Antibiotics in the environment: the possible inadvertent effect on human morbidity & mortality.

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The antibiotics, after being discovered, were considered as the "magic bullets" of medicine; even feedlot animals started growing faster and bigger after mixing antibiotics to animal fodder. In the past decades evidences accumulated, that antibiotic resistance will quickly develop if we consume too much antibiotics even if it is used to fatten animals. The increased energy harvest in antibiotic fed animals was associated with the altered gut flora. In animal models and obese subjects the alteration of the gut flora was associated with elevated LPS plasma level and the production of inflammatory cytokines (e.g. TNFα), which could favor certain chronic disorders, including T2DM. As far as antibiotics can play an important role of altering human gut flora, the extensive utilization of antibiotics in humans and animals alike, which are used as growth promoting agents mixed to animal fodder, can enter the environment and food chain as pharmaceutically active compounds and might have effect on the human gut flora promoting obesity and related insulin resistance with type-2 diabetes. Accumulation of antibiotic molecules in human nutrients and water might contribute in the spread of other, yet to be explained origin of diseases, like autism.

Keywords antibiotics; antibiotic resistance; gut flora; growth promotion; environment; pollution; obesity; type-2 diabetes; autism

1. Introduction

The discovery of antibiotics in the mid-twentieth century has contributed to the increased estimated life span. With such phenomenal progress being made in combating illness, Surgeon General William H. Stewart informed the United States Congress in 1969 that it was time to ''close the book on infectious diseases'' [1]. However, when declaring such a victory, he never could have predicted the increase in emerging, re-emerging, and antibiotic-resistant infectious diseases that were witnessed in the subsequent 30 years [2].

We have only recently started to appreciate that the human body is home to far more than human cells: we harbor at least 100 trillion (10^{14}) microbial cells [3] and a quadrillion viruses in and on us [4]. Collectively, the microbial associates that reside in and on the human body constitute our microbiota, and the genes they encode is known as our microbiome. This complex community contains taxa from across the tree of life, bacteria, eukaryotes, viruses, and at least one archaeon, that interact with one another and with the host, greatly impacting human health and physiology. Only a small minority of these can be cultured, and recently, culture-independent high-throughput sequencing has greatly expanded the repertoire of known microbes both in our bodies and in the environment [5].

2. Antibiotics, gut microbiota, resistance, environmental pollution

Although the microbiota is generally stable within individuals over time, the composition can be altered due to external perturbations. One of the major factors that can perturb the composition of the microbiota is antibiotic use. Antibiotics have a profound effect on the microbiota, and their overuse is linked with an increase in antibiotic-resistant pathogens. There is now compelling evidence of major alterations of the microbiota following treatment with antibiotics [6-8].

In our modern environment, many people are not exposed to the microbiota of our evolutionary past. In the absence of appropriate microbial signals, the immune system does not develop normally [9]. Autoimmune diseases in general, and allergies in particular, have significantly increased in developed countries over the last few years, which has been attributed to a burgeoning list of potential factors [10].

Antibiotics, by changing the composition of the microbiota and altering the development of the immune system, can predispose the host to infections [11].

The use of antibiotics as additives to animal fodder was first considered after the observation, that feeding chickens on tetracyclins promoted the weight gains among the animals [12].

Antibiotics have been used to promote growth and enhance performance of livestock for almost 50 years. Concern about their impact on the development of resistance was first raised in the 1960s and led to the Swann Report [13], that recommended that only antibiotics which 'have little or no application as therapeutic agents in man or animals and will not impair the efficacy of a prescribed therapeutic drug or drugs through the development of resistant strains of organisms' should be permitted for growth promotion. Thus, chlortetracycline, oxytetracycline, penicillin, tylosin (a macrolide related to erythromycin) and the sulphonamides were rejected because their use for growth promotion was considered to pose 'certain hazards to human and animal health'. Of the drugs approved for agriculture, antibiotics are among the most widely administered for animal health and management. Unmetabolized antibiotic substances are often

passed into the aquatic environment in wastewater. Antibiotics used for veterinary purposes or as growth promoters are excreted by the animals and end up in manure. Manure is used as an agricultural fertilizer; thus, the antibiotics seep through the soil and enter ground water. Unlike other classes of drugs, antibiotics are distinctive in that their use precipitates their obsolescence by selecting for resistant microbes. This reality compounds the challenges inherent in the discovery of new antibiotic drugs, which include for example the difficulty in identifying suitable bioactive chemical matter that can traverse microbial membranes.

The term 'antibiotic' is normally reserved for a diverse range of compounds, both natural and semi-synthetic, that possess antibacterial activity. Only in the last few years has the issue of pharmaceuticals in our environment emerged as an important research topic. Most studies since the mid to late 1990s have concentrated on the occurrence and distribution of and veterinary pharmaceuticals in our environment. Because studies have shown these compounds are transported into surface water and ground water from urban and agricultural sources, researchers have begun to conduct effects based studies [14]. The spread and evolution of antibiotic resistance genes, and their acquisition by bacterial pathogens is becoming one of the most important clinical challenges [15].

Concerns over the spread of antibiotic-resistance genes to human and animal pathogens continue to drive the debate. European nations have implemented bans on the use of growth-promoting antibiotics, and the practice in the USA is under increasing regulatory and political scrutiny [16-18].

Antibiotics do more than select for resistant clones. Subpopulations of bacteria can survive lethal doses of antibiotics without becoming resistant by a transient and non-hereditary mechanism, called persistence. A recent work [19] shows that a majority of persisters to the quinolone ciprofloxacin appeared upon exposure to the antibiotic in an SOS-dependent manner. Therefore, persisters are formed by an active and inducible mechanism mediated by the SOS response, which is induced by some antibiotics. This contrasts with the previous view that persisters appear by stochastic means previously to the antimicrobial challenge. Given antibiotic might not only select for resistance to itself, but may also, by increasing the proportion of mutators, indirectly select for the increased probability of resistance to non-related antibiotics. Several antibiotics can increase the mutation rate in differ-ent ways, including oxidative damage, SOS response and general stress responses [20]. The production of reactive oxygen species (ROS) has been postulated as a common step in antibiotic mediated lethality. This common pathway to cell death is mediated by an increased respiration rate, a transient depletion of NADH and the irreversible oxidation of the iron–sulfur clusters, which lead to hydroxyl radical generation via Fenton reactions [21]. However, this mechanism seems to be not strictly necessary, since quinolones and cephalosporins are equally effective in anaerobic conditions. ROS are known to cause damage in key cellular components such as proteins, lipids and DNA. This damage can cause DNA lesions either directly or indirectly, which if not repaired, lead to the accumulation of mutations. Treatment of E. coli with some antibiotics at sublethal concentrations increases ROS levels, which correlate significantly with an increase in mutagenesis [22-23]. This activation triggers the expression of specialized (error-prone) DNA-polymerases able to bypass DNA lesions with reduced fidelity [24]. In fact, it is known that quinolones are mutagenic in bacteria. This is why subinhibitory concentrations of quinolones may increase the frequency of resistance mutations. Ciprofloxacin produces an increase of up to 5-fold in the frequency of rifampin resistant mutants in Streptococcus pneumoniae [25], and carbapenem resistant variants in P. aeruginosa [26].

Therefore, human body and environmental sites exposed to low concentrations of antimicrobials may become antibiotic-induced mutation and recombination hotspots, responsible for phenotypic variation and specifically for the emergence, maintenance and dissemination of antibiotic resistance.

It is clear that antibiotics may act as true promoters of antibiotic resistance. During the past decade, concern has grown about the adverse effects the use and disposal of pharmaceuticals might potentially have on human and ecological health. Research has shown that after passing through wastewater treatment, pharmaceuticals, amongst other compounds, are released directly into the environment [27].

Unused therapeutic drugs are sometimes disposed of into the sewage system. If the drugs are not degraded or eliminated during sewage treatment, in soil or in other environmental compartments, they will reach surface water and ground water, and, potentially, drinking water.

Antibiotics are routinely used at therapeutic levels in livestock operations to treat disease and at sub-therapeutic levels (<0.2 g kg_1) to increase feed efficiency and improve growth rate.

The amount of antibiotics produced in different countries are not exactly known, but according to the UCS (Union of Concerned Scientists), in their report Hogging estimated as 16 million kg of antimicrobial compounds used annually in the US, approximately 70% are used for non-therapeutic purposes [28]. Wise estimated, that the total antibiotic market consumption world-wide lies between 100 000 and 200 000 tons [29]

Ciprofloxacin, for example, was found in concentrations of between 0.7 and 124.5 μg/L in hospital effluent [30]. Ampicillin was found in concentrations of between 20 and 80 μg/L in the effluent of a large German hospital [31]. Antibiotic concentrations calculated and measured in hospital effluents are of the same order of magnitude as the minimum inhibitory concentrations for susceptible pathogenic bacteria. The dilution of hospital effluents by municipal sewage will lower the concentration of antibiotics only moderately, because municipal waste water also contains antibiotic substances and disinfectants from households, veterinary sources and to a minor extent from livestock. Antibiotics have been detected in the μg/L range in municipal sewage, in the effluent of sewage treatment plants

(STPs), in surface water and in ground water [32]. These included quinolones such as ciprofloxacin, sulphonamides, roxythromycin, dehydrated erythromycin and others. If antibiotics are used in animal husbandry, they pass into the soil from manure. Tetracyclines have been detected in concentrations of up to 0.2 μg per kg in soil17 whereas others have been found in the sediment under fish farms [33-34].

Many antibiotics used in the animal food-producing industry are poorly adsorbed in the gut of the animal, resulting in as much as 30–90% of the parent compound being excreted. In addition, antibiotic metabolites can also be bioactive and can be transformed back to the parent compound after excretion. Thus, a significant percentage of the administered antibiotics may be excreted into the environment in active forms. As most of the antibiotics are water-soluble, as much as 90% of one dose can be excreted in urine and up to 75% in animal feces [35].

Antibiotics may be disseminated into the environment from both human and agricultural sources, including excretion, flushing of old and out-of-date prescriptions, medical waste, discharge from wastewater treatment facilities, leakage from septic systems and agricultural waste storage structures. Other pathways for dissemination are via land application of human and agricultural waste, surface runoff and unsaturated zone transport. Once in the environment, like any other organic chemicals, their efficacy depends on their physio-chemical properties, prevailing climatic conditions, soil types and variety of other environmental factors. If antibiotics in the environment are not efficiently degraded, it is possible that these residues may assist in maintaining or developing antibiotic resistant microbial populations [36]. Thus cyclic application of manure on the same location may result in the continuous exposure of soil microbes to antibiotic residues and antibiotic resistant populations of bacteria. This can potentially have deleterious effects in the environment, especially if the residues are transported by surface runoff or leaching through soil and reach nearby rivers or lakes. While it is possible that antibiotics can find their way into the environment from a variety of sources, whether or not there are adverse effects to human, terrestrial and aquatic ecosystems is not well understood. Only in the last few years has the issue of pharmaceuticals in our environment emerged as an important research topic.

It was found that ß-lactam antibiotics, including the sub-groups of penicillins, cephalosporins and, as a marginal fraction carbapenems and others, make up the largest share of human use antibiotics in most countries. They account for approximately 50–70% of total antibiotic use. In most countries, sulphonamides, macrolides, and fluoroquinolones follow in decreasing order of use [37]. In recent years, more and more people face the security problem of tap water in the world. According to an investigation reported by American media on March 9, 2008, tap water contains antibiotics and sedatives in 24 big cities in the U.S.A. Four fluoroquinolone antibiotics (norfloxacin, ciprofloxacin, lomefloxacin, and enrofloxacin) in tap water in Guangzhou and Macao were analyzed using high performance liquid chromatography fluorescence detection. The results showed that all target antibiotics were detected in high rate both in Guangzhou (77.5%) and Macao (100%), ranging from 1.0 to 679.7ng/L (SD ≤ 37.6) in Guangzhou, and from2.0to 37.0ng/L (SD ≤ 2.5) in Macao [38].

3. Obesity, gut flora and metabolic disorders

Experimental models highlight several mechanisms connecting the gut microbiota to obesity and metabolic disorders [39]. The recognition that gut microbiota is important in the regulation of energy extraction from the diet came from the observation that germ-free mice (raised in the absence of microorganisms) were leaner than mice with a normal gut microbiota, even though mice with a normal gut microbiota were fed 30% less calories [40]. Moreover, when germ-free mice were transplanted with gut microbiota harvested from mice with normal gut microbiota they gained 60% body fat and became insulin resistant, despite lower food intake [41]. Most recently, gut microbiota has been linked to low-grade inflammation through activation of innate immunity through the LPS–Toll-like receptor 4 axis. Cani et al demonstrated that mice fed a high-fat diet for 2 to 4 weeks exhibited a significant increase in circulating LPS levels (described as ‘‘metabolic endotoxemia’’) and that these mice became obese and had obesity associated metabolic disorders. Similarly, mice infused with LPS (to reach levels observed in mice that were fed a high-fat diet) also had obesity and obesity-associated metabolic disorders [42-43]. When metabolic endotoxemia was reproduced by subcutaneous infusion of LPS, animals developed the same metabolic abnormalities induced by the high-fat diet, while LPS receptor KO (CD14KO) mice were resistant to the effects of both high-fat diet and LPS infusion. Moreover, CD14KO mice were hypersensitive to insulin even when they were fed a normal diet, suggesting that CD14 may modulate insulin sensitivity in physiological conditions [44]. Obesity has further been shown to be associated with altered gut microbial composition in human subjects and mice. The guts of obese human subjects were shown to have reduced numbers of Bacteroidetes and increased numbers of Firmicutes compared with those of their lean counterparts. Gordon has clearly demonstrated that in obese mice there is a significant reduction of bacteria belonging to the Bacteroidetes family and a proportional increase of Firmicutes and methanogenic Archaea [45]. This entails an increase in the intestinal lumen hydrolysis of non-digestible polysaccharides such as beta-fructans with increase of transport proteins, such as phosphor-transferases. In a few obese human subjects, an increased proportion of fecal Bacteroidetes was found to parallel weight loss on a hypocaloric diet during a 1-year intervention trial.

Recent work has shown that gut bacteria can initiate the inflammatory state of obesity and insulin resistance (IR) through the activity of lipopolysaccharide (LPS), a component of the gram-negative bacterial cell walls, which can trigger the inflammatory process by binding to the CD14 toll-like receptor-4 (TLR-4) complex at the surface of innate

immune cells. The relevance of the TLR-4 pathways for metabolic disease was confirmed by the finding that the deletion of TLR-4 prevented the high-fat diet–induced insulin resistance [46]. The role of LPS in triggering systemic inflammation was subsequently evaluated in healthy human subjects. Anderson et al [47] found a similar grade endotoxemia, increased adipose tumor necrosis factor (TNF) and interleukin (IL)-6 concentrations and promoted IR, and a high-fat, high-carbohydrate meal induced a significant postprandial plasma LPS elevation, accompanied by an increased mononuclear cell expression of TLR-4, nuclear factor-B (NF-B), and suppressor of cytokine signaling-3 (SOCS-3), an adipokine involved in IR. These increases were totally absent after an American Heart Association (AHA) meal rich in fiber and fruit. Taken together, these data support the concept that endotoxinemia may play a key role in the pathogenesis of obesity associated inflammatory state and that food ingestion affects plasma endotoxin levels [48]. Oral antidiabetics, like Metformin and rosiglitazon (RSG) effectively reduced the endotoxin level in patients with T2DM and in the control group also [49].

Clinical observations proved, that children who had been exposed to antibiotics before their 6 month of age were 22% more likely to be overweight, than children who had not been exposed [50].

4. Conclusions

Antibiotics are included in the group of pharmaceutically active compounds (PhACs) considered as environmental emerging contaminants [51]. Several studies have investigated the potential for a range of veterinary medicines to be taken up from soil by plants, and have assessed the potential significance of this exposure route in terms of human health. Soil analyses indicated that, for selected substances, measurable residues of these are likely to occur in soils for at least some months following the application of manure containing these compounds. Some antibiotics are taken up by vegetables such as carrot roots (tubers), lettuce leaves [52-53]. The hypothesis, that antibiotics in our environment might affect human growth/obesity was first published in 2005 [54]. Since then several experimental and clinical observation proved, that antibiotics, through the alteration of gut flora, can influence the development of obesity even in humans, not only in experimental animals. Although no observations were published, that antibiotics in our environment can directly influence human obesity, the association between the consumption of low dose antibiotics given to animal fodder and the increased energy harvest by feedlot animals, resulting in about 20% better “energy consumption”, is clearly established, and the possible mechanisms were described in the literature.

By now, sufficient data were published to conclude, that the accumulation of antibiotics in our environment might provide a permanent, low grade antibiotic pressure on human gut flora, similar to the antibiotic enriched fodder given to animals, which might result in increased body mass index in susceptible persons and can facilitate developing insulin resistance and diabetes (T2DM). The explanation of rapidly spreading epidemic of obesity and diabetes, particularly among youngsters, cannot be explained solely by changed eating habits and the lack of physical activity either.

To prove or reject the association of the massive presence of antibiotic molecules in our environment and the association of the rapidly spreading obesity and diabetes, Framingham-like studies would be necessary, where one part of the population might receive antibiotic free nutrients and water and the result will indicate, whether the number of diabetes were reduced in the antibiotic free group or not. One might speculate, if the appearance of some other unexpected disease were reduced in the antibiotic free group also, like autism, of which the real reason is still to be found, that antibiotic molecules picked up from our environment, can produce different ailments in humans. In the case of autism, the mass appearance of the disease is roughly synchronous with the spread of antibiotic pollution, obesity and T2DM. *Autistic children often suffer of GI disorders, and Kang D-W, Park JG, Illhan ZE reported (Reduced Incidence of Prevotella and other Fermenter Microflora in Autistic Children. Plos One 8(7): e68322. Doi: 10.1371/journal.pone 068322), that among those children the gut microflora is different from healthy control group. It might be due to the effect of antibiotic picked up from the environment.*

Mankind cannot bypass the fact, that accumulation of different molecules in our environment can have some serious effect, yet to be found, on our health.

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