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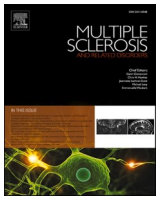

Original article

Antibiotic drug use in the five years preceding the diagnosis of multiple sclerosis

Sonia Darvishi [a], Ewan Donnachie [b], Paula Anne Uibel [a], Martina Flaskamp [a], Christiane Gasperi [a], Alexander Hapfelmeier [c,d,f,1], Bernhard Hemmer [a,e,1,*]

[a] Department of Neurology, Klinikum rechts der Isar, School of Medicine and Health, Technical University of Munich, Munich, Germany
[b] Bavarian Association of Statutory Health Insurance Physicians, Munich, Germany
[c] Institute of General Practice and Health Services Research, School of Medicine and Health, Technical University of Munich, Munich, Germany
[d] Institute of AI and Informatics in Medicine, School of Medicine and Health, Technical University of Munich, Munich, Germany
[e] Munich Cluster for Systems Neurology (SyNergy), Munich, Germany
[f] Munich Center for Health Economics and Policy (M-CHEP), Munich, Germany

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ABSTRACT

Background: Microbiota may play a role in autoimmune disease pathogenesis, including multiple sclerosis (MS). Antibiotic use disrupts the microbiome and may increase the risk of autoimmune diseases. We evaluated the relationship between MS diagnosis and antibiotic, antimycotic and antiviral drug use in the 5 years preceding diagnosis.

Method: Our population-based case-control study used German ambulatory claims data from 2012 to 2022. We defined cohorts of 13,053 MS patients, 22,898 Crohn's disease patients, and 15,037 matched controls without autoimmune diseases, aged 21–70. Logistic and Poisson regression models explored the relationship between MS diagnosis and antimicrobial usage. Two sub-analyses were performed: a separate analysis of patients with clinically isolated syndrome (CIS) and a sensitivity analysis of newly diagnosed MS patients without preceding neurological symptoms.

Results: Patients with MS had higher exposure to antibiotic (Odd Ratio (OR) = 1.27, 95 % CI 1.21–1.33), antimycotic (OR = 1.27, 95 % CI 1.12–1.45), and antiviral drugs (OR = 1.28, 95 % CI 1.15–1.43) in the five years before diagnosis compared to patients with no autoimmune diseases. Similar findings were obtained for the CIS cohort and in the sensitivity analysis. Antibiotic use peaked 5 years before MS diagnosis, declining closer to diagnosis, while antiviral and antimycotic drug use showed the opposite. This effect was not observed in the sensitivity analysis and CIS cohorts. Antibiotic use was higher in Crohn's disease than in MS (OR = 0.86, 95 % CI 0.82–0.90), with no consistent differences in antimycotic and antiviral use.

Conclusions: The association and kinetic of antibiotic use before MS and CIS diagnosis supports the role of microbiota in MS pathogenesis and suggests antibiotic use to be related to the development of autoimmune diseases, including MS. Additional studies are warranted to clarify whether increased antibiotic use is part of the MS prodrome or a true risk factor for MS.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease of the central nervous system (CNS), which afflicts 2.8 million people worldwide (Walton et al., 2020). The pathogenesis of MS still remains poorly understood. The disease emerges from the interplay of genetic and environmental factors (Olsson et al., 2017; Thompson et al., 2018; Bjornevik et al., 2022). Animal models and human studies support the role of microbiota in the pathogenesis of MS and other autoimmune diseases (Thirion et al., 2023; Gödel et al., 2020; Ungaro et al., 2014; Shaw et al., 2010).

Gut microbiota fundamentally impacts the immune system,

* Correspondence author at: Department of Neurology, Klinikum rechts der Isar, School of Medicine and Health, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Germany.
E-mail address: hemmer@tum.de (B. Hemmer).
[1] Both authors contributed equally to the study

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influencing physiological and pathological immune responses (Belkaid and Hand, 2014; Christovich and Luo, 2022). Many factors can influence microbiota composition in early life, including infection, birth delivery mode, antibiotic medication use, environmental stressors, and host genetics (Odamaki et al., 2016; Nagpal et al., 2017; Hasan and Yang, 2019). Antibiotic use can disrupt the fragile balance within the gut microbiota (Cryan et al., 2019). This may increase the risk of autoimmune diseases, particularly chronic inflammatory bowel disease (Ungaro et al., 2014; Shaw et al., 2010). Previous studies have provided inconsistent results between antibiotic usage and the risk of MS (TernÁk et al., 2020; Abdollahpour et al., 2018; Ren et al., 2017; Baldin et al., 2021; Alonso et al., 2006; Nørgaard et al., 2011).

We conducted a large population-based retrospective cohort study to evaluate the frequency of usage of different types of antimicrobial drugs and their relation to the diagnosis of MS in comparison to two control cohorts, that is, a matched cohort of people without autoimmune diseases (No AID) and of persons with a newly diagnosed Crohn's disease, who served as positive control.

2. Materials and methods

2.1. Data

We used German ambulatory claims data, which includes diagnoses coded according to the German version of ICD-10 and prescriptions coded according to the Anatomical Therapeutic Chemical (ATC) code for each quarter, sex (male/female) and age of diagnosis. Data were recorded daily but reported on a quarterly basis. This quarterly report reflects all events (e.g., diagnoses, prescriptions) within a 3-month period. Importantly, no information regarding specific ICD codes or prescriptions and their dates was lost due to the quarterly billing structure. This data was provided by the Bavarian Association of Statutory Health Insurance Physicians (BASHIP) and covered the first quarter of 2012 to the second quarter of 2022. We did not have access to the participants' birth year and district of residence due to privacy/data protection regulations.

The dataset comprised all prescribed antimicrobials. A personal identification number, year and quarter of prescription, and type of drug (ATC code) were available for each antibiotic prescription. We specifically identified prescriptions reporting the ATC code "J01", which corresponds to antibacterial for systemic use. Additionally, we incorporated prescriptions with ATC codes "J02″ and "J05″ for antimycotic and antiviral medications, respectively. Antibiotics were classified into nine categories including any antibiotics (ATC code J01), tetracyclines (J01A), beta-lactam (J01C), beta-lactam other than penicillins (other beta) (J01D), sulfonamides (J01E), macrolides (J01F), aminoglycoside (J01 G), quinolone (J01 M), other antibacterial (J01X), antimycotic (J02) and antiviral (J05). We did not include ATC J04 (drugs for treatment of tuberculosis or lepra) in our analysis due to its extremely low usage across the cohorts, with only 0.1 % in the MS cohort, 0.1 % in the No AID cohort, and 0.2 % in the Crohn's disease cohort.

2.2. Cohorts

We conducted a retrospective cohort study to examine antimicrobial use in a cohort of newly diagnosed persons with MS compared to two control cohorts. Persons in the MS cohort had to have evidence of a recorded visit with a neurologist in the five years preceding the MS diagnosis. We selected persons with Crohn's disease with at least two ICD diagnosis codes K50 in at least two quarterly periods as a positive control cohort. The second control cohort consisted of individuals without any of these two autoimmune diseases. These participants were randomly selected from the BASHIP data source and matched to the MS cohort in a 1:1 ratio based on birth year, sex, and district of residence. A synthetic quarter of the first diagnosis was chosen for these persons based on their matching partner's diagnosis time point.

We included participants diagnosed after 2016 to examine follow-up periods of 5 years before diagnosis. We excluded the quarter immediately before the diagnosis to prevent the evaluation of a period potentially influenced by the onset of the disease. Additionally, we limited the age range to 21 to 70 years to concentrate on the adult population and specific age groups with an ample sample size to ensure robust effects estimation. Finally, we excluded two participants who had used disease modifying therapies (DMTs) in the five years before the diagnosis.

In a sensitivity analysis, we excluded persons with any record of neurologic or cerebrovascular ICD-10 codes and any ICD-10 code possibly associated with MS within the 5 years before the diagnosis of MS from all cohorts (vision problems (H53), MS (G35), disease of vestibular function (H81), neuromuscular dysfunction of bladder (N31), disturbances of skin sensation (R20), abnormalities of gait and mobility (R26), unspecified urinary incontinence (R32), signs involving the genitourinary system (R39), and dizziness and staggering (R42)) (Gasperi et al., 2021a). This sensitivity analysis focused on excluding all patients with confirmed or unconfirmed ICDs, thereby concentrating on healthy individuals who were diagnosed with MS within the subsequent five years. We distinguish between 'confirmed' and 'unconfirmed' diagnoses as classified in the data. Diagnoses indicated as 'confirmed' have been definitively established by the healthcare provider. 'Unconfirmed' diagnoses include those marked as 'suspected' or 'not otherwise specified', reflecting diagnostic uncertainty or lack of detailed specification.

We conducted an additional analysis on patients with clinically isolated syndrome (CIS). These patients were diagnosed with an ICD-10 codes G04 (encephalitis, myelitis, encephalomyelitis, or clinically isolated syndrome (CIS)), G35 (MS), and H46 (optic neuritis) followed by the diagnosis of MS (G35). Our investigation specifically focused on the five-year period before the first coding of G04, G35 and H46. We will refer to this as the CIS analysis. In this analysis, we included patients with unconfirmed G35 codes and confirmed diagnoses of optic neuritis, encephalitis, myelitis, and encephalomyelitis, examining the five years leading up to these ICD diagnoses.

Selection bias and recall bias were avoided by design in this population-based study of claims data. Misclassification bias could not be ruled out, but it was addressed by sensitivity analysis. Potential confounding was accounted for by loose matching and by fitting multiple regression models. Loose matching was used as a first step to extract a homogeneous study population from the database and to reduce potential confounding. For all analyses, including the CIS and sensitivity analyses, we additionally used multiple regression models with the covariates age and sex to further control for potential confounding.

2.3. Analysis

Absolute and relative frequencies present the distribution of antimicrobial usage for the entire 5 years period and each year before diagnosis. Additionally, we present relative frequencies standardized to the age and sex distribution of the MS cohort. Antimicrobial usage is defined as the prescription of any antibiotics, antivirals and antimycotics.

The association of a diagnosis of MS to any antibiotic, antiviral and antimycotic usage within the 5 years before diagnosis was examined using unconditional logistic regression models. That is a proper method to perform for loose-matching data. The binary outcome variable in each model was antimicrobial usage (yes = at least once / no = never). The models included a main effect for the cohorts (MS/Crohn's disease/ No AID), as well as the main effects and interaction effect of sex (male/female) and age categories (21–30, 31–40, …, 61–70 years). Results are reported using odds ratios (ORs) and corresponding 95 % confidence intervals (CIs).

Generalized estimating equations (GEE) with an exchangeable working correlation matrix were used with a similar model specification

but with an additional main effect for time (factor variable with years before diagnosis as separate levels) and an interaction effect between time and cohorts to examine yearly use of any antibiotics in the comparison of cohorts. We performed linear hypothesis tests of differences in the obtained odd ratios of antibiotics usage leading up to the diagnosis.

Additionally, we used a Poisson regression model to compare the count of any antibiotics, antimycotic, and antiviral usage between cohorts. The model was specified like the logistic regression model and used to estimate incidence rate ratios (IRR) and marginal means with 95 % CIs, adjusted for sex and age.

Hypothesis testing was performed at exploratory two-sided 5 % significance levels. All analyses were conducted using R 4.2.1 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

The BASHIP database included 34,475 persons with a first diagnosis of MS, 54,411 with a first diagnosis of Crohn's disease, and 34,408 with No AID between 2012 and 2022. Applying exclusion criteria resulted in sample sizes of 13,053 persons with MS, 22,898 with Crohn's disease, and 15,037 with No AID (Table 1 and Supplementary Fig 1). The Crohn's disease cohort was older and included fewer women than the MS cohort.

Table 1
Descriptive statistics of cohorts.

Main analysis		MS	No AID	Crohn's disease
Size		13,053	15,037	22,898
Sex	Female, n (%)	9,069 (69.5)	10,407 (69.2)	12,587 (55.0)
Age at MS diagnosis (years)	mean ± SD	41.3 ± 12.6	42.35 ±13.1	43.6 ± 13.9
	21–30, n (%)	3,245 (24.9)	3,354 (22.3)	5,358 (23.4)
	31–40, n (%)	3,435 (26.3)	3,860 (25.7)	4,737 (20.7)
	41–50, n (%)	2,849 (21.8)	3,293 (21.9)	4,496 (19.6)
	51–60, n (%)	2473 (18.9)	3,052 (20.3)	5,166 (22.6)
	61–70, n (%)	1,051 (8.1)	1,478 (9.8)	3,141 (13.7)
Sensitivity analysis				
Size		4,728	10,392	14,196
Sex	Female, n (%)	3,170 (67.0)	7,062 (68.0)	7,263 (51.2)
Age at MS diagnosis (years)	mean ± SD	39.9 ± 12.1	41.2 ± 12.7	42.0 ±13.5
	21–30, n (%)	1,312 (27.7)	2,505 (24.1)	3,714 (26.2)
	31–40, n (%)	1,325 (28.0)	2,903 (27.9)	3,247 (22.9)
	41–50, n (%)	1,043 (22.1)	2,228 (21.4)	2,824 (19.9)
	51–60, n (%)	755 (16.0)	1,927 (18.5)	2,878 (20.3)
	61–70, n (%)	293 (6.2)	829 (8.0)	1,533 (10.8)
CIS analysis				
Size		1,461	–	–
Sex	Female, n (%)	1,038 (70.4)	–	–
Age at CIS diagnosis (years)	mean ± SD	40.3 ± 12.2	–	–
	21–30, n (%)	358 (24.5)	–	–
	31–40, n (%)	391 (26.7)	–	–
	41–50, n (%)	322 (22.0)	–	–
	51–60, n (%)	291 (19.9)	–	–
	61–70, n (%)	76 (5.2)	–	-

3.1. Antibiotic

We found that 66.6 % of persons with MS were exposed to any antibiotics at least once in the 5 years before the diagnosis of MS, which was higher in comparison with No AID (61.3 %). Persons with Crohn's disease had the highest antibiotic (68.1 %) consumption among the three cohorts, which further increased to 69.6 % after being adjusted to the age and sex of the MS cohort (Table 2).

Over the course of the observation period from 2012 to 2022, the absolute and age and sex standardized relative frequencies also showed a higher use of antibiotics in MS compared to No AID but a lower use compared to Crohn's disease (Fig 1, Supplementary Fig 2).

In the five years preceding diagnosis, the adjusted OR for persons with MS concerning exposure to any antibiotics compared to other cohorts was 1.27 (95 % CI 1.21–1.33) for No AID and 0.86 (95 % CI 0.82–0.90) for Crohn's disease (Fig 2A). In the CIS analysis, patients with CIS were more likely to be exposed to any antibiotics compared to No AID (OR = 1.33, 95 % CI 1.19–1.50) and less likely compared to Crohn's disease (OR = 0.91, 95 % CI 0.81–1.03) (Fig 2B). Likewise, in the sensitivity analysis, persons with MS were more likely to be exposed to any antibiotics compared to No AID (OR = 1.11, 95 % CI 1.03–1.18) and less likely compared to Crohn's disease (OR = 0.83, 95 % CI 0.77–0.88) (Supplementary Fig 3).

Additionally, the odds of antibiotic use in the five years before diagnosis in persons with MS were higher than persons with No AID for all classes of antibiotics (Supplementary Fig 4A). Furthermore, persons with MS were less frequently exposed to almost all antibiotic drugs than persons with Crohn's disease. In the sensitivity analysis and CIS analysis, similar findings were observed (Supplementary Fig 4B, 3C).

In each year before the diagnosis of MS, the ORs of antibiotic use were higher in MS compared to No AID. Interestingly, the ORs continuously decreased over the years preceding the diagnosis of MS, with the smallest difference observed in the year before and the largest difference 5 years before MS diagnosis (Fig 3). The CIS analysis shows higher antibiotic exposure in the years before diagnosis (Fig 3). Compared to persons with Crohn's disease, persons with MS had lower exposure to antibiotics in the years before diagnosis. The smallest differences were seen 5 years before diagnosis, and the largest was in the year before diagnosis (Supplementary Fig 5C).

Overall, the Poisson regression model showed an association of the MS diagnosis with antibiotic exposure compared to No AID (IRR = 1.25, 95 %CI 1.23–1.27, $p < 0.001$), indicating a 25 % increase in the frequency of antibiotic use in persons with MS in the 5 years before diagnosis (Table 3). The IRR for any antibiotics usage in persons with MS compared to Crohn's disease was 0.86 (95 % CI 0.85–0.87, $p < 0.001$), indicating a 14 % higher antibiotic use in Crohn's disease.

3.2. Antimycotic

3.9 % of individuals diagnosed with MS had been exposed to antimycotic at least once within the five years prior to their MS diagnosis (Table 2). The standardized relative frequency was higher in MS compared to No AID (3.1 %) but lower compared to Crohn's disease (4.4 %).

Persons with MS had a higher exposure to antimycotic (OR 1.27, 95 % CI 1.12–1.45) compared to persons with No AID (Fig 2). This increase remained consistent in the sensitivity and CIS analyses (OR = 1.28, 95 % CI 1.05–1.57) and (OR = 1.40, 95 % CI 1.06–1.82), respectively.

In the years before the diagnosis, the ORs of antimycotic use were higher in MS compared to No AID (Supplementary Fig 5A). Interestingly, we observed a different trend compared to antibiotic use. The ORs were highest in the year before diagnosis and decreased in the preceding years, with almost no difference between groups in the fifth year before diagnosis.

Table 2
Frequency of antibiotics use in the 5 years before diagnosis.

Main analysis		MS n (%)	No AID n (%)	Crohn's disease n (%)
Size		13,053	15,037	22,898
	Any antibiotics	8,698 (66.6)	9,192 (61.1)	15,593 (68.1)
	Tetracyclines	1,742 (13.3)	1,548 (10.3)	3,211 (14.1)
	Beta lactam	3,729 (28.6)	3,824 (25.4)	7,070 (30.8)
	Other beta[a]	3,789 (29.0)	3,888 (25.9)	7,082 (30.9)
	Sulfonamides	1,138 (8.7)	977 (6.5)	1,891 (8.2)
	Macrolides	3,657 (28.0)	3,745 (24.9)	6,913 (30.2)
	Aminoglycosides	13 (0.09)	2 (0.01)	11 (0.05)
	Quinolone	2,792 (21.4)	2,645 (17.6)	5,639 (24.6)
	Other antibacterial	1,662 (12.7)	1,470 (9.8)	2,251 (9.8)
	Antimycotic	508 (3.9)	458 (3.0)	829 (3.6)
	Antiviral	723 (5.5)	663 (4.4)	1,296 (5.7)
Standardized relative frequency				
	Any antibiotics	66.6 %	61.3 %	69.6 %
	Antimycotic	3.9 %	3.1 %	4.4 %
	Antiviral	5.5 %	4.3 %	6.1 %
Sensitivity analysis				
Size		4,728	10,392	14,196
	Any antibiotics	2,782 (58.8)	5,847 (56.2)	8,719 (61.4)
	Tetracyclines	471 (9.9)	919 (8.8)	1,626 (11.5)
	Beta lactam	1,167 (24.7)	2,400 (23.1)	3,818 (26.9)
	Other beta	1,125 (23.8)	2,367 (22.8)	3,711 (26.1)
	Sulfonamides	297 (6.3)	547 (5.3)	872 (6.1)
	Macrolides	1,139 (24.1)	2,315 (22.2)	3,672 (25.9)
	Aminoglycosides	6 (0.1)	1 (0.01)	4 (0.03)
	Quinolone	787 (16.6)	1,538 (14.8)	2,806 (19.7)
	Other antibacterial	456 (9.6)	840 (8.1)	998 (7.0)
	Antimycotic	154 (3.3)	262 (2.5)	390 (2.7)
	Antiviral	216 (4.6)	408 (3.9)	636 (4.5)
CIS analysis				
Size		1474	15,037	22,898
	Any antibiotics	999 (67.8)	9,192 (61.1)	15,593 (68.1)
	Tetracyclines	190 (12.9)	1,548 (10.3)	3,211 (14.1)
	Beta lactam	430 (29.2)	3,824 (25.4)	7,070 (30.8)
	Other beta	439 (29.8)	3,888 (25.9)	7,082 (30.9)
	Sulfonamides	135 (9.1)	977 (6.5)	1891 (8.2)
	Macrolides	418 (28.4)	3,745 (24.9)	6,913 (30.2)
	Aminoglycosides	0	2 (0.01)	11 (0.05)
	Quinolone	321 (21.8)	2,645 (17.6)	5,639 (24.6)
	Other antibacterial	199 (13.5)	1,470 (9.8)	2,251 (9.8)
	Antimycotic	63 (4.3)	458 (3.0)	829 (3.6)
	Antiviral	75 (5.1)	663 (4.4)	1,296 (5.7)

[a] This class comprises beta-lactam antibacterials other than penicillins.

The Poisson regression model revealed a strong relation of antimycotic exposure to an MS diagnosis compared to No AID (IRR = 1.26, 95 % CI 1.16–1.38, $p < 0.001$). This suggests a notable 26 % increase in the frequency of antimycotic usage among individuals diagnosed with MS in the 5 years preceding diagnosis (Table 3).

We observed a difference between the MS and Crohn's disease cohorts in the exposure to antimycotic drugs in the five years preceding diagnosis, but the difference did not reach significance (Fig 2).

3.3. Antiviral

In our study, 5.5 % of persons with MS had been exposed to antiviral drugs at least once within the five years preceding their MS diagnosis. The standardized relative frequency in persons with No AID was 4.3 % and in Crohn's disease 6.1 % (Table 2).

During the five years before diagnosis, persons with MS showed an OR of 1.28 (95 % CI 1.15–1.43) for antiviral exposure compared to the No AID cohort (Fig 2A). Similar findings were obtained in CIS analysis (OR = 1.17, 95 %CI 0.91–1.49) and the sensitivity (OR = 1.18, 95 % CI 1.01–1.41) and (Fig 2B, Supplementary Fig 3). A difference between the two groups was only observed two years before diagnosis but not confirmed in the sensitivity analysis (Supplementary Fig 5B). Also, no statistically significant difference between MS and No AID cohorts was seen in the IRR analysis.

While we did not observe consistent differences in the use of antiviral drugs between the MS and Crohn's disease cohorts (Supplementary Fig 3, Supplementary Fig 5B), the IRR indicates lower antiviral use in MS compared to Crohn's disease (0.88; 95 % CI 0.82–0.94, $p < 0.001$) (Table 3).

4. Discussion

In this retrospective study, we examined the relation of various types of antibiotics, antimycotics and antivirals to the diagnosis of MS, their frequency of usage, and the annual usage patterns. This examination was compared to two control cohorts: matched persons with No AID and Crohn's disease. We utilized ambulatory claims data that accounts for 85 % of the Bavarian population. We conducted a conservative sensitivity analysis to assess the robustness of our results. Therefore, we excluded persons with any ICD records that could be interpreted as symptoms of MS in the 5 years preceding the MS diagnosis.

Additionally, we conducted a separate analysis in patients with CIS. Our findings, from the main analysis, sensitivity analysis and CIS analysis, consistently show that individuals diagnosed with MS or CIS had higher exposure to various antibiotics, including all classes of antibiotics, when compared to participants with No AID in the 5 years preceding their MS diagnosis. Interestingly CIS had a higher likelihood of antibiotic exposure than those in the main and sensitivity analyses. Furthermore, the proportion of patients diagnosed with CIS compared to MS at disease onset, based on the 2017 McDonald criteria, aligns with the low rate of CIS observed in our study (11 %) (Schwenkenbecher et al., 2019). Persons with Crohn's disease had an even higher exposure to antibiotics than persons with MS in the 5 years before diagnosis. The number of persons exposed to antiviral and antimycotic drugs was much lower compared to antibiotics. Nevertheless, we also observed a higher exposure to antimycotic and antiviral substances in MS compared to No AID in the years preceding the diagnosis. However, the kinetic varied among the substances. The difference between MS and No AID decreased from year five to year one before MS diagnosis for antibiotics while it increased for antiviral and antimycotic substances.

In Germany, all antibiotics are available only on prescription, and about 85 % of antibiotic prescriptions are for outpatients (Zeidan et al., 2012). Germany has a relatively low outpatient antibiotic use compared to the rest of Europe (European Centre for Disease Prevention and Control, 2020). A statistically significant decline was observed over the 2011–2020 period for the EU/EEA and eight individual countries, including Germany (European Centre for Disease Prevention and Control, 2020).

Our study possesses several key strengths, including its substantial size, a well-defined population, and a systematic population-based approach. The inclusion of carefully chosen control cohorts, including

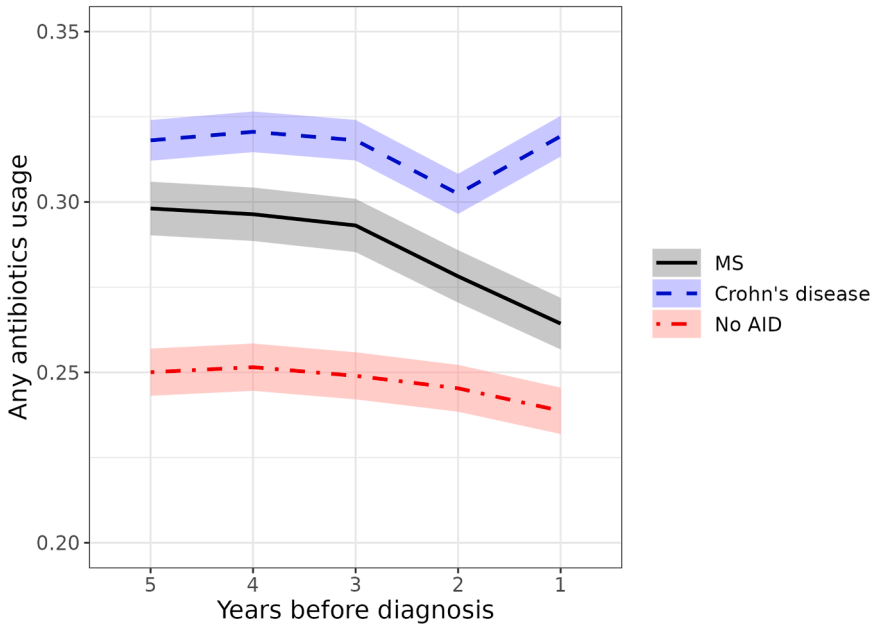

Fig. 1. Relative frequency of antibiotics usage 5 years before diagnosis. Standardized values are displayed for MS, Crohn's disease, and persons without autoimmune diseases.

Crohn's disease as a positive control, enhances the robustness of our comparisons. However, Wijnands et al. (Wijnands et al., 2019) observed that the use of anti-infectives (ATC J) was more common in the five years preceding a first demyelinating event compared to a matched population cohort. While differences in study design exist, their approach is broadly comparable to our CIS analysis.

To our knowledge, this is the first study to systematically investigate the exposure of individuals with MS to all classes of antibiotics, antivirals, and antimycotic drugs in the years preceding diagnosis, encompassing the main analysis, conservative sensitivity analysis, and CIS-specific analysis. Furthermore, our study employed diverse modelling methods alongside robust sensitivity analyses to ensure the reliability of our findings. These strengths collectively enhance the credibility and generalizability of our findings.

Inconsistent findings have been reported in the literature with respect to the association of several classes of antibiotics and the risk of MS. Three case-control studies indicated a reduced risk of experiencing a first MS attack through antibiotic use (Ren et al., 2017; Alonso et al., 2006; Sipilä et al., 2023). Also, a recent study from Finland with a large cohort size reported no association between the use of antibiotics and MS risk (Sipilä et al., 2023). However, among these studies, only one claimed to be population-based, while the other had short timelines, smaller sample sizes and were based on questionnaires or self-reporting. Conversely, two studies reported an increased use of antibiotics before MS diagnosis. A case-control population-based study conducted in Denmark reported an approximate 20 % increased risk of MS associated with the use of penicillin and a roughly 30 % increased risk linked to the use of most other types of antibiotics (Nørgaard et al., 2011). Additionally, an Italian study showed a significant association between antibiotic usage in the three years before diagnosis and a heightened risk of MS (Baldin et al., 2021).

Our findings further substantiate the relationship between antibiotic drug use and MS risk in the main analysis, the sensitivity analysis and the CIS analysis We underscore the validity of our approach by confirming the strong association of antibiotic use with Crohn's disease. Moreover, we provide evidence that the strongest association is observed in the years distant from the diagnosis. The observation that CIS patients had an even higher likelihood of antibiotic exposure than MS patients in the years preceding diagnosis are with this finding. However, in CIS, no kinetic effect was observed, as the temporal pattern of antibiotic use remained unchanged in the period leading up to the diagnosis. We also demonstrate increased use of antiviral and antimycotic substances in the years preceding MS diagnosis. However, the kinetic is different from antibiotic use, with the largest differences in the year just before diagnosis.

The mechanism behind these associations remains uncertain. Several scenarios could be discussed.

The increased use of antimicrobial substances in MS could be attributed to a higher rate of infections in persons who later develop MS. A link between infections and the occurrence of autoimmune diseases, including MS, is supported by animal models and human studies. However, in our own and other studies, no association of infections with the MS diagnosis was observed. We and others even noted a lower rate of respiratory infections during the five years preceding MS diagnosis, making it unlikely that an increased rate of infections is responsible for the increased rate of antimicrobial use in the years preceding MS diagnosis (Sipilä et al., 2023; Gasperi et al., 2021b).

The use of antimicrobial substances could also be related to the prodromal phase of MS or subclinical disease activity that has not yet led to the MS diagnosis (Gasperi et al., 2021b; Marrie et al., 2022). Persons in the time period before diagnosis experience nonspecific or neuro-psychiatric symptoms, which lead to altered healthcare behavior and possibly also increased use of substances. While this could explain the increased use of antiviral and antimycotic drugs, it seems unlikely for antibiotic drugs, given the findings in our study. The rate of prodromal symptoms is highest in the year before diagnosis but decreases in the more distant years before diagnosis. By contrast, the difference in the use of antibiotic drugs is highest 5 years before diagnosis and decreases thereafter. Thus, the occurrence of prodromal symptoms alone is unlikely to explain the increased use of antibiotics before diagnosis.

The most likely explanation for the increased use is the effect of antimicrobial substances on the microbiome. It is well known that antibiotic drugs have profound effects on the microbiome, particularly the gut microbiome (Cryan et al., 2019). Gut microbiota interact with intestinal and immune cells, particularly in the small intestine and colon (Correale et al., 2022). It is important to note that this intricate interplay affects peripheral immune function and extends to the CNS (Christovich and Luo, 2022). The microbiome's importance in MS was shown in

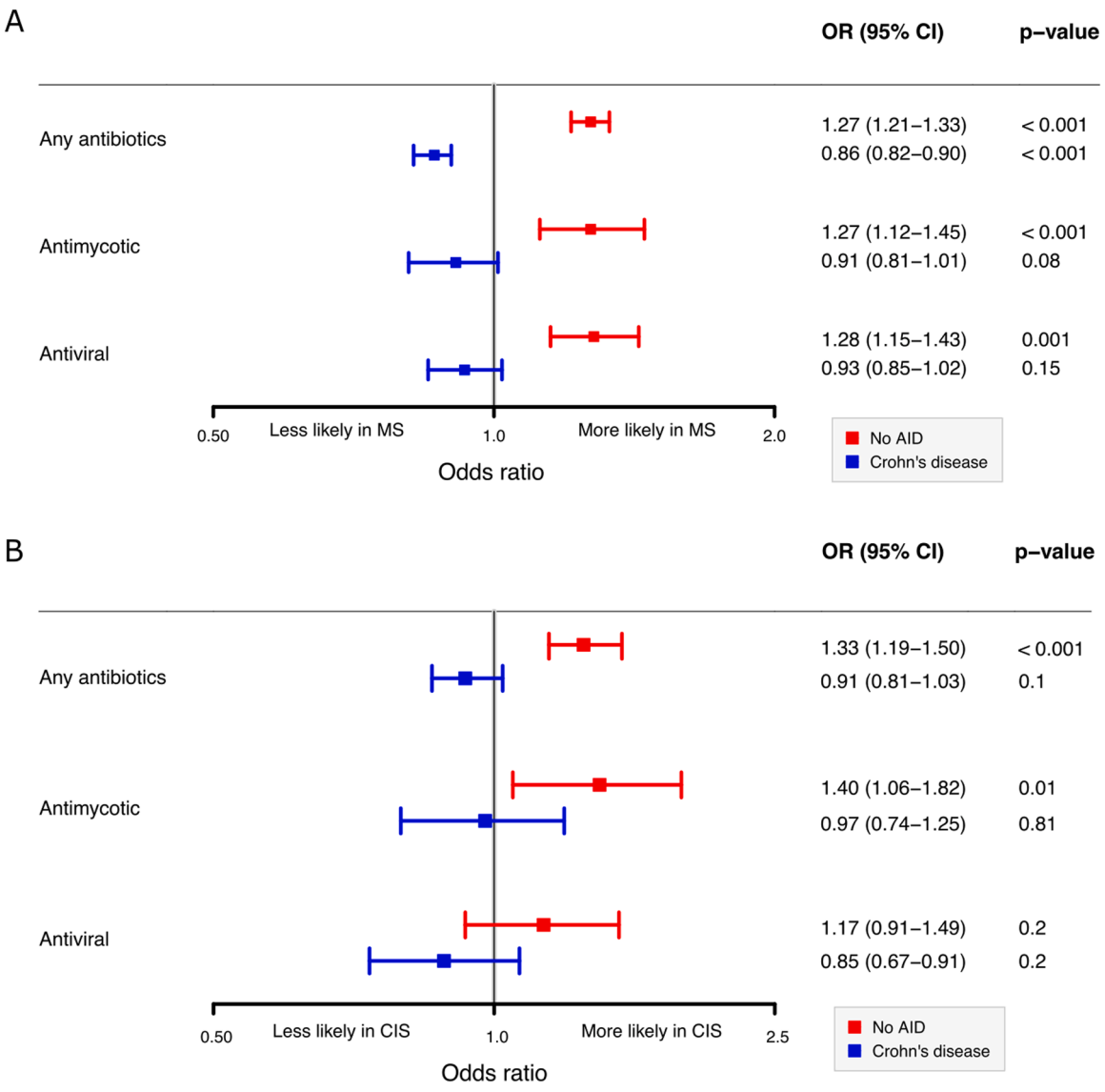

Fig. 2. Antibiotic, antimycotic, and antiviral drug use (at least once) in the five years before diagnosis for MS cohort vs. control cohorts from (A) the main and (B) CIS analysis.

experimental animal models and recent studies on the microbiome in MS (Christovich and Luo, 2022; Correale et al., 2022; Grigg and Sonnenberg, 2017).

Our study provides evidence for increased use of all classes of antimicrobials preceding the diagnosis of MS. While this study shows an association between antibiotic use and MS diagnosis, causation cannot be inferred. Future studies with prospective cohort designs, longer observation periods, and detailed microbiota analyses are warranted to clarify whether increased antibiotic use is part of the MS prodrome or a true risk factor for MS and to explore the underlying mechanisms of these associations.

Our study has several limitations. Despite the high standards of the BASHIP database, errors and incorrect coding cannot be eliminated. We also lacked information on the number of treatment days and actual consumption in our study. Additionally, it only contains approximately 85 % of the overall population, which could enable sufficient generalizability of our findings. The 15 % not encompassed by the study data primarily consists of individuals with private health insurance, such as civil servants, the self-employed, and those with incomes exceeding a specific threshold. The influence of these factors on the presented results is considered relatively insignificant.

Data availability

For data protection reasons, the authors cannot distribute the underlying data. Interested researchers may contact the corresponding author or the BASHIP to request access.

Ethics approval

In this retrospective cohort study, we analyzed anonymous claims data held by the BASHIP. Approval by an ethical standards committee on human experimentation (institutional or regional) for any experiments using human participants was not needed according to the Guidelines and Recommendations for Good Practice of Secondary Data Analysis. Approval was obtained from the responsible data protection officer of the BASHIP. Likewise, there was no need for written informed consent from participants.

CRediT authorship contribution statement

Sonia Darvishi: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal

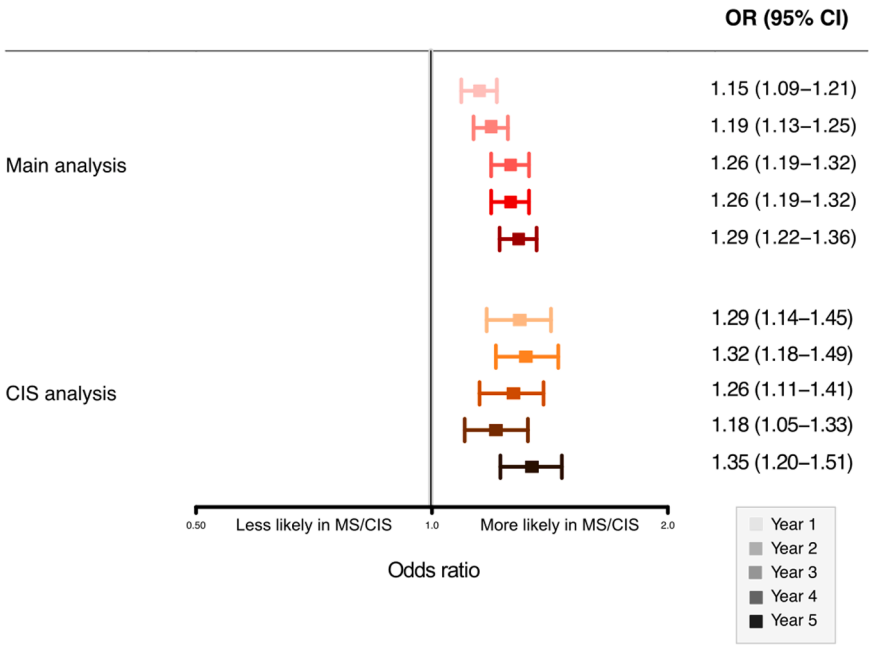

Fig. 3. Antibiotic drugs use per year for the MS and CIS cohort vs. No AID cohort in the 5 years preceding the diagnosis.

Table 3
Incidence rate ratios in the 5 years before diagnosis for MS vs. control cohorts.

	Predictor	Mean[a] count (95 % CI)	IRR	95 % CI	p-value
Any antibiotics					
	MS	2.29 (2.26–2.31)	–	–	–
	No AID	1.83 (1.81–1.85)	1.25	(1.23, 1.27)	< 0.001
	Crohn's disease	2.65 (2.63–2.67)	0.86	(0.85, 0.87)	< 0.001
Antimycotic					
	MS	0.062 (0.058–0.067)	–	–	–
	No AID	0.049 (0.046–0.053)	1.26	(1.16, 1.38)	< 0.001
	Crohn's disease	0.059 (0.056–0.063)	1.05	(0.98, 1.14)	0.1
Antiviral					
	MS	0.102 (0.096–0.108)	–	–	–
	No AID	0.097 (0.092–0.102)	1.04	(0.97, 1.12)	0.2
	Crohn's disease	0.113 (0.109–0.118)	0.88	(0.82, 0.94)	< 0.001

[a] Marginal means adjusted for age and sex.

analysis, Data curation, Conceptualization. **Ewan Donnachie:** Writing – review & editing, Data curation. **Paula Anne Uibel:** Writing – review & editing. **Martina Flaskamp:** Writing – review & editing. **Christiane Gasperi:** Writing – review & editing. **Alexander Hapfelmeier:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Bernhard Hemmer:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

Bernhard Hemmer has served on scientific advisory boards for Novartis; he has received research grants from Hoffmann La Roche for multiple sclerosis research. He has received honoraria for counseling (Gerson Lehrmann Group). He holds part of two patents; one for the detection of antibodies against KIR4.1 in a subpopulation of served as a DMSC member for AllergyCare, Sandoz, Polpharma, Biocon and TG therapeutics; his institution patients with multiple sclerosis and one for genetic determinants of neutralizing antibodies to interferon. None of the conflicts are relevant to the topic of the study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2025.106366.

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