SciencePG
Science Publishing Group

Antibiotic-modified Microbiome Might Be Responsible for Non-contagious World-wide Epidemics

Gábor Ternák

Chair of Migration Health, Faculty of Medicine, University of Pécs, Pécs, Hungary

Email address:
gabor.ternak@aok.pte.hu

To cite this article:
Gábor Ternák. Antibiotic-modified Microbiome Might Be Responsible for Non-contagious World-wide Epidemics. *American Journal of Bioscience and Bioengineering.* Vol. 7, No. 2, 2019, pp. 34-39. doi: 10.11648/j.bio.20190702.12

Abstract: The rapid, pandemic-like, spread of certain noncontagious diseases (NCDs), like (childhood) obesity, type2 diabetes mellitus, particularly in children, autism, Alzheimer diseases, etc. prompted the researches to try to find appropriate explanation for this phenomenon. The well-known association between antibiotic enriched fodder and the weight gain of food animals, through the modification of the gut flora, clearly raises the possibility of similar relationship in human beings as well, which was described in several publications. The role of obesity in the development of diabetes is a well-known also. The antibiotic consumption pattern in the World clearly demonstrated the extensive utilization of the broad spectrum antibiotics, starting about three decades ago, and the appearance of still active degradation products in the environment, which might reenter into humans and animals again, and as the result of the alteration of human microbiome, could induce the pandemic-like NCDs. The yet to be explained rapid increase of prevalence of autism and Alzheimer diseases observed in the past three decades is alarming. According to a CDC survey, the prevalence of autism in the surveyed population was estimated as 16.8 per 1,000 (one in 59) children aged 8 years, which is considered as a 150% increase from the year 2000. The prevalence of Alzheimer's disease in Europe was estimated at 5.05% (95% CI, 4.73-5.39). Microbiome alterations were observed in both cases and obesity is present in the 30% of autism as well. Putting together the mosaics from relevant publications, it might be concluded that we face the long term side effects of antibiotic consumption/pollution manifesting, through the alteration of microbioms as the pandemic appearance of certain NCDs. The restoration of the "normal" gut flora by fecal microbiota transfer (FMT) might be an option to influence those conditions.

Keywords: Antibiotics, Microbiome, Type 2 Diabetes Mellitus (T2D), Obesity, Autism, Alzheimer Disease, Antibiotic Pollution, Fecal Microbiota Transfer (FMT)

1. Introduction

The discovery of antibiotics have greatly contributed to the extension of the average human life expectancy and it was the greatest therapeutic change in medical history, significantly altering mortality and morbidity indicators related to infections. From the introduction of Salvarsan to the latest discoveries of broad spectrum antibiotics, we quickly had to face the facts of the rapidly developing and spread of antibiotic resistant strains, and the appearance of the antibiotic pollution in the environment. The accidental observation that antibiotic enriched fodder augment the weight gain and growth of food animals resulted the more rapid spread of multi-resistant bacteria among humans, actually increased further the antibiotic load on the

environment and hence on the population as well. One might speculate that antibiotic consumption and "antibiotic enriched" environment might have a similar effect on human beings as well; augmenting obesity and growth.

Antibiotics are capable modifying the human (animal) natural gut flora (microbiome) and this phenomenon attracted considerable research efforts as it was suspected that the altered gut flora might play role, or associated with the development of different diseases. Particular attentions were paid to the discovered, so called, gut-brain axis (GBA), because altered microbiome might be related to certain behavioral changes, like autism and other neurological disorders.

Considering the huge amount of scientific observations described in thousands of publications, it might be concluded

that antibiotic-modified microbiome can act as a causative agent in the development of non-contagious world-wide epidemics as obesity, particularly in children, the related type2 diabetes mellitus, autism, Alzheimer disease and other conditions.

2. Issues for Consideration

2.1. Trends in Antibiotic Consumption (Human, Non-human)

In the year 2000, antibiotic production in the United States totaled 50 million pounds, which is around 75 thousand metric tons approximately. Accurate figures are hard to obtain, but assuming this level of production for the past 20 years, it can be estimated that one billion pounds were made during this time. When we consider that the United States is not the principal antibiotic manufacturer (China, India and other countries are heavily involved), the quantity of antibiotics produced and used worldwide may be at least three times greater. In an extensive survey [1] it was found that between 2000 and 2015, antibiotic consumption, expressed in defined daily doses (DDD), increased 65% (21.1–34.8 billion DDDs), and the antibiotic consumption rate increased 39% (11.3–15.7 DDDs per 1,000 inhabitants per day). It is of importance to notice that the highest increase was observed in low and middle income countries (LMICs). The antibiotic consumption rate increased from 8.2 to 13.6 DDDs per 1,000 inhabitants per day (63%) in India, from 5.1 to 8.4 DDDs per 1,000 inhabitants per day (65%) in China, and from 16.2 to 19.6 DDDs per 1,000 inhabitants per day (21%) in Pakistan. While antibiotic consumption in the three leading high income countries (HICs) marginally increased, the highest-consuming LMICs saw large increases. In 2015, the leading HIC consumers of antibiotics were the United States, France, and Italy. The antibiotic consumption rate of broad-spectrum penicillin is the most commonly consumed class of antibiotics (39% of total DDDs in 2015), increased 36% between 2000 and 2015 globally.

The greatest increase was in LMICs (56%), although the antibiotic consumption rate in HICs increased 15%. While the antibiotic consumption rate of the next three most consumed classes - cephalosporins (20% of total DDDs), quinolones (12% of total DDDs), and macrolides (12% of total DDDs) - all increased overall, the antibiotic consumption rate decreased in HICs. In LMICs, the antibiotic consumption rate increased 399, 125, and 119% for cephalosporins, quinolones, and macrolides, respectively, while the antibiotic consumption rate of these three drugs in HICs decreased by 18, 1, and 25%, respectively.

Since the accidental discovery of the growth promoting effect of antibiotics in animals, the utilization of antibiotics mixed to animal fodder was extensively used in the agriculture. The exact magnitude of antibiotics fed to animals as growth promoting factor is difficult to estimate, but different reports guess that it might be the 80% of the total antibiotic production of the world. As it was communicated by Thomas P. Van Boeckel et al, in 2013 [2], the global consumption of all antimicrobials in food animals was estimated at 131,109 tons (95% confidence interval (CI) (100,812 to 190,492 tons) and is projected to reach 200,235 tons (95% CI (150,848 to 297,034 tons) by 2030. Consumption levels varied considerably between countries, ranging from 8 mg/population correction unit (PCU) (a kilogram of animal product) in Norway to 318 mg/PCU in China. As the largest consumer of veterinary antimicrobials, both in relative (per PCU) and in absolute terms, China has an important leadership role with regard to its response to antimicrobial resistance (AMR) and has already set precedents in phasing out drugs that are last resorts for human infections but are still in use in Europe in animal husbandry [2]. In European countries, amounts of total antibiotics applied per kg of animal produced lie between 18 mg /Norway/ and 188 mg /Netherlands/ [3].

As the pathomechanism of antibiotic-induced weight gain is concerned in animals and humans, it was proven that augmented energy harvest from the antibiotic enriched fodder is related to the antibiotic modified flora, of the intestine. The modified flora induces mild inflammatory changes and increases the LPS level, which promotes the absorption of more nutrients through increased intestinal permeability. LPS itself can augment the food absorption in experimental animals also. Similar pathomechanism was observed in the cases of antibiotic induced obesity in humans, particularly in children, as well [4, 5, 6, 7, 8].

2.2. Antibiotic Pollution of the Environment

Antibiotics used by humans are being excreted into the toilet unchanged in 30-90% of the cases. Globally, two-thirds of antibiotics produced are used in animals. They secrete them onto land and into slurry pits, which can run off into rivers, lakes or groundwater. Direct discharges from manufacturing, either during the actual production of the active ingredient, or during formulation, can be exceedingly high. Larson et al (2007) reported that samples taken from effluents of a major manufacturer in India contained 31000 microgram/L of ciprofloxacin which is one thousand times higher than the therapeutic level [9]. Antibiotic utilization for clinical or farming purposes selects resistant microorganisms. It is thus predictable that residues from hospitals or farms will contain both types of pollutants: antibiotics and resistance genes. The metabolic fate of antibiotics depends on their chemical properties, functional groups and the reactive atoms in the structures. Various types of antibiotics can therefore be treated differently by the body and thus precede different types of metabolites in the excretion (urine/feces) [10, 11, 12].

2.3. Gut Flora Alteration by Antibiotics

It has been estimated that the microbes in our bodies collectively make up to 100 trillion cells, tenfold the number of human cells, and suggested that they encode 100-fold more unique genes than our own genome [13]. The majority of

microbes resides in the gut, have a profound influence on human physiology and nutrition, and are crucial for human life. Furthermore, the gut microbes contribute to energy harvest from food, and changes of gut microbiome may be associated with bowel diseases or obesity etc. Thousands of species are found in the gut microbiome, and the majority of these species belong to six bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia [14]. Gut microbiota are highly dynamic and have substantial interindividual and intraindividual variation. People with different genotypes, geographic locations, lifestyles, and ages have distinct gut microbiota, and microbiomic differences even exist between monozygotic twins [15, 16]. Microbiomic differences begin to emerge after we are born. An infant is inoculated with microbiota immediately after delivery, the microbiome changes dramatically during the first year of life, then becomes adult-like at age 2,5 years, and subsequently remains relatively stable until old age [17].

Antibiotics directly target the microorganisms that make up the microbiome; therefore, the composition of human and animals' gut microbiomes could be changed even by very low concentrations of some antibiotics. Thus, it is believed that environmental antibiotic-induced gut microbiota dysbiosis is tightly linked to human health. As Jakobsson et al (2010) pointed out the gut microbiota are very essential for host health. They participate in the regulation of many physiological functions. The gut microbiota resides in our intestinal mucus layer and even participates in shaping the mucus layer. The composition of the gut microbiota regulates the colon mucus barrier [18]. The effects of antibiotics on human gut microbiota can persist for several years. Reported treatment with clarithromycin and metronidazole persistently changed the gut microbiota composition for up to 4 years [19]. After infants finished antibiotic treatment, although some aspects of microbiota composition recovered to pretreatment levels, the abundance of some bacterial species was permanently altered [20]. This kind of change is detrimental for babies because it could perturb their early development. Exposure to some antibiotics could increase the severity of certain disease. For example, *Clostridium difficile*, a major cause of antibiotic-induced diarrhea, could greatly increase the morbidity and mortality of hospitalized patients.

Experimental data and clinical observation with antibiotic treatment indicated the development or the amelioration of certain nonmicrobial-driven diseases [21-24].

3. Major, Non-contagious Emerging Epidemics (Pandemics)

3.1. Obesity and Type 2 Diabetes Mellitus (T2D)

The rapid, "epidemic-like" spread of obesity, particularly among children, prompted several nations to introduce certain measures to tackle the problem. According to OECD data the share of children who are overweight or obese at age 15 ranges from 10% in Denmark to 31% in the United States. Despite policies put in place in OECD countries for a number of years, the number of 15-year-olds who report to be

overweight or obese has steadily increased since 2000 in the majority of countries, according to the Health Behavior in School-aged Children survey [25]. A more detailed analysis covering children aged 3 to 17 at several points in time shows relatively stable rates in France up to 2012, while trends have been somewhat upward again for both boys and girls in England since 2012, and since 2011 for boys in the United States, OECD latest Obesity Update says. In the majority of countries, women are more obese than men – however, in most OECD countries for which data are available, male obesity has been growing more rapidly. Obesity has been rising more rapidly in less educated men and in average-educated women, in most countries. However, in the United States, rates have been increasing most rapidly among high-educated people. Studies demonstrated that this tendency started approx. from the 1980-th, nearly four decades ago. The U.S. came in number one with a 38.2% rate, as far ahead of second place Mexico (32.4%). New Zealand was third at 30.7%, and Hungary was fourth at 30%. (Japan was last at 3.7 %.) [26, 27]. Today, more than 1.7 billion adults worldwide are overweight, and 312 million of them are obese. In addition, at least 155 million children worldwide are overweight or obese.

Comparing large databases of childhood and adult obesity with antibiotic consumption in different countries in Europe, significant association was found between the consumption of broad spectrum antibiotics expressed in DDD/1000 inhabitants/day and childhood obesity, particularly with the consumptions of macrolides. Similar association was not observed with narrow spectrum antibiotics and in adults [28].

Diabetes is a major public health problem that is approaching epidemic proportions globally. Worldwide, the prevalence of chronic, non-communicable diseases is increasing at an alarming rate. About 18 million people die every year from cardiovascular disease, for which diabetes and hypertension are major predisposing factors. A diabetes epidemic is underway. Each year 7 million people develop diabetes and the most dramatic increases in T2D have occurred in populations where there have been rapid and major changes in lifestyle, demonstrating the important role played by lifestyle factors and the potential for reversing the global epidemic [29].

T2D has attained the status of a global pandemic, spreading from affluent industrialized nations to the emerging economies of Asia, Latin America, and Africa. There is significant global variation in susceptibility to T2D, with Pacific Islanders, Asian Indians, and Native Americans being considerably more prone to develop the disorder. Although genetic factors may play a part, the rapidity with which diabetes prevalence has risen among these populations reflects the far-ranging and rapid socioeconomic changes to which they have been exposed over the past few decades [30]. Long considered a disease of the affluent "Western" countries of Europe and North America, T2D has now spread to every corner of the world. Indeed, there are now more people with diabetes residing in the "emerging" economies than in the industrialized nations [31]. In developing nations,

the prevalence of diabetes is undoubtedly higher among urban versus rural populations [32] although it is also rapidly increasing in rural areas [33]. T2D, which was once considered a rare condition in the pediatric population, now accounts for about 15% to 45% of all newly diagnosed cases of diabetes in children and teenagers. The epidemic of T2D in the pediatric age group is the result of a variety of factors, the most important of which appears to be an increase in the rate of obesity in children [34].

3.2. Autism

Autism spectrum disorder (ASD) is a developmental disability defined by diagnostic criteria that include deficits in social communication and social interaction, and the presence of restricted, repetitive patterns of behavior, interests, or activities that can persist throughout life. According to a CDC survey [35], the prevalence of autism in the surveyed population was estimated as 16.8 per 1,000 (one in 59) children aged 8 years, which is considered as a 150% increase from the year 2000. In 1970, only 1 child in 10,000 was diagnosed with autism. The worldwide population prevalence is about 1% [36]. Although several reasons were implicated as causative, or precipitating factors for autism, including genetics, prenatal and perinatal risk factors [37], toxic metals [38], dioxins [39], but it can be considered as an important observation that over 1/3 of the autistic children are overweight and modified gut flora was detected with the abundance of certain clostridial species (*Clostridium bolteae*), which prompted the speculations of producing vaccines against autism [40]. Other observations also supported the possible role of different bacteria [41, 42]. The discovery of the so called gut-brain axis also underlined the importance the gut microbes in the development of autism and other cognitive disorders [43].

3.3. Alzheimer Disease (AD)

A disease of unknown etiology, Alzheimer's disease (AD) is the most common type of dementia. As the elderly population grows worldwide, the number of patients with AD also increases rapidly [44] 5,7 million USA citizens live with AD, the death from Alzheimer increased by 123% since 2000, which is the 6-th leading cause of death in the USA, more than breast and prostate cancer combined, and every third elderly will die of AD or other dementia. The prevalence of Alzheimer's disease in Europe was estimated at 5.05% (95% CI, 4.73-5.39). The prevalence in men was 3.31% (95% CI, 2.85-3.80) and in women, 7.13% (95% CI, 6.56-7.72), and increased with age. The incidence of Alzheimer's disease in Europe was 11.08 per 1000 person-years (95% CI, 10.30-11.89). Broken down by sex, it was 7.02 per 1000 person-years (95% CI, 6.06-8.05) in men and 13.25 per 1000 person-years (95% CI, 12.05-14.51) in women; again these rates increased with age [45]. The leading hypothesis on AD pathogenesis has been framed within the 'amyloid cascade' construct. Pathologically, Alzheimer disease consists of amyloid-b plaques,

neurofibrillary tangles containing phosphorylated tau (p-tau), and cerebral angiopathy. Although there is a crude correlation between cognitive performance and mean plaque count, at least a quarter of persons dying with significant amounts of plaque in their brain are not cognitively impaired [46, 47]. Epidemiological evidence supports the observation that subjects with type 2 diabetes (T2D) are at higher risk to develop Alzheimer's disease (AD). However, whether and how these two conditions are causally linked is unknown [48], but the common denominator might be the altered gut flora is both cases. Recent studies have suggested the role of an infectious component in the pathogenesis of Alzheimer's disease (AD). In light of this, research has focused on some bacteria constituting the intestinal microbial flora which can produce amyloid. Once generated, the latter hypothetically triggers a systemic inflammatory response which compromises complex brain functions, such as learning and memory. Clinical studies have shown that, in cognitively impaired elderly patients with brain amyloidosis, there is lower abundance in the gut of E. rectale and B. fragilis, two bacterial species which have an anti-inflammatory activity, versus a greater amount of pro-inflammatory genera such as Escherichia/Shigella [49].

4. Conclusion

In recent decades, the global balance of communicable diseases (CD) versus non- communicable diseases (NCD) has tilted towards the latter; the World Health Organization (WHO) estimates that 60 percent of all deaths globally are attributable to NCDs, and that 80 percent of these occur in low- and middle-income countries. The phenomenon of rapid, pandemic-like spreading of certain NCDs (childhood obesity, type 2 DM, particularly in children, autism, Alzheimer disease, etc.), which were never seen before in human history, prompted researchers to try to find the exact reasons, but only several, and very divergent causative agents were suspected. Our definitive knowledge that antibiotics, through the modification of the intestinal flora, promotes weight gain in food animals, strongly supports the possibility of having the same inadvertent effect on human beings as well, which was confirmed later by several publications [50].

In one of our previous paper [28] we have identified strongly significant association between childhood obesity in Europe, and the consumption rate of broad spectrum antibiotics, which actually started to be widespread about three decades ago.

The alteration of the human intestinal microbiome was observed in several, very different diseases as well, which might not be the consequence, but the etiology of those conditions. Current knowledge of the microbiome, the concept of dysbiosis and results of preliminary research suggest that there is an association between gastrointestinal bacterial disruption and certain disorders [51]. The discovery of the relationship between the human gut microbiota and certain neurological disorders (gut-brain axis), might be another proof for the above hypothesis [43].

As one of the strongest agents to influence the composition of the gut flora are the antibiotics, it might be suspected that the continuous presence, and increase (by circulation) of broad spectrum antibiotics in the environment and in the medical therapy has reached a certain level of saturation, which started modifying the human microbiome and as a consequence, pandemic-like NCDs appeared. It is of significance that those NCDs are started parallel surfacing pandemics approximately at the same time when the wide-spread use of broad spectrum antibiotics commenced.

When we start putting together the different mosaics, it appears that we experience the global side effects of antibiotic consumption/pollution which is resurfaced through the modified microbiome induced pandemic-like NDCs.

The only alternative, which might arise, is the restoration of the "normal" gut flora by using fecal microbiome transfer (FMT). The process of stool transfer from healthy donors to the sick, known as fecal microbiota transplantation (FMT), has an ancient history. However, only recently, researchers started investigating its applications in an evidence-based manner.

References

[1] Eili Y Klein, Elena Martinez, Suraj Pant, Sumanth Gandra, Simon A. Levin, Herman Goossens, et al (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015, Proceedings of the National Academy of Sciences, March 2018 DOI: 10.1073/pnas.1717295115.

[2] Van Boeckel TP, Glennon EE, Chen D, Gilbert M, Robinson TP, Grenfell BT, et al (2017). Reducing antimicrobial use in food animals, Science Vol. 357, Issue 6358, pp. 1350-1352, DOI: 10.1126/. science.aao1495.

[3] Grave K, Torren-Edo J, Mackay D (2010). Comparison of the sales of veterinary antibacterial agents between 10 European countries. J Antimicrob Chemother, 65: 2037-2040.

[4] Andrade MJ1, Jayaprakash C, Bhat S, Evangelatos N, Brand A, Satyamoorthy K. (2017). Antibiotics- Induced Obesity: A Mitochondrial Perspective, Public Health Genomics, 20. 257–273.

[5] L. Charles Bailey, Christopher B. Forrest, Thomas ichards, Alice Livshits, Patricia DeRusso (2014): Association of Antibiotics in Infancy With Early Childhood Obesity, JAMA Pediatr. 168, 1063–1069. doi: 10.1001/jamapediatrics.2014. 1539

[6] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al (2007). Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56, 1761-72.

[7] Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57, 1470-8.

[8] Del Fiol FS, Balcão VM, Barberato-Fillho S, Lopes LC, Bergamaschi CC (2018). Obesity: A New Adverse Effect of Antibiotics?, Front. Pharmacol. 9: 1408. doi: 10.3389/fphar.2018.01408).

[9] Larsson DG, de Pedro C, Paxeus N. (2007): Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater. 30, 148(3): 751-5.

[10] Wang J, Mao D, Mu Q, Luo Y (2015). Fate and proliferation of typical antibiotic resistance genes in five full-scale pharmaceutical wastewater treatment plants, Science of the Total Environment 526, 366–373.

[11] Livermore, D. M. (2005), Minimizing antibiotic resistance. Lancet Infect. Dis. 5, 450-459.

[12] Teuber, M., (2001). Veterinary use and antibiotic resistance. Curr. Opin. Microbiol. 4, 493-499.

[13] Junjie Qin, Ruiqiang Li, Jeroen Raes, Manimozhiyan Arumugam, Kristoffer Solvsten Burgdorf, Chaysavanh Manichanh, et al (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59-65.

[14] Paul B. Eckburg, Elisabeth M. Bik, Charles N. Bernstein, Elizabeth Purdom, Les Dethlefsen, Michael Sargent et al (2015). Diversity of the human intestinal microbial flora. Science 308, 1635-1638.

[15] Goodrich, Julia K., Waters, Jillian L., Poole, Angela C., Sutter, Jessica L., Koren, Omry, Blekhman, Ran; et al. (2014). Human genetics shape the gut microbiome. Cell 159, 789 -799.

[16] Tanya Yatsunenko, Federico E. Rey, Mark J. Manary, Indi Trehan, Maria Gloria Dominguez- Bello, et al (2012). Human gut microbiome viewed across age and geography. Nature 486, 222-227.

[17] Clemente JC, Ursell LK, Parfrey LW, Knight R.(2012). The impact of the gut microbiota on human health: an integrative view. Cell 148, 1258-1270.

[18] Hedvig E Jakobsson, Ana M Rodríguez-Piñeiro, André Schütte, Anna Ermund, Preben Boysen, Mats Bemark, et al (2015). The composition of the gut microbiota shapes the colon mucus barrier. EMBO Rep.16, 164-177, 2016.

[19] Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. (2010). Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5, e9836.2010.

[20] Fouhy F, Guinane CM, Hussey S, Wall R, Ryan CA, Dempsey EM, et al (2012). High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob. Agents Chemother. 56, 5811-5820.

[21] Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al (2014). Altered profile of human gut microbiome is associated with cirrhosis and its complications. J. Hepatol. 60, 940-947.

[22] Bajaj JS, Heuman DM, Sanyal AJ, Hylemon PB, Sterling RK, Stravitz RT, et al (2013). Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. PLoS One 8, e60042.

[23] Lisowska, A., Wojtowicz, J., Walkowiak, J. (2009). Small intestine bacterial overgrowth is frequent in cystic fibrosis: combined hydrogen and methane measurements are required for its detection. Acta Biochim. Pol. 56, 631e634.

[24] Shannon L. Russell, Matthew J. Gold, Lisa A. Reynolds, Benjamin P. Willing, Pedro Dimitriu, Lisa Thosrson , et al (2015). Perinatal antibiotic-induced shifts in gut microbiota have differential effects on inflammatory lung diseases. J. Allergy Clin. Immunol. 135, 100-109.

[25] Jo Inchley, Dorothy Currie, Taryn Young, Oddrun Samdal, Torbjørn Torsheim, Lise Augustson, et al (2016). Growing up unequal.   HBSC   2016   study http://www.euro.who.int/en/health-topics/Life- stages/child-and-adolescent-health/health-behaviour-in-school-aged-children-hbsc/hbsc-international-reports/growing-up-unequal.-hbsc-2016-study-20132014-survey.

[26] http://www.czechcompete.cz/economic-policy/health-care/oecd-obesity-update-2017-obesity- rates- are- expected-to-increase-further.

[27] https://www.forbes.com/sites/brucelee/2017/06/13/global-obesity-epidemic-new-study- shows-how-big-a-problem-its-become/#342ef0b72a2c.

[28] Gabor Ternak, Zs Edel, Zs Feiszt, J. Szekeres, B. Visegrady, I. Wittmann (2015). Selective Association between Cephalosporin, Quinolone, Macrolide, and Penicillin Antibiotic Consumption and Childhood Obesity in Europe, Health, 7, 1306-1314.

[29] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3068646/.

[30] Ranjit Unnikrishnan[1], Rajendra Pradeepa[1], Shashank R. Joshi and Viswanathan Mohan (2017). Type 2 Diabetes: Demystifying the Global Epidemic, Diabetes 66(6), 1432-1442.

[31] http://www.diabetesatlas.org/resources/2017-atlas.html.

[32] Anjana RM, Pradeep R, Deepa M, Datta, Sudha V, Unnikrishnan R, Bhansali A, et al (2011). ICMR–INDIAB Collaborative Study Group Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study. Diabetologia 54, 3022– 3027pmid: 21959957.

[33] Zabetian A, Sanchez IM, Narayan KM, Hwang CK, Ali MK (2014). Global rural diabetes prevalence: a systematic review and meta-analysis covering 1990–2012. Diabetes Res Clin Pract 104: 206–213pmid: 24507869.

[34] José F. Cara, The Epidemic of Type 2 Diabetes Mellitus in Children, https://www.medscape.org/viewarticle/447138.

[35] Jon Baio, Lisa Wiggins, Deborah L., Christensen Matthew J Maenner, Julie Daniels, Zachary Warren, et al (2018). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014, MMVR Surveillance Summaries / April 27, 2018 / 67(6); 1–23.

[36] Meng-Chuan Lai, Michael V Lombardo, Simon Baron-Cohen (2014). Autism, Lancet, Volume 383, Issue 9920, 8–14 March 2014, Pages 896-910.

[37] Lord C, Elsabbagh M, Baird G, Veenstra-Vanderweele J

(2018). Autism spectrum disorder, Lancet. 2018 Aug 11; 392 (10146): 508-520. doi: 10.1016/S0140-6736(18)31129-2. Epub 2018 Aug 2.

[38] Bjørklund G, Skalny AV, Rahman MM, Dadar M, Yassa HA, Aaseth J, et al (2018). Toxic metal (loid)-based pollutants and their possible role in autism spectrum disorder, Environmental Research Volume 166, 234-250.

[39] Guo Z, Xie HQ, Zhang P, Luo Y, Xu T, Liu Y, et al (2018), Dioxins as potential risk factors for autism spectrum disorder, Envionment International, 121, 906-915.

[40] Pequegnat B, Sagermann M, Valliani M, Toh M, Chow H, et al. (2013). A vaccine and diagnostic target for Clostridium bolteae, an autism associated bacterium, Vaccine 31, 2787-2790.

[41] James B Adams, Leah J Johansen,, Linda D Powell, David Quig and Robert A Rubin (2011). Gastrointestinal flora and gastrointestinal status in children with autism; comparisons to typical children and correlation with autism severity, BMC Gastroenterology 11, 2. http://www.biomedcentral.com/1471-230X/11/22,

[42] Heberling CA[1], Dhurjati PS, Sasser M.. (2013). Hypothesis for a systems connectivity model of autism spectrum disorder pathogenesis: Links to gut bacteria, oxidative stress, and intestinal permeability. Medical Hypotheses 80 264-270.

[43] Amar Sarkar, Siobhán Harty, Soili M Lehto, Andrew H. Moeller, Timothy G. Dinan, Robin I. M. Dunbar, et al (2018). The Microbiome in Physiology and Cognitive Neuroscence, Trends in Cognitive Science, Vol. 22, No. 7. https://doi.org/10.1016/j.tics.2018.04.006 611 ©.

[44] https://www.alz.org/media/Documents/alzheimers-facts-and-figures-infographic.pdf.

[45] H. Niua, I. Álvarez-Álvarez F. Guillén-Grimaa, I. Aguinaga-Ontosoa (2017). Prevalencia e incidencia de la enfermedadde Alzheimer en Europa: metaanálisis. Neurología. 32, 523—532.

[46] Morley, JE., Farr, SA., Nguyen, AD. (2018): Alzheimer Disease, Clin Geriatr Med 34 591–601.

[47] Miho Uematsu, Ayako Nakamura, Momoko Ebashi, Katsuiku Hirokawa, Ryosuke Takahashi, and Toshiki Uchihara (2018) Brain stem tau pathology in Alzheimer's disease is characterized by increase of three repeat tau and independent of amyloid b. Acta Neuropathol Commun 6, 1.

[48] Hao K, Di Narzo AF, Ho L, Luo W, Li S, Chen R, et al (2015): Shared genetic etiology underlying Alzheimer's disease and type 2 diabetes, Molecular Aspects of Medicine, Volumes 43–44, 66-76.

[49] Mancuso, C., Santangelo, R. (2018). Alzheimer's disease and gut microbiota modifications: The long way between preclinical studies and clinical evidence, Pharmacological Research, Volume 129, 329-336.

[50] Ternak, G. (2005). Antibiotics may act as growth/obesity promoters in Humans as an inadvertent result of antibiotic pollution ? Medical Hypotheses, Volume 64, Issue 1, 14-16.

[51] Hani Sbahi, Jack A Di Palma (2016). Faecal microbiota transplantation: applications and limitations in treating gastrointestinal disorders, BMJ Open Gastro2016; 3:e000087. doi:10.1136/bmjgast-2016- 000087.