001	1.64 (1.24–2.16) P<0.0001
TMP/SMX	0	128,308 (91.82%)	410,732 (93.22%)	Ref.	Ref.
	1	7,876 (5.64%)	21,019 (4.77%)	1.17 (1.14–1.2) P<0.0001	0.97 (0.91–1.03) P=0.31
	2–5	3,212 (2.3%)	7,998 (1.82%)	1.28 (1.22–1.33) P<0.0001	1.12 (1.03–1.22) P=0.007

Antibiotic type	Number of antibiotic courses	Cases (N=139,732)	Controls (N=440,587)	Crude OR	Adjusted OR*,1
	>5	336 (0.24%)	838 (0.19%)	1.29 (1.14–1.47) P<00.001	1.1 (0.86–1.4) P=0.
Tetracyclines	0	127,458 (91.22%)	408,529 (92.72%)	Ref.	Ref.
	1	7,611 (5.45%)	19,855 (4.51%)	1.22 (1.18–1.25) P<0.0001	1.08 (1.01–1.15) P=0.02
	2–5	3,590 (2.57%)	9,273 (2.1%)	1.23 (1.18–1.28) P<0.0001	1.04 (0.95–1.13) P=0.42
	>5	1,073 (0.77%)	2,930 (0.67%)	1.18 (1.1–1.26) P<0.0001	1.28 (1.1–1.48) P=0.001
Imidazole	0	134,909 (96.55%)	428,520 (97.26%)	Ref.	Ref.
	1	3,782 (2.71%)	9,686 (2.2%)	1.2 (1.16–1.25) P<0.0001	1.04 (0.96–1.13) P=0.33
	2–5	969 (0.69%)	2,248 (0.51%)	1.33 (1.23–1.44) P<0.0001	1.05 (0.9–1.23) P=0.5
	>5	72 (0.05%)	133 (0.03%)	1.71 (1.28–2.29) P<0.0001	1.36 (0.75–2.48) P=0.31
Anti-fungals	0	137,722 (94.98%)	421,447 (95.66%)	Ref.	Ref.
	1	3,855 (2.76%)	10,127 (2.3%)	1.21 (1.16–1.25) P<0.0001	1.04 (0.96–1.13) P=0.34
	2–5	2,552 (1.83%)	7,311 (1.66%)	1.11 (1.06–1.16) P<0.0001	1.05 (0.96–1.16) P=0.27
	>5	603 (0.43%)	1,702 (0.39%)	1.13 (1.02–1.24) P=0.01	0.98 (0.82–1.18) P=0.85
Anti-virals	0	136,862 (97.95%)	432,631 (98.19%)	Ref.	Ref.
	1	2,514 (1.8%)	6,857 (1.56%)	1.11 (1.06–1.17) P<0.0001	0.97 (0.88–1.07) P=0.52
	2–5	271 (0.19%)	847 (0.19%)	1.02 (0.88–1.17) P=0.83	0.86 (0.66–1.13) P=0.28
	>5	85 (0.06%)	252 (0.06%)	1.08 (0.84–1.38) P=0.57	1.05 (0.67–1.66) P=0.82

*
Adjusted for BMI, smoking, coronary artery disease, hyperlipidemia treated with statins, last glucose level before index date, and number of respiratory infections before index date.

1
After the Bonferroni correction, the p-value needed to show statistical significance was 0.006.

Table 4

Diabetes risk among subjects with type 1 diabetes as a function of the number of specific antibiotic courses

Antibiotic type	Number of antibiotic courses	Cases (N=15,505)	Controls (N=61,418)	Crude OR	Adjusted OR*,[1]
Penicillins	0	8,723 (56.26%)	38,025 (61.91%)	Ref.	Ref.
	1	2,333 (15.05%)	9,469 (15.42%)	1.19 (1.13–1.26) P<0.0001	1.02 (0.86–1.22) P=0.8
	2–5	3,186 (20.55%)	10,810 (17.6%)	1.54 (1.46–1.62) P<0.0001	1.25 (1.06–1.48) P=0.007
	>5	1,263 (8.15%)	3,114 (5.07%)	2.35 (2.17–2.55) P<0.0001	1.41 (1.11–1.78) P=0.004
Cephalosporines	0	13,953 (89.99%)	56,900 (92.64%)	Ref.	Ref.
	1	933 (6.02%)	2,941 (4.79%)	1.38 (1.27–1.49) P<0.0001	1.06 (0.85–1.31) P=0.626
	2–5	518 (3.34%)	1,415 (2.3%)	1.63 (1.46–1.82) P<0.0001	1.63 (1.26–2.11) P<0.0001
	>5	101 (0.65%)	162 (0.26%)	2.85 (2.21–3.68) P<0.0001	1.99 (1.09–3.66) P=0.03
Macrolides	0	13,071 (84.3%)	54,049 (88.0%)	Ref.	Ref.
	1	1,402 (9.04%)	4,499 (7.33%)	1.35 (1.27–1.44) P<0.0001	1.1 (0.91–1.32) P=0.33
	2–5	837 (5.4%)	2,455 (4.0%)	1.51 (1.39–1.65) P<0.0001	1.18 (0.95–1.46) P=0.14
	>5	195 (1.26%)	415 (0.68%)	2.12 (1.78–2.53) P<0.0001	1.55 (1.01–2.38) P=0.04
Quinolones	0	14,858 (95.83%)	59,782 (97.34%)	Ref.	Ref.
	1	415 (2.68%)	1,260 (2.05%)	1.36 (1.21–1.52) P<0.0001	1.04 (0.79–1.36) P=0.78
	2–5	181 (1.17%)	338 (0.55%)	2.24 (1.86–2.7) P<0.0001	1.61 (1.06–2.45) P=0.03
	>5	51 (0.33%)	38 (0.06%)	5.52 (3.62–8.41) P<0.0001	2.1 (0.86–5.21) P=0.1
TMP/SMX	0	14,050 (90.62%)	56,716 (92.34%)	Ref.	Ref.
	1	914 (5.89%)	3,125 (5.09%)	1.21 (1.12–1.31) P<0.0001	0.93 (0.76–1.14) P=0.47
	2–5	463 (2.99%)	1,388 (2.26%)	1.41 (1.26–1.57) P<0.0001	1.12 (0.86–1.44) P=0.4

Antibiotic type	Number of antibiotic courses	Cases (N=15,505)	Controls (N=61,418)	Crude OR	Adjusted OR*,1
	>5	78 (0.5%)	189 (0.31%)	1.74 (1.33–2.28) P<0.0001	1.18 (0.63–2.21) P=0.6
Tetracyclines	0	14,424 (93.03%)	57,585 (93.76%)	Ref.	Ref.
	1	652 (4.21%)	2,171 (3.53%)	1.21 (1.1–1.32) P<0.0001	1.18 (0.94–1.49) P=0.15
	2–5	319 (2.06%)	1,194 (1.94%)	1.08 (0.95–1.23) P=0.24	0.95 (0.71–1.29) P=0.76
	>5	110 (0.71%)	468 (0.76%)	0.95 (0.77–1.17) P=0.63	1.08 (0.65–1.78) P=0.77
Imidazole	0	14,934 (96.32%)	59,655 (97.13%)	Ref.	Ref.
	1	385 (2.48%)	1,341 (2.18%)	1.17 (1.04–1.32) P=0.008	0.88 (0.67–1.15) P=0.35
	2–5	176 (1.14%)	403 (0.66%)	1.81 (1.5–2.17) P<0.0001	1.19 (0.82–1.73) P=0.37
	>5	10 (0.06%)	19 (0.03%)	2.22 (1.03–4.78) P=0.04	1.75 (0.37–8.2) P=0.48
Anti-fungals	0	14,725 (94.97%)	59,072 (96.18%)	Ref.	Ref.
	1	463 (2.99%)	1,469 (2.39%)	1.28 (1.15–1.43) P<0.0001	1.22 (0.92–1.61) P=0.17
	2–5	256 (1.65%)	764 (1.24%)	1.37 (1.19–1.59) P<0.0001	1.06 (0.76–1.49) P=0.72
	>5	61 (0.39%)	113 (0.18%)	2.25 (1.64–3.08) P<0.0001	1.29 (0.64–2.61) P=0.48
Anti-virals	0	15,252 (98.37%)	60,649 (98.75%)	Ref.	Ref.
	1	208 (1.34%)	648 (1.06%)	1.27 (1.08–1.49) P=0.003	1.27 (0.89–1.82) P=0.18
	2–5	32 (0.21%)	88 (0.14%)	1.47 (0.98–2.2) P=0.07	1.1 (0.49–2.49) P=0.82
	>5	13 (0.08%)	33 (0.05%)	1.55 (0.81–2.94) P=0.18	1.07 (0.24–4.81) P=0.93

*
Adjusted for obesity (BMI>30), ever smoking, coronary artery disease, hyperlipidemia treated with statins, last glucose level before index date, number of urinary tract, skin and respiratory infections before index date.

1
After the Bonferroni correction, the p-value needed to show statistical significance was 0.006.