Dysbiosis in Alzheimer’s disease might be triggered by certain classes of antibiotics with time-lapse: New insights in the pathogenesis?

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Abstract

**Background and objectives:** Alzheimer's disease (AD) is a progressive neurodegenerative illness, responsible for 60-70% of all dementias, affecting over 50 million people worldwide, and nearly 11 million in European countries. Several putative factors are identified in the literature as causative agents or risk factors for the development of AD. The amyloid cascade hypothesis has been the main hypothesis about the pathophysiology of AD for decades. Recent studies raised the possible role of dysbiosis in the development of AD which prevents memory loss. The amyloid-β (Aβ) deposition might be considered as an inflammatory reaction to certain molecular products arising from the altered microbiome. Based on the above observations, it has been suspected, that antibiotic consumption patterns of different antibiotic classes might be associated with the prevalence of AD in European countries.

**Methods:** Antibiotic consumption (ECDC) for 1997-2007, 2008-2018, and as the whole 1997-2018 period, have been compared to the AD prevalence for 2018 expressed in percentage of the population and statistically analyzed by Pearson calculation.

**Results:** A significant positive correlation has been found between the AD prevalence (2018) and the average quinolone consumption for the year 1997-2007 ($p: 0.044$). A similar association was not observed for the entire 22 years (1997-2018) of the average quinolone consumption, and the years 2008-18, indicating 10-20 years of time-lapse between the antibiotic exposure and the development of AD. The ratio of broad-spectrum and narrow-spectrum antibiotics (B/N) estimated in the ECDC database for the years of 2008-2018 showed a strong positive association with AD prevalence (2018) ($p: 0.026$) and a positive correlation tendency for the entire 22 years 1997-2018 ($p: 0.063$), but none for the years 1997-2007 ($p: 0.241$). Broad-spectrum, beta-lactamase sensitive penicillin (J01CA) consumption showed a positive (non-significant) correlation with the prevalence of AD for the years 2008-2018 ($p:0.080$).

**Discussion:** Our study indicated the possible sequential role of certain classes of antibiotics in the development of dysbiosis leading to amyloid deposits of AD, which strengthen the possible role of different mediator molecules (short-chain fatty acids, lipopolysaccharides, etc.) produced by the altered microbiome in the development of AD.

Introduction
Diagnosed by the German psychiatrist and neuropathologist, Aloise Alzheimer in 1906, Alzheimer's disease (AD) is the most prevalent form of dementia in the aging population\(^1\). AD is considered the sixth major cause of death in the USA (Alzheimer's: America's Sixth Leading Cause of Death). AD patients exhibit the gradual decline of cognitive abilities and memory functions until the development of incapability to perform routine daily functions \(^2, 3\) The number of prevalent cases of dementia doubled from 1990 to 2016, while the age-standardized data of population aging and growth remained about the same. According to the 2016 Global Burden of Diseases (GBD) data, the prevalence of Alzheimer's disease in Europe stands at 5.05%. Regarding all cases, the prevalence was about half in men compared to women (3.31% vs. 7.13%). The number of newly diagnosed AD patients in Europe was 11.08 per 1000 person-years \(^3, 4, 5\). According to the WHO report (September 2020), around 50 million people have dementia, and there are nearly 10 million new cases every year. Alzheimer's disease is the most common form of dementia and may contribute to 60–70% of the cases \(^6, 7\).

In the early '90s, the amyloid cascade hypothesis has been the main hypothesis about the pathophysiology of AD \(^8, 9\). The putative etiologies and risk factors are extensively reported in the relevant publications \(^10\). Recent studies \(^11-13\) raised the possible role of dysbiosis in the development of AD, and antibiotics entering the human body, either as therapeutic agents or as environmental pollutants, considered strong agents capable of modifying the gut microbial flora. Now, it is suspected that the development of AD starts, with dysbiosis and inflammatory changes in the gut, 10-20 (30) years before the actual development of AD, involving different mechanisms, primarily mediator molecules, (gut-brain axis /GBA/) produced by the intestinal microbiome. It is more likely that infections, infectious agents, and their toxic products may be a trigger factor for neurodegenerative processes, mainly through the disruption of the functioning of the immune system, which is associated with excessive synthesis and accumulation of Aβ, hyperphosphorylation of tau protein, and induction of chronic inflammation in the brain \(^14, 15\).

Intestinal microflora, which contains up to 95% of all human microbiome bacteria, forms the microbiome-gut axis that provides two-way communication through cytokine, immunological, hormonal, and neuronal signals \(^16, 17\).

**Hypothesis/objectives**

The possible role of dysbiosis in the development of AD raises the concept that external factors, including antibiotics, might trigger this process. Antibiotics are one of the most powerful agents triggering profound changes in the composition of gut flora, particularly early in life, when the gut microbiota has not yet been fully established, and hence, they might contribute to the development of dysbiosis. Dysbiosis might contribute to the pathomechanisms of different metabolic, neurodegenerative, and other diseases \(^18-22\) including AD.

As far as different classes of antibiotics might trigger different dysbiosis, including the ones promoting AD, we have hypothesized that the AD-associated dysbiosis might be related to the qualitative and quantitative consumption of different classes of antibiotics and reflected in the prevalence figures of AD in European countries. The higher consumption rate of certain classes of antibiotics, through the modification of gut flora, might be responsible for the higher prevalence of AD.

To evaluate the relevance of our hypothesis, we have compared the average yearly antibiotic consumption data of different European countries to the calculated prevalence of AD published as a percentage of the population in European countries, in the Dementia Yearbook of 2020 for 2018 \(^7\).

**Methods**
Based on the ECDC yearly reports of antibiotic consumption in the community from 1997 to 2018 at ATC (Anatomical Therapeutic Chemical classification) level three and level four in the group of J01C (penicillin), average yearly antibiotic consumption was calculated for 30 European countries. The average total yearly systemic antibiotic consumption (J01) was expressed in Defined Daily Dose/1,000 inhabitants/day (DID) and the relative share of major antibiotic groups (J01A /tetracycline/, J01C /penicillin/, J01D /cephalosporin/, J01F /macrolide/, J01M /quinolone/) in DID was calculated as a percentage of the total (J01) consumption. At ATC level four of the penicillin group consumption (J01CE /narrow spectrum, beta-lactamase sensitive penicillin/, J01CF /narrow spectrum, beta-lactamase resistant penicillin/, J01CA /broad-spectrum, beta-lactamase sensitive penicillin/, and J01CR /broad-spectrum, beta-lactamase resistant combination penicillin/) was estimated as yearly average consumption in the years 1997-2018. Concerning the long process of the development of AD, we have suspected that the role of antibiotic consumption, if such association exists, does not follow immediately the increase of AD prevalence, but several years later. Testing the relevance of this hypothesis, we have calculated the average yearly antibiotic consumption separately for the years 1997-2007 and 2008-2018. Prevalence data of AD was obtained from the Dementia in Europe Yearbook 2020 and appeared as a percentage of the population. Pearson correlation was used to calculate significance. A strong correlation was considered when p values were ≤ 0.05. The data for comparisons are shown in Tables 1-3. Scatter diagrams showed the association between the quinolone (J01M), broad-spectrum, beta-lactamase sensitive penicillin (J01CA), and the broad spectrum/beta-lactamase inhibitor combination penicillin (J01CR) consumption and the prevalence of AD 2018. Similarly, diagrams were plotted to compare the ratio of broad-spectrum and narrow-spectrum (B/N) antibiotics to AD prevalence (Diagrams 1-5.).

Results

Using Pearson calculation, a strong positive correlation has been found between the AD prevalence (2018) and the average quinolone consumption for the year 1997-2007 (Pearson p: 0.044). We did not find a similar association for the entire 22 years (1997-2018), and the average quinolone consumption for 2008-18 and the prevalence of AD, indicating 10-20 years of time-lapse between the antibiotic exposure (quinolone) and the development of AD. The ratio of broad-spectrum and narrow-spectrum antibiotics (B/N) estimated in the ECDC database for the years of 2008-2018 showed a strong positive association with AD prevalence (2018) (p:0.026) and a positive correlation tendency for the entire 22 years of 1997-2018 (p: 0.063), but none for the years 1997-2007 (p:0.241). Broad-spectrum, beta-lactamase sensitive penicillin (J01CA) demonstrated positive, moderate (p: 0.08), correlation tendencies for 2008-18 (shown in Table 3, and Diagram 4.) also. Interestingly, no similar association has been observed on comparing broad-spectrum, beta-lactamase combined (J01CR) penicillin and the prevalence of AD (Diagram 5.).

Discussion
The microbial community of the gut includes trillions of bacteria with an estimated biomass of 1.5-2 kg and this is the largest organ in the body composed of 1,000–1,200 cell types (species) that encode 150-fold more genes (microbiome) than we have in our genome. The gut microbiota plays a fundamental role in human health, as it evolved specific functions that complement human metabolism and physiology, and several external factors are capable of influencing its composition, reducing the diversity of the microbiome, and paving the way for different diseases\textsuperscript{24,25}.

Antibiotics are considered the most effective external factors producing perturbance of the microbial flora. Common features of post-antibiotic dysbiosis include a loss of taxonomic and functional diversity combined with reduced colonization resistance against invading pathogens, which harbors the danger of antimicrobial resistance\textsuperscript{26}.

Many studies reported a decrease in bacterial diversity with antibiotic treatment. An extensive meta-analysis including 129 studies indicated that amoxicillin, cephalosporin, macrolides, clindamycin, quinolones, and sulphonamides decreased the abundance of \textit{E. coli} in the gut flora. Even the short-term effect of broad-spectrum antibiotics on the gut flora was profound, with a loss of diversity and drastic shifts in community composition. In addition, antibiotics considerably reduced the abundance of bacterial taxa with important metabolic functions, such as the production of butyrate and other molecules\textsuperscript{27}.

Experimental studies using the most frequently prescribed quinolone, ciprofloxacin (CPFX), indicated that high CPFX treatment impaired tight junction molecules \textit{Ocln/ZO-1} level and down-regulated antibacterial genes expression (\textit{reg3γ}, \textit{pla2g2a}, and \textit{defb1}). Further, the high CPFX treatment increased pro-inflammatory cytokine IL-1β in the intestinal tract decreased IL-17A of the duodenum but increased IL-17A of the colon at day 37\textsuperscript{28}.

Enteric bacteria may have a major impact on the immune system, brain development, and behavior, as they can produce several neurotransmitters and neuromodulators like serotonin, kynurenine, catecholamine, etc., as well as amyloids. However, brain destructive mechanisms, that can lead to dementia and AD, start with the intestinal microbiome dysbiosis, development of local and systemic inflammation, and dysregulation of the GBA. Increased permeability of the gut epithelial barrier results in an invasion of different bacteria, viruses, and their neuroactive products that support neuroinflammatory reactions in the brain\textsuperscript{14}. The alteration of the microbiome in AD had been reported, indicating the increased \textit{Bacteroides} and decreased \textit{Bifidobacterium} presence in the fecal samples of AD patients, and they observed correlations between levels of differentially abundant gut microbiota and cerebrospinal fluid (CSF) biomarkers of AD pathology also\textsuperscript{29}.

Microbial amyloids or LPS can activate toll-like receptors (TLRs) in the gut, leading to the release of pro-inflammatory and/or anti-inflammatory cytokines, which results in the imbalance of the immune system, contributing to the progression of AD pathologies and cognitive decline\textsuperscript{30}.

The AD is mostly sporadical, and no appropriate causative factors identified yet. The genetic background might be responsible only for 1-5% of the cases and is considered as familial AD\textsuperscript{2,31}. Other possible causes of AD are mentioned in the literature\textsuperscript{2,3,32-34}. The hypothesis that AD may be associated with specific dysbiosis of microbes in the intestine is observed in animal models also\textsuperscript{35}. In studies with so-called germ-free rodents, a characteristic reduction of the $\text{Aβ}$ pathology was observed, which came back again when the mice were exposed to the gut microbiota of the control mice\textsuperscript{35}.

Even though the amyloid-$\beta$ (A\textsubscript{β}) is still considered as the most important causative agent of AD, the failures of A\textsubscript{β}-centric therapies call for different therapeutic approaches. Neuroinflammation is considered a possible driving force of neurodegeneration\textsuperscript{36}. Microglia plays a crucial role in maintaining the homeostasis of brain tissue and secreting different
neurotrophic factors, cytokines, chemokines, etc. after infection and cell injury, which triggers inflammatory changes and immune response followed by tissue repair. Large meta-analyses have confirmed the presence of elevated pro-inflammatory cytokines (PICs) and other inflammatory molecules in the serum and whole blood of AD patients, indicating a higher inflammatory status, evidenced by elevated levels of interleukin 6 (IL-6), IL-12, tumor necrosis factor-alpha (TNF-α), IL-1β and IL-18, etc., compared with age- and sex-matched healthy controls.

Studies in acute and chronic animal models of AD showed a possible beneficial effect of doxycycline. In the case of the animal model investigating the effects of short-term doxycycline therapy, memory recovery was also demonstrated. According to a new concept, antimicrobial protection can be another important factor in the development of AD. Even rifampicin exhibited certain "brain-protective" properties. This theory emphasizes that the accumulation of Aβ in the brain is only an epiphenomenon that represents the immune response to the accumulation of harmful bacteria. The key point of this theory is that Aβ peptide "represents a natural antimicrobial agent", however during the development of the AD Aβ accumulates in the brain due to a constant inflammation caused by the microbiome of the gut.

The classical amyloid cascade model for Alzheimer’s disease (AD) has been challenged by several findings and accumulating evidence proves the possibility that AD starts in the intestine (dysbiosis). Molecular pathomechanisms leading to augmented inflammatory process associated with the development of amyloid and tau pathology, mitochondrial dysfunction, etc. are reported in the literature. Microbiome-derived proteins, lipoproteins, and nucleic acids provide essential microorganism-specific gene products which support microbial structure, function, and viability. Many of these are the same components shed from the different Gram-negative bacterial species into surrounding bio-fluids, which eventually enter the systemic circulation. The abundance of short-chain fatty acids (SCFAs), is often low in the gut of older adults with AD. It has been demonstrated that inhibition of free fatty acid receptor 2 (FFAR2) signaling increases amyloid-beta (Aβ) stimulated neuronal toxicity.

Our analyses support the probability that antibiotic-modified microbiome might play part in the development of AD in addition to other putative factors. It is important to note that this effect might develop 10-15-20 years later after the suspected initial antibiotic exposure and microbiome modification (dysbiosis) possibly triggering a self-perpetual, slow-motion chain of molecular mechanisms ending up in AD and other dementias.

We have found that the average quinolone (J01M) consumption for the years 1997-2007 in the countries included in the study, showed a remarkable positive association with the prevalence of AD (and other dementias) estimated for 2018, indicating an about 10-year lapse between the antibiotic insult and the development of AD. Our comparative study identified certain classes of antibiotics (quinolone /J01M/, and broad-spectrum, beta-lactamase sensitive penicillin /J01CA/), which might alter the microbiome in a favorable way for the development of AD, while broad-spectrum, combination penicillin did not show a similar association, indicating the possible ameliorating effect of beta-lactamase inhibitors in the development of AD. This effect might appear in a sequential pattern indicating the possible time lapse between the antibiotic insult and the development of AD. The cumulative effect of broad-spectrum antibiotics might enhance the development of AD also.

The limitation of our study is that the role of antibiotic consumption patterns cannot be observed at the individual level for AD patients compared to a control group. We assume that different antibiotics might induce different changes in the composition of the gut microbiome, which alters the production of different mediator molecules influencing the development of different diseases, including AD.
A positive correlation tendency was observed between the ratios (consumption) of the broad and narrow spectrum antibiotics. The table below presents the consumption of broad and narrow-spectrum antibiotics for various countries in Europe for the year 1997.

Table 1. Yearly, average antibiotic consumption for 1997–2018 expressed in percentage (%) of the total, systemic antibiotic consumption (J01, 100%) estimated as Defined Daily Dose/1000 inhabitants/Day (DID) per country. ATC codes: J01A: tetracycline, J01C: penicillin, J01CA: broad-spectrum, beta-lactamase sensitive penicillin, J01CR: broad-spectrum, beta-lactamase sensitive penicillin combined with beta-lactamase inhibitors, J01CE: narrow spectrum, penicillinase sensitive penicillin, J01CF: narrow spectrum, beta-lactamase resistant penicillin, J01D: cephalosporin, J01F: macrolide, J01M: quinolone, J01B/N: the ratio of the broad and narrow-spectrum antibiotics. A positive correlation tendency was observed between the ratios (consumption) of broad/narrow-spectrum antibiotics and AD prevalence (p: 0.063).
A positive correlation was observed between the consumption of quinolone (J01M) and penicillin, J01C: broad spectrum antibiotics. Table 2. Yearly, average antibiotic consumption for 1997-2007 expressed in percentage (%) of the total, systemic antibiotic consumption (J01, 100%) estimated as Defined Daily Dose/ 1000 inhabitants/ Day (DID) per country. ATC codes: J01A: tetracycline, J01C: penicillin, J01CA: broad-spectrum, beta-lactamase sensitive penicillin, J01CR: broad-spectrum, beta-lactamase sensitive penicillin combined with beta-lactamase inhibitors, J01CE: narrow spectrum, penicillinase sensitive penicillin, J01CF: narrow spectrum, beta-lactamase resistant penicillin, J01D: cephalosporin, J01F: macrolide, J01M: quinolone, J01 B/N: the ratio of the broad and narrow-spectrum antibiotics. A positive correlation was observed between the consumption of quinolone (J01M) and AD prevalence (p: 0.044).
| Country          | Defined Daily Dose/1000 inhabitants/day (J01) | Average B/N 2008-2018 | Prevalence of AD and other dementias in % of the population 2018 |
|------------------|--------------------------------------------|------------------------|---------------------------------------------------------------|
| Austria          | 12.45                                      | 6.57                   | 1.66                                                          |
| Belgium          | 22.52                                      | 65.89                  | 1.69                                                          |
| Bulgaria         | 17.54                                      | 20.50                  | 1.54                                                          |
| Croatia          | 17.86                                      | 6.92                   | 1.6                                                          |
| Cyprus           | 26.77                                      | 28.19                  | 1.17                                                          |
| Czech Republic   | 16.51                                      | 3.95                   | 1.41                                                          |
| Denmark          | 13.23                                      | 5.26                   | 1.51                                                          |
| Estonia          | 10.23                                      | 10.83                  | 1.74                                                          |
| Finland          | 16.16                                      | 36.42                  | 1.83                                                          |
| Germany          | 13.23                                      | 5.19                   | 1.91                                                          |
| Greece           | 32.22                                      | 242.24                 | 1.99                                                          |
| Hungary          | 13.65                                      | 34.17                  | 1.49                                                          |
| Iceland*         | 18.76                                      | 14.53                  | 0.813                                                         |
| Ireland          | 19.51                                      | 4.77                   | 1.09                                                          |
| Italy            | 22.23                                      | 141.13                 | 2.12                                                          |
| Latvia           | 10.75                                      | 10.05                  | 1.74                                                          |
| Lithuania        | 14.97                                      | 7.34                   | 1.74                                                          |
| Luxembourg       | 23.35                                      | 39.86                  | 1.25                                                          |
| Malta            | 19.18                                      | 116.99                 | 1.38                                                          |
| Netherlands      | 9.62                                       | 7.86                   | 1.49                                                          |
| Norway           | 15.28                                      | 1.41                   | 0.19                                                          |
| Poland           | 20.39                                      | 28.70                  | 1.38                                                          |
| Portugal         | 17.79                                      | 30.27                  | 1.88                                                          |
| Romania          | 26.14                                      | 12.10                  | 1.43                                                          |
| Slovakia         | 20.33                                      | 8.67                   | 1.15                                                          |
| Slovenia         | 11.68                                      | 2.86                   | 1.65                                                          |
| Spain            | 18.72                                      | 5.22                   | 1.83                                                          |
| Sweden           | 12.74                                      | 4.66                   | 1.56                                                          |
| UK               | 16.95                                      | 2.73                   | 1.56                                                          |

Table 3. Yearly average antibiotic consumption for 2008-2018 expressed in percentage (%) of the total, systemic antibiotic consumption (J01, 100%) estimated as Defined Daily Dose/1000 inhabitants/Day (DID) per country. ATC codes: J01A: tetracycline, J01C: penicillin, J01CA: broad-spectrum, beta-lactamase sensitive penicillin, J01CR: broad-spectrum, beta-lactamase sensitive penicillin combined with beta-lactamase inhibitors, J01CE: narrow spectrum, penicillinsensitive penicillin, J01CF: narrow spectrum, beta-lactamase resistant penicillin, J01D: cephalosporin, J01F: macrolide, J01M: quinolone, J01B/N: the ratio of the broad and narrow-spectrum antibiotics. A positive, strong correlation was observed between the ratios (consumption) of broad/narrow-spectrum (B/N) antibiotics and AD prevalence ($p: 0.026$). Positive correlation tendency has been found between broad-spectrum, beta-lactamase sensitive penicillin (J01CA) and AD ($p: 0.080$).
Diagram 1. Association observed between the average quinolone consumption (J01M) 1997-2007 and the prevalence of AD 2018.

Diagram 2. No association was observed between the average quinolone consumption (J01M) 2008-18 and the prevalence of AD 2018.
Diagram 3. A positive association was found between the AD prevalence (2018) and the high consumption ratio of broad/narrow (B/N) spectrum antibiotics 2008-2018.

Diagram 4. A positive, moderate correlation was found between the AD prevalence (2018) and the average consumption of broad-spectrum, beta-lactamase sensitive penicillin (J01CA) 2008-2018.
Diagram 5. No correlation was observed between the AD prevalence (2018) and the average consumption of broad-spectrum, beta-lactamase resistant combination penicillin (J01CR) 2008-2018, indicating the possible ameliorating effect of beta-lactamase inhibitors on the development of AD.

Figure 1. The possible sequence of events in the development of AD

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