The effect of past antibiotic exposure on diabetes risk

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Abstract

Objective: Gut microbiota influence metabolic pathways related 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 UK. The cases were defined as those with incident diagnosis of diabetes. For every case, four 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% CIs were estimated using conditional logistic regression. The risk was adjusted for BMI, smoking, last glucose level, and number of infections before index-date, as well as past medical history of coronary artery disease and hyperlipidaemia.

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 two to five 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 more than 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.

European Journal of
Endocrinology
(2015) 172, 639–648

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 to atherosclerosis and cancer (1, 2, 3, 4).

In the past decades, obesity, insulin resistance and type 2 diabetes have become a worldwide epidemic (5). Recent studies have emphasised 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).

Clinical Study	B Boursi and others	Antibiotic exposure and diabetes risk	172:6	640
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Diet may alter the microbiota composition, influencing the body's ability to utilise energy from food. Changes in the relative abundance of *Bacteroidetes* and *Firmicutes*, the two dominant bacterial phyla in the human gut, have been 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 carbohydrates (7, 10, 11). Similarly, in the Ob/Ob mice model, there was reduced abundance of *Bacteroidetes* and increase in *Firmicutes* compared with 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 colonisation of germ-free mice with obese mice microbiota (15).

Prolonged inflammation was also associated with insulin resistance and diabetes. Several works suggest that gut microbiota is involved in chronic intestinal inflammation (5), increased gastro-intestinal permeability and metabolic endotoxaemia, contributing to the development of insulin resistance (16). An increase in the level of bacterial 16S rDNA in blood was associated with higher diabetes risk in humans (17). In addition, 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, affect metabolism (2). Those small molecules were also shown to regulate intestinal gluconeogenesis, thus influencing diabetes risk (20, 21).

Two recent studies have 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 faecal transplantation from lean donors, insulin sensitivity of recipients with metabolic syndrome increased along with the levels of butyrate-producing intestinal microbiota (22). In post-gastric bypass individuals, diabetes can resolve even before any change in weight, possibly due to a 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 glycaemic control independent of food intake and changed the hormonal, inflammatory and metabolic status of the host (27). Recent works in humans have shown that antibiotic exposure among infants during the first year of life was associated with an increased the 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 of approximately ten million patients under the care of general practitioners in 570 practices. THIN includes information on patient demographics, socioeconomic status, medical diagnoses, laboratory 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 who received medical care from 1995 to 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. The 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 1 year before 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 hyperlipidaemia treated with statins; number of previous urinary, skin and respiratory infections; and last glucose measure before or at diagnosis date. All covariates were measured before the index date.

Statistical analyses

The baseline characteristics of cases and controls were compared using χ^2 test for categorical variables and *t*-test for continuous variables. The primary analysis was a conditional logistic regression to estimate ORs and 95% 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 nine treatment groups (seven 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 minimise 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 did not adjust to last glucose value and the number of infections before index date as they occurred after exposure. In an additional sensitivity analysis, we evaluated the association between antibiotic exposure and auto-immune type 1 diabetes compared with 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, hyperlipidaemia and coronary artery disease, as part of the metabolic syndrome. In addition, cases had higher rate of infections before diagnosis date compared with 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 five antibiotic courses of penicillins (Table 2). In the multivariate analysis, a single antibiotic prescription more than 1 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 two to five 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 five courses of quinolones. For two to five 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 five 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 the 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). In additionally, among subjects on 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 five 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 analysed, there was an association only between exposure to more than five courses of penicillin or two to five 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 five courses.

Table 1 Characteristics of cases and controls.

	Cases (n=208 002)	Controls (n=815 576)	Crude OR
Age (years, s.d.)	60.5 (16.0)	60.2 (16.0)	NA
Sex (% male, n)	53.3% (110 831)	53.3% (434 574)	NA
Follow-up duration (years, s.d.)	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)
Hyperlipidaemia 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% (7954)	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, s.d.)	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 ^{a,b}
Penicillins	0	110 539 (53.14%)	486 880 (59.7%)	References	References
	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%)	References	References
	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	9266 (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	2021 (0.97%)	4735 (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%)	References	References
	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	3131 (1.51%)	7404 (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%)	References	References
	1	9803 (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	4675 (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%)	2113 (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%)	References	References
	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	9652 (4.64%)	30 700 (3.76%)	1.31 (1.28–1.34) P<0.0001	1.1 (1.05–1.15) P<0.0001
	>5	1928 (0.93%)	5486 (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%)	References	References
	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	6527 (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	1854 (0.89%)	6270 (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%)	References	References
	1	7129 (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	2202 (1.06%)	6245 (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%)	References	References
	1	6833 (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	4683 (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	1142 (0.55%)	3734 (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%)	References	References
	1	4351 (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%)	1756 (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

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

^bAfter 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 ^{a,b}
Penicillins	0	80 349 (57.5%)	283 591 (64.37%)	References	References
	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	8074 (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%)	References	References
	1	8112 (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	3663 (2.62%)	7985 (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%)	References	References
	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	7274 (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	1367 (0.98%)	2583 (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%)	References	References
	1	4963 (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	1968 (1.41%)	4447 (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%)	References	References
	1	7876 (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	3212 (2.3%)	7998 (1.82%)	1.28 (1.22–1.33) P<0.0001	1.12 (1.03–1.22) P=0.007
	> 5	336 (0.24%)	838 (0.19%)	1.29 (1.14–1.47) P<0.0001	1.1 (0.86–1.4) P=0.
Tetracyclines	0	127 458 (91.22%)	408 529 (92.72%)	References	References
	1	7611 (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	3590 (2.57%)	9273 (2.1%)	1.23 (1.18–1.28) P<0.0001	1.04 (0.95–1.13) P=0.42
	> 5	1073 (0.77%)	2930 (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%)	References	References
	1	3782 (2.71%)	9686 (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%)	2248 (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%)	References	References
	1	3855 (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	2552 (1.83%)	7311 (1.66%)	1.11 (1.06–1.16) P<0.0001	1.05 (0.96–1.16) P=0.27
	> 5	603 (0.43%)	1702 (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%)	References	References
	1	2514 (1.8%)	6857 (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

^aAdjusted for BMI, smoking, coronary artery disease, hyperlipidaemia treated with statins, last glucose level before index date, and number of respiratory infections before index date.

^bAfter 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 ^{a,b}
Penicillins	0	8723 (56.26%)	38 025 (61.91%)	References	References
	1	2333 (15.05%)	9469 (15.42%)	1.19 (1.13–1.26) P<0.0001	1.02 (0.86–1.22) P=0.8
	2–5	3186 (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	1263 (8.15%)	3114 (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%)	References	References
	1	933 (6.02%)	2941 (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%)	1415 (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%)	References	References
	1	1402 (9.04%)	4499 (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%)	2455 (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%)	References	References
	1	415 (2.68%)	1260 (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%)	References	References
	1	914 (5.89%)	3125 (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%)	1388 (2.26%)	1.41 (1.26–1.57) P<0.0001	1.12 (0.86–1.44) P=0.4
	> 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%)	References	References
	1	652 (4.21%)	2171 (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%)	1194 (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%)	References	References
	1	385 (2.48%)	1341 (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%)	References	References
	1	463 (2.99%)	1469 (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%)	References	References
	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

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

^bAfter the Bonferroni correction, the P value needed to show statistical significance was 0.006.

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 two to five courses and 1.37 (95% CI 1.19–1.58, $P < 0.0001$) for more than five 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 glycaemic 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 one million subjects, of them 208 002 diabetic patients, with a follow-up of up to 18 years. Only individuals who 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 minimised 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 analysed only antibiotic prescriptions given

more than 1 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 analyse 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, hyperlipidaemia 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 ~30% of the study population, because unlike prescription or medical diagnosis information in THIN, recording of BMI data was not mandated by the general practices. We analysed 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 were ~22% compared with the reported 26% of the UK population in 2010 (HSCIC: Statistics on Obesity, Physical Activity and Diet – England, 2012).

The cases were defined according to at least one Read code for diabetes mellitus. Because many codes did not specify the type of diabetes that was diagnosed, in addition to specific type 1 diabetes codes, we included age <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 did not include information regarding the microbiota composition of cases and controls and thus cannot infer causality. However, as change in the microbiota was previously demonstrated in humans in the entire spectrum of pathologies from obesity to insulin resistance and diabetes and as 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 β -cells of the pancreas is 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 changed our results towards 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 with 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). As we performed multiple comparison for exposure to various pre-defined antibiotic groups, we used the conservative Bonferroni 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 could not rule out over adjustment, decreasing the risk towards 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. These 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, which 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 glycaemic control.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

The work was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR000003. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Author contribution statement

Dr Y-X Yang and Dr B Boursi had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. Y-X Yang, B Boursi, K Haynes and R Mamtani contributed to conception and design of the study; Y-X Yang and B Boursi acquired the data; Y-X Yang, B Boursi, K Haynes and R Mamtani contributed to analysis and interpretation of data, drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

Acknowledgements

The authors would also like to thank Nadir Arber MD MSc MHA, Head, The Integrated Cancer Prevention Center, Tel-Aviv University, Israel for reviewing the manuscript. Dr B Boursi would like to thank the Djerassi family for supporting his post-doctoral fellowship.

This work was performed in partial fulfilment of the requirements for a PhD degree of B Boursi, Sackler Faculty of Medicine, Tel-Aviv University, Israel

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Received 30 December 2014

Revised version received 22 February 2015

Accepted 27 February 2015