Inverse association between use of broad spectrum penicillin with beta-lactamase inhibitors and prevalence of type 1 diabetes mellitus in Europe

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Increasing incidence of type 1 diabetes is supposed to be induced by environmental factors. Microbiome modulated by antibiotics seems to serve as one of the environmental factors which could influence the development of T1DM. Mitochondria, as autochthonous environmental bacteria living in our cells, and other bacteria share many common enzymes including beta-lactamases and it is supported by evidence that some beta-lactamase inhibitors are able to interact with counterpart enzymes. Thus, antibiotics may utilize two different pathways influencing the development of T1DM; one through modulation of microbiome and a second one via the interaction of mitochondrial enzymes. Data of consumption of penicillin (both narrow and broad spectrum) and beta-lactamase inhibitors in 30 European countries were collected from the database of the European Centre for Disease Prevention and Control. These data were correlated with the prevalence reported by the International Diabetes Federation (2019) referring to type 1 diabetes in Europe. No correlation was found between total penicillin consumption or use of broad spectrum penicillin and the prevalence of type 1 diabetes. Nevertheless, broad spectrum penicillin, in combination with beta-lactamase inhibitor, was in inverse correlation with the prevalence of type 1 diabetes (r = −0.573, p = 0.001). On the other hand, narrow spectrum penicillin was in positive correlation with type 1 diabetes (r = 0.523, p = 0.003). Prevalence of type 1 diabetes showed an inverse correlation with the use of beta-lactamase inhibitors and a positive one with that of narrow spectrum penicillin. Such a detailed analysis has not so far been provided referring to the penicillin group. In the background of this association either microbiomal or direct mitochondrial effects can be supposed.

Abbreviations

ECDC European Centre for disease prevention and control
IDF International diabetes federation
T1DM Type 1 diabetes
TEDDY The environmental determinants of diabetes in the young

T1DM is caused by an autoimmune reaction in which the body’s immune system attacks the insulin-producing beta cells of the pancreas. The causes of this destructive process are not fully understood but a likely explanation is that the combination of genetic susceptibility and environmental triggers, such as viral infections, can initiate the autoimmune reaction. The disease process typically starts at a young age, earlier than 18 years. The rate of T1DM has increased conspicuously in most countries by about 3% per annum. The cause of this rise has been only partially identified and a seasonal sinusoidal pattern was described.

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Recently, there has been an increasing interest in the effect of intestinal homeostasis on the development of type 1 diabetes mellitus (T1DM). Microbial dysbiosis with altered permeability of the gut barrier has been documented in T1DM subjects. Accumulating scientific evidence indicates the probable role of antibiotic-modified microbiome in the development of immunological disturbances leading in this way to T1DM.

To examine the possible association between antibiotic consumption patterns and the development of diabetes, a large database of antibiotic consumption (European Centre for Disease Prevention and Control, ECDC database) was compared with the European data issued by the International Diabetes Federation (IDF) regarding the prevalence of T1DM (patients with diabetes younger than 18 years), as it appeared in the Diabetes Atlas of IDF, 9th edition (2019).

Another focus of this study was the role of beta-lactamase inhibitors, because they, such as clavulanic acid and sulbactam, are prescribed for patients in combination with penicillin, for instance ampicillin. Interestingly, 18 different types of beta-lactamase-like metalloproteins have been detected in human cells in the mitochondria. Moreover, using in vitro experiments, an inhibition of some of these mitochondrial enzymes was proved by the beta-lactamase blocker sulbactam. These data suggest that beta-lactamase inhibitors may have a direct effect on the human body.

It is suspected that penicillin and beta-lactamase consumption patterns of different European countries might be reflected by the prevalence figures of T1DM.

**Materials and methods**

**Databases.** Data of cumulative yearly consumptions of penicillin (J01C) and different penicillin subgroups (broad spectrum, penicillinase sensitive penicillin (J01CA), broad spectrum penicillin combined with penicillinase (beta-lactamase) inhibitors (J01CR), narrow spectrum, penicillinase sensitive penicillin (J01CE) and narrow spectrum penicillinase resistant penicillin (J01CF)) in 30 European countries were obtained from databases of the European Centre for Disease Prevention and Control (ECDC). Combined groups were also calculated: total broad spectrum penicillin (J01CA + J01CR) and total narrow spectrum penicillin (J01CE + J01CF). Three time-periods were observed: 1997–2007; 2008–2018 and the combination of these (1997–2018). The total amount of antibiotic use was expressed in Defined Daily Dose (DID) (/1000 population/day). Average yearly consumption data of penicillin and the above mentioned penicillin subgroups were calculated and expressed in percentage of the relative average share of the total amount (in DID). Antibiotic consumption was estimated at ATC levels 3 and 4.

Prevalence of T1DM in the 0–19-year age group in certain countries were extracted from the Diabetes Atlas 2019 of the International Diabetes Federation and was calculated for 100,000 inhabitants. Principally, we have got the same results using the Diabetes Atlas 2017, estimation method and limitations of which are described elsewhere.

**Statistics.** Spearman correlation was used to calculate the association between cumulative yearly consumption of the penicillin subgroups and prevalence of childhood and adolescent T1DM, as distribution of data of T1DM prevalence and most of the penicillin subgroup consumptions were not normal according to Kolmogorov–Smirnov’s test. A correlation was considered as significant in case p value was <0.05.

**Results**

Total antibiotic use and cumulative yearly consumptions of penicillin and penicillin subgroups of 30 European countries for periods 1997–2007; 2008–2018 and 1997–2018 are shown in Tables 1, 2, 3, respectively. Table 3 also contains data of T1DM prevalence in the 0–19-year age group.

Correlations of T1DM prevalence and use of penicillin subgroups are represented in Table 4. Correlations of T1DM prevalence in 2019 were investigated with penicillin use in the three mentioned time periods (1997–2007, 2008–2018 and 1997–2018) separately. Similar results were obtained along these different periods, and results similar to the IDF Diabetes Atlas 2019, using the IDFA Diabetes Atlas 2017, as well (data not shown).

No association was observed with total penicillin use (Fig. 1a).

Further analysing the penicillin group at ATC level 4, no correlation was found with broad spectrum, penicillinase sensitive penicillin use (Fig. 1b), while strong negative associations were observed with the use of broad spectrum penicillin combined with penicillinase (beta-lactamase) inhibitors (Fig. 1c) and with total broad spectrum penicillin consumption (Fig. 1d).

On the contrary, T1DM prevalence showed strong positive correlations with consumption of narrow spectrum, penicillinase sensitive penicillin (Fig. 2a), narrow spectrum penicillinase resistant penicillin (Fig. 2b) and with total narrow spectrum penicillin use (Fig. 2c).

**Discussion**

The main findings of this study are, that (1) using broad spectrum penicillins is not associated with the prevalence of T1DM, (2) use of broad spectrum penicillins with beta-lactamase inhibitors is in inverse correlation with the prevalence of T1DM, (3) a positive correlation can be found between the use of both penicillinase sensitive and resistant narrow spectrum penicillins as well as the prevalence of T1DM.

Our results strongly support the idea that penicillin consumption patterns in different countries may influence the epidemiology of T1DM. The unsolved “mystery” of the high incidence of T1DM in Scandinavian countries might be associated with the consumption of penicillins. The prevalence of T1DM in Denmark, Finland, Norway and Sweden calculated for 100,000 inhabitants is the highest in Europe, with 54.35, 130.92, 70.66, and 85.09 cases, respectively. In contrast, a quite high narrow spectrum penicillin consumption of 41.028, 9.623, 27.577,
38.205%, and by far the lowest use of broad spectrum penicillin combined with beta-lactamase inhibitor, 2.510, 4.500, 0.000, 1.300% was observed in these countries, respectively. The consumption of a relatively high rate of narrow spectrum penicillin and a very low rate of beta-lactamase inhibitor combinations may contribute to the high prevalence of T1DM in this region of Europe.

In our study there was no correlation between the prevalence of T1DM and the use of broad spectrum penicillin neither in the 1997–2007, nor in 2008–2018 nor in the whole of 1997–2018 period (Table 4). On the contrary, significant inverse associations could be observed between the prevalence of T1DM and the consumption of broad spectrum penicillin combined with beta-lactamase inhibitor in all three observational periods (Table 4). Interestingly, narrow spectrum penicillin clearly shows a positive correlation with the prevalence of T1DM. These facts suggest that not penicillin itself, but the beta-lactamase inhibitors are able to result in an inverse correlation between the prevalence of T1DM and the consumption of different types of penicillin products.

Seasonality of community antibiotic use is a well-known phenomenon with an especially clear picture in the case of penicillins. Data of antibiotic sales extracted from the International Medical Services Health’s Xponent database showed for the United States in the period between 1999 and 2007 at least a three-fold higher number of prescriptions of aminopenicillins in January than in July16. Similarly, analysis of about 98 million outpatient antibiotic prescriptions in the United States between 2013 and 2015 showed that amoxicillin with clavulanate was 78% (95% confidence intervals: 68% to 129%) more likely to be prescribed in February than in September17. Report on outpatient antibiotic use in more than one million Swiss adults showed around 3 defined daily doses (DDDs) of amoxicillin use (with or without clavulanate) per 1000 inhabitants per day in August–September as opposed to about 5 DDDs in the period of December to March18. It can be assumed reasonably that the above-outlined seasonal peaks in the use of antibiotics may originate from prescriptions aimed to treat upper respiratory infections that may well be of viral origin in the cold and influenza season.

Table 1. Cumulative yearly penicillin consumption (ECDC database 1997–2007). Penicillin consumption (1997–2007), expressed in % of the total consumption (SUM) in DID, according to the ECDC (European Centre for Disease Prevention and Control) database. ATC code: aJ01; bJ01C; cJ01CA; dJ01CR; eJ01CA + J01CR; fJ01CE; gJ01CF; hJ01CE + J01CF.
Seasonality in the manifestation of T1DM was consequently reported in children living in several European countries. In a three-year-long Dutch study including 676 children diagnosed with T1DM per year, the annual incidence rates per hundred thousand children were 6.6 and 6.4 in winter as opposed to 4.9 in spring and 5.4 in summer (p < 0.03)\(^1\). In 5422 children diagnosed with T1DM in Romania between 1996 and 2015, the maximum monthly incidence value was recorded in January (more than 450) as opposed to the minimum value recorded in June (around 300, p < 0.001)\(^2\). In 2174 Polish children diagnosed with T1DM between 2010 and 2014, the odds of diagnosis being made between June and August was 57% (95% confidence intervals: 51% to 67%, p < 0.0001) of the odds of diagnosing the disease between December and February\(^2\). In a multicentre study summarising data obtained in 23,063 diabetic children at 48 European centres, nearly 10% of the patients were diagnosed in January as compared to 7% diagnosed in June (p < 0.04)\(^2\).

T1DM is more prevalent in countries preferring the use of more antibiotics and vaccinations and improving hygiene standards, decreasing in these way the incidence of infections. This is the so-called “hygiene hypoth-esis”\(^2\). In a recent study, in The Environmental Determinants of Diabetes in the Y oung\(^2\) HLA genotyped high risk newborns from the high risk countries (Finland and Sweden), and also from Germany and the USA were investigated. In reports of early life (3 months and 4 years of age) antibiotic use and development of autoim-unity were assessed. No association between the use of penicillin or beta-lactamase plus penicillin and beta-cell autoimmunity (insulin autoantibody, glutamic acid decarboxylase autoantibody, tyrosine phosphatase IA-2 autoantibody) could be found. We also proved that total penicillin use is not in correlation with the prevalence of T1DM, probably due to the bidirectional effect of the increase with narrow and decrease with broad spectrum penicillin with beta-lactamase. In TEDDY they did not separate narrow spectrum penicillins as a group, and

| Country (DID 100%) (%) | Total antibiotic consumption\(^a\) (%) | Total penicillin\(^b\) (%) | Broad spectrum, penicillinase sensitive penicillin\(^c\) (%) | Broad spectrum penicillin combined with penicillinase inhibitors\(^d\) (%) | Total broad spectrum penicillin\(^e\) (%) | Narrow spectrum, penicillinase sensitive penicillin\(^f\) (%) | Narrow spectrum penicillinase resistant penicillin\(^g\) (%) | Total narrow spectrum penicillin\(^h\) (%) |
|------------------------|--------------------------------------|--------------------------|-------------------------------------------------|-------------------------------------------------|-----------------------------|-------------------------------------------------|-------------------------------------------------|-----------------------------|
| Austria 12.45          | 38.61                                | 6.48                     | 25.52                                           | 32.00                                           | 6.76                        | 0.07                                            | 6.82                                            |                                            |
| Belgium 22.52          | 46.10                                | 21.28                    | 23.27                                           | 44.55                                           | 0.21                        | 1.13                                            | 1.34                                            |                                            |
| Bulgaria 17.54         | 31.83                                | 19.28                    | 11.57                                           | 30.84                                           | 1.36                        | 0.00                                            | 1.36                                            |                                            |
| Croatia 17.86          | 42.57                                | 11.74                    | 26.46                                           | 38.19                                           | 4.32                        | 0.05                                            | 4.37                                            |                                            |
| Cyprus 26.77           | 34.94                                | 10.39                    | 24.35                                           | 34.74                                           | 0.32                        | 0.09                                            | 0.41                                            |                                            |
| Czech Rep 16.51        | 35.88                                | 7.16                     | 16.67                                           | 23.83                                           | 11.61                       | 0.23                                            | 11.85                                            |                                            |
| Denmark 15.24          | 63.09                                | 20.54                    | 4.21                                            | 24.75                                           | 29.46                       | 8.73                                            | 38.19                                            |                                            |
| Estonia 10.23          | 31.73                                | 17.36                    | 12.38                                           | 29.74                                           | 2.05                        | 0.01                                            | 2.06                                            |                                            |
| Finland 16.16          | 29.75                                | 16.24                    | 5.27                                            | 21.51                                           | 7.87                        | 0.22                                            | 8.09                                            |                                            |
| France 23.62           | 50.54                                | 29.30                    | 19.30                                           | 48.60                                           | 0.67                        | 1.17                                            | 1.84                                            |                                            |
| Germany 13.23          | 25.15                                | 16.72                    | 2.56                                            | 19.28                                           | 5.87                        | 0.08                                            | 5.94                                            |                                            |
| Greece 32.22           | 29.26                                | 13.66                    | 14.70                                           | 28.35                                           | 0.87                        | 0.00                                            | 0.87                                            |                                            |
| Hungary 13.65          | 33.95                                | 7.03                     | 24.39                                           | 31.42                                           | 2.61                        | 0.00                                            | 2.61                                            |                                            |
| Iceland 18.76          | 47.87                                | 16.45                    | 14.36                                           | 30.81                                           | 11.66                       | 5.56                                            | 17.21                                            |                                            |
| Ireland 19.51          | 47.34                                | 14.22                    | 20.87                                           | 35.09                                           | 6.24                        | 6.73                                            | 12.98                                            |                                            |
| Italy 22.23            | 45.40                                | 12.90                    | 31.85                                           | 44.75                                           | 0.09                        | 0.04                                            | 0.04                                            |                                            |
| Latvia 10.75           | 38.21                                | 26.26                    | 11.51                                           | 37.77                                           | 0.64                        | 0.01                                            | 0.65                                            |                                            |
| Lithuania 14.97        | 45.78                                | 33.26                    | 9.37                                            | 42.63                                           | 3.27                        | 0.04                                            | 3.31                                            |                                            |
| Luxembourg 23.35       | 37.14                                | 12.95                    | 22.53                                           | 35.48                                           | 0.18                        | 0.89                                            | 1.07                                            |                                            |
| Malta 19.18            | 33.08                                | 2.94                     | 30.15                                           | 33.09                                           | 0.38                        | 0.24                                            | 0.62                                            |                                            |
| Netherlands 9.62       | 32.42                                | 13.47                    | 11.38                                           | 24.85                                           | 2.82                        | 4.36                                            | 7.17                                            |                                            |
| Norway 15.28           | 40.04                                | 13.73                    | 0.04                                            | 13.76                                           | 22.09                       | 4.00                                            | 26.10                                            |                                            |
| Poland 20.39           | 33.08                                | 17.11                    | 14.76                                           | 31.87                                           | 1.07                        | 0.03                                            | 1.10                                            |                                            |
| Portugal 17.79         | 46.70                                | 9.35                     | 34.06                                           | 43.41                                           | 0.10                        | 3.24                                            | 3.34                                            |                                            |
| Romania 26.14          | 43.56                                | 16.74                    | 21.42                                           | 38.16                                           | 2.86                        | 2.41                                            | 5.27                                            |                                            |
| Slovakia 20.33         | 30.50                                | 5.70                     | 17.64                                           | 23.34                                           | 6.67                        | 0.00                                            | 6.67                                            |                                            |
| Slovenia 11.68         | 59.92                                | 19.39                    | 24.26                                           | 43.66                                           | 15.04                       | 1.26                                            | 16.30                                            |                                            |
| Spain 18.72            | 54.78                                | 21.07                    | 31.94                                           | 53.01                                           | 0.45                        | 1.09                                            | 1.54                                            |                                            |
| Sweden 12.74           | 49.89                                | 8.54                     | 1.63                                            | 10.17                                           | 27.86                       | 11.67                                            | 39.53                                            |                                            |
| UK 16.945              | 38.412                               | 20.719                   | 4.614                                           | 25.333                                          | 4.764                       | 8.160                                            | 12.923                                            |                                            |

| Table 2. Cumulative yearly penicillin consumption (ECDC database 2008–2018). Penicillin consumption (2008–2018), expressed in % of the total consumption (SUM) in DID, according to the ECDC (European Centre for Disease Prevention and Control) database. ATC code: \(\text{aJ01; bJ01C; cJ01CA; dJ01CR; eJ01CA + J01CR; fJ01CE; gJ01CF; hJ01CE + J01CF.}\)
Table 3. Prevalence of T1DM for 0–19 years and penicillin consumption (ECDC database 1997–2018). Prevalence data of T1DM for 0–19 years calculated for 100,000 inhabitants/country (Diabetes Atlas edition 9) compared to penicillin consumption (1997–2018), expressed in % of the total consumption (SUM) in DID, according to the ECDC (European Centre for Disease Prevention and Control) database. ATC code: aJ01; bJ01C; cJ01CA; dJ01CR; eJ01CA + J01CR; fJ01CE; gJ01CF; hJ01CE + J01CF.

| Country      | T1DM prevalence (0–19 years) (/100,000 inhabitants) | Cumulative yearly antibiotic consumption (ECDC database 1997–2018) |
|--------------|----------------------------------------------------|------------------------------------------------------------------|
|              | Total antibiotic consumption^a (%)                 | Total penicillin^b (%)                                           |
|              | (DID 100%) (%)                                     | Broad spectrum, penicillinase sensitive penicillin^c (%)          |
|              |                                                    | Broad spectrum penicillin combined with penicillinase inhibitors^d (%) |
|              |                                                    | Total broad spectrum penicillin^e (%)                             |
|              |                                                    | Narrow spectrum, penicillinase sensitive penicillin^f (%)         |
|              |                                                    | Narrow spectrum penicillin resistant penicillin^g (%)             |
|              |                                                    | Total narrow spectrum penicillin^h (%)                            |
| Austria      | 32.89                                              | 12.124                                                          |
| Belgium      | 36.94                                              | 21.962                                                          |
| Bulgaria     | 15.54                                              | 17.385                                                          |
| Croatia      | 32.37                                              | 18.594                                                          |
| Cyprus       | 33.02                                              | 26.946                                                          |
| Czech Rep    | 38.39                                              | 15.013                                                          |
| Denmark      | 54.35                                              | 14.182                                                          |
| Estonia      | 46.11                                              | 10.406                                                          |
| Finland      | 130.92                                             | 16.791                                                          |
| France       | 41.83                                              | 24.877                                                          |
| Germany      | 39.53                                              | 12.895                                                          |
| Greece       | 29.87                                              | 30.423                                                          |
| Hungary      | 36.46                                              | 14.962                                                          |
| Iceland      | 36.17                                              | 19.473                                                          |
| Ireland      | 66.21                                              | 18.252                                                          |
| Italy        | 26.4                                               | 22.005                                                          |
| Latvia       | 13.5                                               | 10.581                                                          |
| Lithuania    | 51.18                                              | 15.885                                                          |
| Luxembourg   | 35.75                                              | 23.009                                                          |
| Malta        | 43.18                                              | 18.833                                                          |
| Netherlands  | 42.74                                              | 9.341                                                          |
| Norway       | 70.66                                              | 15.295                                                          |
| Poland       | 33.16                                              | 18.770                                                          |
| Portugal     | 24.69                                              | 18.341                                                          |
| Romania      | 14.75                                              | 24.144                                                          |
| Slovakia     | 46.9                                               | 21.516                                                          |
| Slovenia     | 28.37                                              | 13.186                                                          |
| Spain        | 33.07                                              | 17.264                                                          |
| Sweden       | 85.09                                              | 13.805                                                          |
| UK           | 57.78                                              | 15.259                                                          |

Table 4. Correlations of antibiotic consumption and prevalences of T1DM (0–19 years). ATC code: aJ01; bJ01C; cJ01CA; dJ01CR; eJ01CA + J01CR; fJ01CE; gJ01CF; hJ01CE + J01CF. r values of significant correlations are given in boldface.
Figure 1. Association of consumption of total penicillin (a), broad spectrum, penicillinase sensitive penicillin (b), broad spectrum penicillin combined with penicillinase inhibitors (c) and total broad spectrum penicillin (d) with prevalence of T1DM (0–19 years). Total broad spectrum penicillin represents broad spectrum, penicillinase sensitive penicillin + broad spectrum penicillin combined with penicillinase inhibitors.
TEDDY did not use the hard end point of the overt T1DM either, what we, however, implicated. Moreover, they rather targeted the surrogate endpoints of immunological positivity. Correlations obtained in our study do not strictly reveal causative association, but they raise two possible explanations for these results. On the one hand, antibiotics induced changes of the microbiome, and on the other, direct interactions of beta-lactamase inhibitors with the human beta-lactamase enzymes could be supposed to be in the background of this phenomenon. We are currently investigating the correlations with consumption of other antibiotics, as well.

Regarding microbiome, in an animal model of T1DM (non-obese diabetic, NOD-mouse) in the early postnatal period, vancomycin treated animals presented a lower rate of diabetes and one microbe of the gut became dominant. Nevertheless, in the same NOD-model, vancomycin and a combined antibiotic treatment using streptomycin, colistin, and ampicillin increased the incidence of T1DM. Moreover, transplantation of microbiome of NOD to diabetes resistant mice induced insulitis, however, germ-free NOD-mice attest metabolic and immunologic disturbances. Another experiment on the mouse-model revealed that early postnatal, low dosage of penicillin treatment caused microbiota perturbation, which was long-lasting, resulting in metabolic

![Diagram](https://example.com/diagram.png)

**Figure 2.** Association of consumption of narrow spectrum, penicillinase sensitive penicillin (a), narrow spectrum penicillinase resistant penicillin (b) and total narrow spectrum penicillin (c) with prevalence of T1DM (0–19 years). Total narrow spectrum penicillin represents narrow spectrum, penicillinase sensitive penicillin + narrow spectrum penicillinase resistant penicillin. Antibiotic consumptions were expressed in natural logarithm, regarding their distribution.
changes of the host. Summarizing the animal-based data, they are contradictory regarding gut microbiome and antibiotics and T1DM. In humans, more and more observational studies and several review papers point at bacterial changes in prediabetes and in overt T1DM, and the development of leaky gut leading to the dysregulation of the immune system resulting in beta-cell destruction. All these results strongly suggest an association between microbiome and T1DM, but there is no answer to whether all these changes are causes or consequences (the chicken-egg question). Moreover, there is no convincing evidence supporting the role of antibiotic therapy in the development of T1DM.

Regarding beta-lactamase hypothesis, it is interesting that mitochondria of the human cells show some bacterial features supporting the hypothesis assuming that during eukaryogenesis the fermentative, anaerobic host archaeon interacted with the aerobic, organotrophic bacterial partner, which became endosymbiont developing the intracellular mitochondria. Thus, human mitochondria express more than a dozen of different types of beta-lactamase enzymes characteristic of bacteria, of which some are inhibited by sulbactam. All these beta-lactamase enzymes are zinc containing proteins, which metal is necessary for insulin biosynthesis and storage in the beta cells. One of these beta-lactamase enzymes is a human mitochondrial endoribonuclease (LACTB2), which is essential for the functioning of mitochondria. Moreover, LACTB2 regulates mitochondrial fat metabolism by organizing mitochondrial membrane and regulates complex I being a member of the mitochondrial electron transport chain. All these data taken into consideration, it can be hypothesized that beta-lactamase inhibitors may have a direct effect on human cells preventing or slowing down the processes leading to T1DM, ever, direct evidence is lacking.

The strengths of this study are the Europe-wide nature, the clear significance of associations, and long exposure to antibiotic treatment (1997–2018). Our cohort consisted of whole population data. We assume that the parental exposition (pregestational, gestational as well) to different types of stressors including antibiotic treatment may affect the development of T1DM in children. Furthermore, our population-wide data involve not only cases with genetically determined high risk but also incidental T1DM patients. This approach provides a broader view on risk factors of a population, particularly referring to the role of antibiotic consumption developing T1DM. Other studies were also performed to clarify the role of environmental factors in the development of T1DM. The TEDDY study was a prospective 6 centre investigation involving Europe and the United States focusing on children younger than four years with a risk of T1DM due to family involvement or carrying high-risk HLA-DR, DQ genotypes. Consequently, the population studied had a genetically determined risk to develop T1DM, and those who had an incident of T1DM were not involved in this study. This may explain why there was no association between antibiotic consumption and the development of T1DM in the TEDDY programme.

The main limitations are the retrospective approach of the study, and that the antibiotic consumption in the ECDC database is characteristic for the overall population of the countries, not only of those who are younger than 18 years of age.

Concluding, use of beta-lactamase inhibitors seem to be in inverse, while that of narrow spectrum penicillin in positive association with T1DM.

Further studies are urgently needed to verify the possible causative associations between T1DM and beta-lactamase inhibitors in order to introduce these results in clinical routine aiming to prevent further escalation of the prevalence of T1DM.

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Author contributions
I.W. designed the study. G.T. and K.B. performed the data collection and S.K., S.N. performed data analyses and created the figures and tables. G.T., S.K., T.D., G.S. and I.W. took part in the data interpretation and drafted the paper’s language and style. I.W. is the guarantor of this work.

Competing interests
The authors declare no competing interests.

Additional information

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