Impact of antibiotics in early life on development of the intestinal microbiota

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Abstract

The dynamic gut microbial microbiota is susceptible to disturbances before stabilizing into a mature microbiome community after the first 3 years of life. One of the factors that is associated with marked changes in the gut microbiota composition, is the use of antibiotics. This can have immediate effects such as infections of opportunistic pathogens and adds disadvantages of the increased resistant organisms in the gut microbiota. Microbiome alterations by antibiotics can also have indirect effect on health in the long-term and has been associated with an increased risk of a wide range of diseases and syndromes, such as obesity, diabetic, inflammatory bowel disease, asthma and allergies. It has been shown that antibiotic exposure decreases the gut microbiota diversity and affects gene expression, protein activity and overall metabolisms. The mutualistic microbes in the intestinal gut tract have important functions such as nutrient absorption and modulation, and the development of the mucosal immune system. Therefore, antibiotic exposure can alter basic physiological processes. It is important to understand the impact of antibiotics on the development of the gut microbiome and for the increasing concern that antibiotics may have long-term consequences. Strategies are needed to minimize the negative consequences of antibiotics. Probiotics, prebiotics and fecal microbiota transplantations are aimed to restore the imbalanced gut microbiota and seem promising strategies.

1. Introduction

The gut microbiome is formed into a mature microbial community in the first 3 years of life (Yatsunenko et al., 2012). The surface of the gastrointestinal tract gets coated with microbes, which consists of bacteria, archaea, viruses and fungi. The gut microbiota of the newborn has relatively few species and lineages, but will increase in diversity rapidly during the first years of life (Palmer et al., 2007). Several important colonizers determine the communities on the surface of the infants' gut hours after birth: microorganisms from the maternal vaginal, fecal, skin microbiome and the environment (Penders et al., 2006). The contributions of those factors are different when the baby is born by caesarian section. The delivery mode determines the gut microbiota in the first few hours of life, whereby the gut microbiome of babies born vaginally have resemblance with the maternal vagina microbiome. Babies that are born by caesarian section get their initial microbes from the skin of people who touch them after birth (Dominguez-Bello et al., 2010; Koenig et al., 2011). It has been shown that babies born by caesarian section have an increased risk for certain immune related disorders, such as asthma and allergies (Bager et al., 2008; Negele et al., 2004). Indicating that this could be due diminished exposure to maternal vaginal microbes during birth. Breast-feeding and introduction of solid foods influence establishment of the infant's gut microbiota (Azad et al., 2013; Penders et al., 2013; Yatsunenko et al., 2012). It has been shown that lack of breastfeeding predispose to asthma (Azad and Kozyrskyj, 2011). Before stabilizing into a mature bacterial community after the first years of life, the dynamic gut microbial community is susceptible to disturbances such as illness, antibiotic treatment and dietary changes due the low diversity and high instability (Koenig et al., 2011; Bokulich et al., 2016). The still vulnerable infant gut mi-

crobiota could be disrupted and this can affect health later in life. Analysis of the gut microbiota of a single infant during the first two and a half years of his life revealed discrete steps of bacterial succession associated with life events. The study showed therefore that the microbial community succession was nonrandom and indicates that the composition of colonization in early-life influences the communities found later in life (Koenig et al., 2011).

The gastrointestinal tract has important functions such as nutrient absorption and modulation, and the development of the mucosal immune system (Sudo et al., 1997; Falk et al., 1998; Hooper and Gordon, 2001; Kelly et al., 2007; Ishikawa et al., 2008; Martino et al., 2008). The state of the microbial communities is therefore linked with the host growth and immune development during early life (Renz et al., 2012). The stable adult-like gut microbial community is also important as barrier against pathogenic microorganisms or overgrowth of opportunistic microorganisms (Bernet et al., 1994; Lievin et al., 2000; Taguchi et al., 2002). The factors and life events that influence the colonization of the gut in early-life have effect on the development of the microbiome and host. However antibiotics generally have a low-risk profile, these medications can especially disrupt conserved functions of the microbiota during critical developmental times. Based on these important functions it is necessary to understand the development of the gut microbiome and the effect that certain factors have on the community composition. This is needed for the increasing concern that antibiotics may have long-term consequences (Blaser and Falkow, 2009).

One of the factors that is associated with marked changes in the gut microbiota composition, is the use of antibiotics in childhood (Korpela et al., 2016). Broad-spectrum antibiotics can cause rapid drops in taxonomic richness, diversity and evenness (Dethlefsen et al., 2008; Dethlefsen and Relman, 2011). Furthermore, studies have marked the critical role of commensal bacteria in human health. Researchers have given more attention to external factors such as antibiotic exposure. Those studies were focused on antibiotic exposure in adults and revealed decreased microbial diversity (Jakobsson et al., 2010; Dethlefsen et al., 2008; Dethlefsen and Relman, 2011). The same results have been shown in mice (Nobel et al., 2015).

In fact, antibiotics are the most common prescription drugs given to the pediatric populations in western countries Sturkenboom et al. (2008). Children in the U.S. receive about three antibiotic courses in the first two years of life and 10 courses by the age of 10 (Hicks et al., 2013). The gut microbiome has a certain degree of resilience after ending of the antibiotic treatment, however the original state is often not totally recovered. The antibiotic-induced changes in the gut microbiome can remain only for the short-term but also for years Dethlefsen et al. (2008); Dethlefsen and Relman (2011). Since antibiotics can have an effect on the gut microbiome, this gives concern in the high use of antibiotics in humans and especially in the first years of life.

Here I will answer the following question: to what extent does the early antibiotic exposure affect the gut microbiota development? Here, the relationships between antibiotic exposures in early-life and the development of human intestinal microbiome are reviewed, addressing (1) the effect of antibiotics on the composition and function of the gut microbiota (2) and the impact of antibiotic-induced microbiota changes on health, immunity and metabolism. In addition, solutions to restore the altered gut microbiota will be discussed.

2. Gut bacteria related to healthy gut microbiome

A healthy microbiome can be described as a perturbation that departs from ecologic stability and has the ability to resist structure change under stress or can rapidly return to the 'healthy' state following a stress-related change (Bäckhed et al., 2012). Finding properties that distinguish healthy from unhealthy microbiomes could support the diagnosis of microbiome-related diseases. Moreover, this

could provide a target for sustaining and improving health of individuals with disrupted gut microbiota.

The concept that humans have a core microbiome, suggests that every individual shares some of the same microbes (Consortium et al., 2012). The intestinal microbiota of newborns is characterized by a relative dominance of the phyla *Proteobacteria* and *Actinobacteria*. After stabilizing into a mature gut microbiota state, the gut is consistently dominated by *Bacteroidetes* and *Firmicutes*, whereby the *Proteobacteria* and *Actinobacteria* stay present in the human gut (Qin et al., 2010; Bäckhed, 2011; Eckburg et al., 2005). Commensal indigenous microbiota could also be beneficial for the health, such as some predominance bacteria in the colon: *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, and *Clostridium* (Zhang et al., 2015). Antibiotics can target and inhibit microorganisms in a variety of ways, which could damage commensal gut bacteria while controlling pathogenic bacteria. Colonization of commensal bacteria with a health-promoting role can be reduced, such as the *Bifidobacteria* and *Lactobacilli*. (Blaser, 2011).

3. Effect of antibiotics on the gut microbiome

The effects of antibiotics in adults on the gut microbiome dysbiosis have been well characterized (Bokulich et al., 2016). However, antibiotic use in children is prevalent in most parts of the world and is increasing, little is known about the impact of antibiotics on development of the gut microbiome in early life and the effects on long-term health in general (Fouhy et al., 2012). The consequences of the disturbances in the gut microbiome on host physiology are not well understood. To what extent the early antibiotic exposure impacts gut microbiota development requires more studying. Among the studies who did examine this, are Ishikawa et al. (2008) and Fouhy et al. (2012). They showed effects in the infants' microbiota already within one week and within two months after birth. The diversity was reduced and composition of the microbiome was changed in those infants. They also showed a reduced colonization of *Bifidobacterium* and increases of pro-inflammatory *Proteobacteria*. Infants that were not treated with antibiotics, but whose mothers received antibiotics during the delivery showed the same results (Ishikawa et al., 2008). Recently, Yassour et al. (2016) confirmed the reduced gut microbiota diversity in antibiotic-treated children. They followed the development of the gut microbiome in infants that received multiple courses of antibiotics during the first 3 years of life. Less diversity in bacterial species and strains was found, with some species often dominated by single strains. Indicating that antibiotic exposures in early-life lead to prolonged effects on host metabolic characteristics. Another recent study in this issue showed decreased stability in the gut of antibiotic-treated children during the first 2 years of life (Bokulich et al., 2016).

Not only changes in the composition of taxa in the gut microbiome has been revealed, but also effect in the gene expression, protein activity and overall metabolisms due antibiotic exposure. Those effects on the gut microbiota have been investigated by multi'omic data types (reviewed in Franzosa et al. (2015)). These changes in gene expression, protein activity and overall metabolisms due to antibiotics can occur in a much faster pace than replacement of taxa (Pérez-Cobas et al., 2013). In addition, studies showed that alterations in the gut microbiota due to antibiotic exposures can drive the functionality of the microbiota towards disease conditions and change the physiological state and activity (Hernández et al., 2013; Maurice et al., 2013). Indicating that the effect of antibiotic treatment on the functioning of gut microbiome can have impact on the physiological processes and can therefore give complications in the long-run.

4. Alterations on the immune and metabolic health due to an antibiotic-treated gut microbiota

The antibiotic exposure in early-life can have potential immediate effects on health, such

as opportunistic pathogens that cause acute diseases due the increased susceptibility to infections. Antibiotic exposure in children can also have indirect effect on health in the long-term and has been associated with an increased risk of a wide range of diseases and syndromes. Infants and children that receive antibiotic treatment have a higher risk of getting obesity, diabetes, inflammatory bowel disease, asthma and allergies (Azad et al., 2014; Kilkkinen et al., 2006; Arrieta et al., 2015; Metsälä et al., 2013). Those studies revealed that during the first years of life, development of a stable and healthy gut microbiome is important and will influence the health in the long-term.

Although, it is known that antibiotic treatment can have impact on the human gut microbiota, it is difficult to determine the extent of impact because the response of each individual is unique (Goodrich et al., 2014). The community succession varies among individuals and for humans does also apply that the genetic differences affect the composition of the microbiota (Dethlefsen et al., 2008; Dethlefsen and Relman, 2011). Several studies showed alterations in the composition in gut microbiota, but it is not possible to reveal the consequences for an individual with respect to the loss of bacteria (Schulfer and Blaser, 2015). Moreover, the functional redundancy in the human gut microbiota indicates that antibiotic exposure often does not result in gastrointestinal symptoms (Dethlefsen and Relman, 2011). However, the gut microbiome in early life is susceptible to disturbances due the low diversity and high instability, making it more likely that antibiotic treatment will influence the composition of gut microbial communities later in life and therefore the health of an individual. Moreover, the immune system remains developing after birth, influenced by the gut microbiota (Zeissig and Blumberg, 2014). The relationship of the host with the symbiotic bacteria is therefore especially important during the early years of life. Mouse models have provided evidence that early-life administration of antibiotics is related to immune-related and metabolic diseases, possibly due changes in the gut microbial composition (Cho et al., 2012; Russell et al., 2012; Cox et al., 2014). Differences between the early development of gut microbiota of antibiotic-treated and non-treated infants have been shown (Ishikawa et al., 2008; Fouhy et al., 2012).

4.1. *Increased susceptibility to infections*

The increased susceptibility to immediate intestinal infections can be a consequence of the changes in the community composition due antibiotics. Importantly, dysbiosis of the gut adds disadvantages of the increased resistant organisms in the microbiota, which could result in infection. Infections are caused by new acquired pathogens or from overgrowth of opportunistic organisms that were already present in the intestinal microbiota. Antibiotic-associated diarrhea (AAD) is associated with the administration of antibiotics. AAD due to nosocomial pathogens is frequent and can cause life-threatening infections. The development of AAD is associated with organisms such as *Klebsiella pneumoniae*, *Staphylococcus aureus* and most of all, *Clostridium difficile* (Wilcox, 2003; Young and Schmidt, 2004; Song et al., 2008; Rupnik et al., 2009; Sekirov et al., 2010; Chen et al., 2013). For example, a mouse model revealed that substantial losses of microbial diversity due to antibiotics could results in an increased risk for a chronic infection with *C. difficile* (Lawley et al., 2009).

The increased risk of infections due antibiotic altered gut microbiota in infants has been studied. Madan et al. (2012) and Mai et al. (2013) showed that high antibiotic exposure with broad-spectrum antibiotics in premature infants changes the gut microbiota composition and is associated with the risk of sepsis. Prolonged use of antibiotics in premature infants decreased the gut microbial diversity and overall acquired a predominance of *Staphylococcus*, an opportunistic pathogen (Madan et al., 2012).

4.2. *Compromised immune homeostasis and tolerance*

Increased risk for immunological diseases is associated with early-life antibiotic use. Atopic, inflammatory and autoimmune diseases have been associated with gut microbiota dysbiosis. Studies also showed that antibiotic-induced dysbiosis is linked with the risk for those diseases (Francino, 2014). Review studies have focused on the association between the microbiota and host immunity. Certain species of the gut microbiota have been shown to regulate the immune function, whereby one important aspect is that the T cell population in the gut can be influenced by the metabolites of the microbiota and the composition of the microbiota itself. One specific and frequently studied product of bacteria are the short-chain fatty acids. These bacterial metabolites have been shown to influence regulatory T cells in the gut (Kamada and Núñez, 2014). Moreover, the importance of the presence of gut microbiota in early life in relationship with immunity has been shown. Germ-free mice that have never been exposed to microbes, showed impaired immune function and increased immunoglubin E (IgE) levels (Cahenzli et al., 2013). The IgE concentration is a hallmark of autoimmune disorders. This study showed that the IgE levels can be restored with colonization of the gut with a healthy mouse's gut microbiota. However, restoration could only take place when microbes were given in early life.

Several studies have shown links between to the community composition in the gut and atopic diseases during infancy and early childhood (Kuvaeva et al., 1984; Wold, 1998; Penders et al., 2006; Wang et al., 2008; Bisgaard et al., 2011; Abrahamsson et al., 2012). In addition, it has been shown that maternal intake of antibiotics during pregnancy increased the risk for developing several atopic diseases in early infancy (Jędrychowski et al., 2006). The use of broad-spectrum antibiotics in early-life revealed a stronger association with asthma. Indicating that decrease in bacterial diversity in the gut microbiota due antibiotics can contribute to the development of this disease (McKeever et al., 2002). Epidemiological studies showed differences of the gut microbiome between asthmatic and non-asthmatic infants (Penders et al., 2007). Besides the early life antibiotic exposure, epidemical data showed that treatment during pregnancy is associated with an increased risk of asthma (Marra et al., 2009; Martel et al., 2009; Murk et al., 2011). The risk of asthma increased with the number of courses of antibiotics prescribed during the first year of life (Marra et al., 2009). A more recent study showed that macrolide use has effects on the developing microbiota of children and is associated with long-term distortions in composition and function of the gut microbiota. They found that macrolides use in early life is linked with an increased risk of asthma (Korpela et al., 2016). Moreover, other allergic outcomes have been associated with early intake of antibiotics (Risnes et al., 2011). However, it is not known whether microbial variation is the cause or effect of these diseases. Recently, increased interest emerged for the role of the gut microbiota in the development of immune tolerance to food. Murine models provided a substantial evidence that gut microbiome is associated with a major role in food allergy and tolerance, but human studies have shown contradictory findings (Rachid and Chatila, 2016).

Studies showed links of the gut microbiota composition with inflammation and autoimmunity. For necrotizing enterocolitis (NEC), a devastating inflammatory disease primarily seen in premature infants, a different gut microbiota composition before onset of this disease has been revealed. There was a low abundance noninflammatory *Bifidobacterium* and a low bacteria diversity (Mai et al., 2013). These findings suggest that there is an association between the pattern of gut microbial species and NEC. The disruption of intestinal colonization due antibiotic use in early life has also been linked with an increasing risk of Crohn's disease (Hildebrand et al., 2008). In this study, the antibiotic therapy between birth and the age of 5 indicated that antibiotics has influence on

the development of immunological tolerance. Antibiotic exposure in children with new-onset Crohn's disease revealed changes in the microbiota and triggered a major reduction in certain bacteria species. Importantly, there was a reduction of *Bacteroidales* and *Erysipelotrichaceae*, which are associated with noninflammatory conditions (Gevers et al., 2014). Indicating that antibiotics amplify the microbial dysbiosis that is associated with Crohn's disease. In the case of Irritable Bowel Syndrome (IBS), alterations and a reduced diversity in the gut microbiota have been detected (Vanner, 2008; Yamini and Pimentel, 2010; Durbán et al., 2012). Observational studies showed an association between antibiotic exposure during infancy or childhood and a subsequent diagnosis of IBD (Ng et al., 2013). There is limited evidence that adults have an increased risk for IBD due to antibiotic exposure.

4.3. *Deregulated metabolism*

The gut microbiota is important in the regulation of host metabolism, especially the energy homeostasis and adiposity. The gut microbiota dysbiosis has been associated with several metabolic disorders. Antibiotic exposure in early life showed changed composition of the gut microbiota in mice, which resulted in increased weight in mice and also humans (Cox et al., 2014; Trasande et al., 2013). The study by Trasande et al. (2013)) is an example that helps to determine whether early variations of the gut microbiota due antibiotic in infants can be associated with metabolic or systemic conditions later in life. This retrospective study showed that exposure to antibiotics during the first 6 months of life is associated with consistent increased body mass. The associated was not consistent when antibiotic exposure was later in infancy, indicating that antibiotics have the highest consequences in the first months of life. Experimental work in mice showed that early-life antibiotic exposure can result in obesity, whereby the mice had normal dietary intake (Cho et al., 2012). This study showed that after the antibiotic exposure had stopped and microbiota recovered, the mice had an increased fat, lean and total mass even after 26 weeks. Although mice models studies do not represents the human metabolism, the observations are consistent with the role of gut microbiota in the host metabolism during development in early-life. An experiment using germ-free mice revealed the increased weight gain in mice due to altered microbiota and not due antibiotics per se (Cox et al., 2014). The study of Nobel et al. (2015) showed that early-life therapeutic-dose pulsed antibiotic treatment (PAT) of commonly prescribed classes leads to short-term increased weight and bone growth in mice and longer-term alterations in gut microbiome diversity, composition and metagenomics content. All above named studies suggest that early-life antibiotic exposures could have long-term developmental metabolic effects, supported by human epidemiological studies (Cox et al., 2014; Bailey et al., 2014; Ajslev et al., 2011; Azad et al., 2014; Trasande et al., 2013).

Antibiotics have recently been associated with the risk for type 1 diabetes. In addition, there has been a growing recognition of the role of the gut microbiome in type 1 diabetes (Knip and Siljander, 2016). An epidemiological study from a large UK population, revealed that exposure to certain antibiotic groups increases the risk for diabetes in adults, especially after multiple courses or sustained exposure. The participants used repeatedly penicillin, cephalosporins, macrolides, or quinolones (Boursi et al., 2015). A case-control study in children showed that diabetes 1 is associated with compositional changes in gut microbiota (Murri et al., 2013). Moreover, a decreased intestinal alpha-diversity in infants has been shown to precede type 1 diabetes onset (Kostic et al., 2015). Importantly, recent studies have been shown that the gut microbiota influences stem cells and brain function. Therefore, the altered gut microbiota could have a relation with more disorders (Foster and Neufeld, 2013; Serino et al., 2014)

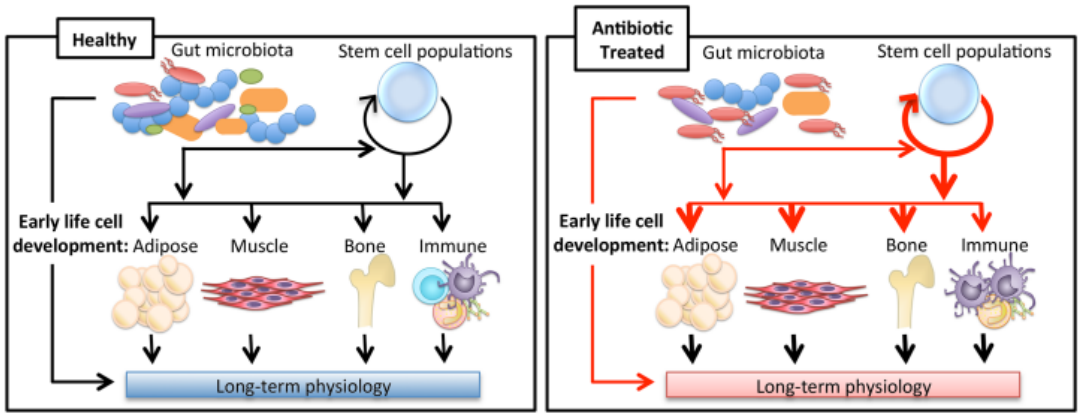

Figure 1: *Impact of antibiotic-altered gut microbiota on long-term physiology. The gut microbiota influences the development of the host's immune system and is also implicated in adipose, muscle and bone tissue growth. Recently, evidence showed that the stem cell population is influenced by the gut microbiota. Indicating that the altered gut microbiota may change the course of these developmental pathways, which has consequences for the long-term physiology. (Blaser, 2015)*

5. Strategies to restore the altered gut microbiota

Although, the intestinal microbiota is less stable and more variable in microbial communities during infancy than older children, the disruption of the gut microbiota in infants and children is both common. The process of the dynamic microbiome recovery in early-life is still uncharacterized (Nobel et al., 2015). To minimize the negative consequences of gut microbial dysbiosis, strategies that will diminished the imbalance are needed. Therapeutic strategies such as probiotics, prebiotics and fecal microbiota transplantations have gained popularity and will further be explained (Nguyen et al., 2015). Those strategies are aimed to reestablish the gut microbiota or restore the dysbiosis. Since the gut microbiota is highly variable between individuals and in time, it is difficult to understand which microbes and metabolic pathways are essential. Besides, reduction of the antibiotic exposure is necessary, however these medications will continue to be essential and especially in early-life.

5.1. *Probiotics*

Probiotics is the most widely used approach, which are live microorganisms that confer a health benefit to the host. They can affect the composition or function of the commensal microbiota and result in altering host epithelial and induce immunological responses, but how probiotics exactly do this is not understood. More research is needed to understand the mechanisms underlying the beneficial effects of probiotics.

Clinical trials indicate that some intestinal diseases with intestinal dysbiosis have resulted in clinical benefits with probiotic interventions, such as antibiotic-associated diarrhea, NEC, pouchitis, ulcerative colitis and IBS (Ringel et al., 2012). There is insufficient evidence to support the use of probiotics in Crohn's disease (Lichtenstein et al., 2016). Moreover, some probiotics have been shown to have beneficial metabolic effects in experimental models and human studies (Wang et al., 2014; Chorell et al., 2013; Kadooka et al., 2010; Kumar et al., 2012; Luoto et al., 2010). Probiotics have not established to prevent any noncommunicable diseases (West, 2014).

It has been shown that the incidence of allergy at the age of 5 was reduced in children

born by cesarean section by using of Lactobacillus probiotics from birth until age of 6 months. However, these results were not shown in vaginally delivered children (Kuitunen et al., 2009). The probiotic approach has been shown efficiency in preventing severe necrotizing enterocolitis in preterm infants (AlFaleh and Anabrees, 2014). Probiotics containing *Lactobacillus*, or in combination with *Bifidobacterium*, were effective in severe NEC cases and could therefore be very potential in CS-delivered infants and needs further exploring . Most studies have been used single or several strains of *Lactobacilli* and *Bifidobacteria* for the treatment and prevention of allergic diseases. Some probiotics had immunomodulatory effects, which have been mostly shown in experimental models but also in human studies (West, 2014; Fiocchi et al., 2012).

Importantly, the timing of probiotic treatment for promoting immune tolerance seems to be critical (West, 2014). Prevention against allergy has been shown to be effective when a combination of prenatal and postnatal probiotic treatment is given (West, 2014; Fiocchi et al., 2012). Prenatal microbial exposure increases the prevention of allergies by starting treatment in the second trimester of pregnancy. This might also have effects on asthma development. So far, asthma has not been prevented by probiotic interventions (West, 2014).

5.2. *Prebiotics*

Prebiotics are nondigestible food components and are also used to restore the dysbiosis in gut microbiota. They enter the colon where they provide for nutrients for specific bacteria, mainly *Bifidobacteria* and *Lactobacilli* (Distrutti et al., 2016). Short-chain carbohydrates are a common used prebiotic. Experimental data and human studies have shown that prebiotics have a beneficial effect in different diseases, including infections, allergies, pregnancy-related disorders, metabolic disorders, hepatic and gastrointestinal diseases, IBD and chronic constipation. There is insufficient evidence to support the use of prebiotics in IBS (Distrutti et al., 2016). It is not clear, whether prebiotics promote colonial stability or induces population shifts that are beneficial.

5.3. *Fecal microbiota transplantation*

There has been an increasing interest for fecal microbiota transplantation (FMT), whereby the fecal material from a healthy person is transplanted to the patient with an altered gut microbiota. This is a promising strategy as a treatment for a large spectrum of diseases, especially diseases associated with microbiota dysbiosis (Konturek et al., 2015). FMT gained attention when it was first used in the treatment for *C. difficule*-induced diarrhea and was also confirmed by other studies to be effective for recurrent *Clostridium Difficile* infection (CDI) (Kassam et al., 2013; Mattila et al., 2012). The study of Fischer et al. (2015) showed that FMT treatment for severe and complicated *C. difficile* infected patients, with or without selected use of vancomycin, leads to effective outcome. Indicating this is due to increased gut microbiota diversity, i.e. an increase in anti-inflammatory *Firmicutes* and a decrease in pro-inflammatory *Proteobacteria*. Besides, FMT is an interesting strategy for: chronic constipation, IBD, recurrent metabolic syndrome, multiple sclerosis, autism and chronic fatigue syndrome (Konturek et al., 2015).

6. Discussion

Research has shown that the use of antibiotics in early life could cause gut microbial dysbiosis. Given that the gastrointestinal tract has important function such as nutrient adsorption and modulation, and the development of the mucosal immune system, it follows that antibiotic exposures can have impact on those functions by affecting the microbiota composition. It has been shown that antibiotic exposure also decreases the gut microbiota diversity. Moreover, antibiotic exposures have shown to effect the gene expression, protein activity and overall metabolisms. Indicating that antibiotic treatment has effect on the phys-

iological processes. Altered gut microbiota can have potential immediate effects on health, such as the increases susceptibility to opportunistic pathogens. Moreover, dysbiosis of the gut microbiome adds disadvantages of the increased resistant organisms in the gut microbiota, which gives challenge for control when infections occur. Antibiotic exposure in early life has also been shown to have an indirect and negative impact on health in the long-term and has been associated with a wide range of diseases and syndromes. Increased risk for immunological diseases is linked with early-life antibiotic use. Atopic, inflammatory and autoimmune diseases have been associated with gut microbiota dysbiosis during infancy and early childhood. The gut microbiota dysbiosis has also been associated with several metabolic disorders, whereby research has shown increased risk of obesity and diabetes due to an antibiotic altered gut microbiome. According to these findings, strategies are needed to minimize the negative consequences of antibiotics. Already for some time, therapeutic strategies such as probiotics, prebiotics and fecal microbiota transplantations are aimed to restore the imbalanced gut microbiota. Some commensal bacteria in the gut have shown to have a health-promoting role, such as the *Bifidobacteria* and *Lactobacilli*. Health-promoting bacteria (probiotics) are used to affect the composition of the gut microbiota and this seems to be a promising strategy. It has been shown that prebiotics have a beneficial effect in different diseases. There has been an increasing interest for FMT and seems a promising strategy for diseases associated with microbiota dysbiosis.

The high use of antibiotics to pediatric populations gives concern, since antibiotics can affect the gut microbiome in the first years of life. High use of antibiotics is partly based on the low-risk profile, but could affect health in the long-term. Research has shown that an altered gut microbiota in early life is associated with an increased risk for a variety of diseases (Azad et al., 2014; Kilkkinen et al., 2006; Hviid et al., 2010; Arrieta et al., 2015; Metsälä et al., 2013). Experimental models in early life have provided evidence that these associations are causal, as shown by Cho et al. (2012), Cox et al. (2014) and Nobel et al. (2015). The higher effects of antibiotics in early life appear to be confirmed. For infants this is not remarkable, since the infant's gut has a low diversity and high instability and therefore susceptible to disturbances (Koenig et al., 2011; Bokulich et al., 2016). Indicating that infants and children have higher risks for developing metabolic disorders due to antibiotic exposure, which is supported by human epidemiological studies.

Longer treatments of antibiotics during infancy seem to have higher consequences for health. For example, the risk of asthma increases with the number of courses of antibiotic prescribed during the first year of life (Marra et al., 2009). When longer antibiotic courses are given it takes longer for the gut microbiome to return to ecologic stability. Therefore, long treatment courses should be prevented to minimize microbial dysbiosis. The impact of early-life antibiotic exposures can be mitigated with strategies to restore the microbial dysbiosis. The chance of an altered gut microbiota in early-life is higher and therefore those strategies should be applied as soon as possible. Even if the gut microbial dysbiosis would not be the (main) cause of the before mentioned diseases, it would be sensible to try to restore the microbiome dysbiosis of children before occurrence of diseases.

To what extent the early antibiotic exposure can effect gut microbiota is shown in several studies. Studies showed that the diversity was reduced and composition of the microbiome was changed infants and children (Yassour et al., 2016; Bokulich et al., 2016). Often, studies revealed a reduced colonization health-promoting-bacteria (Ishikawa et al., 2008). Indicating that specific strains have unique functions in the developing intestine. Displacement could lead to prolonged effects on host-microbial interactions.

Antibiotic exposures can have impact on the gut microbiome and health in the long-term, however factors such as mode of birth and infant nutrition have been shown to influ-

ence the development of the gut microbiome during early-life. For example, babies born by caesarian section have increased risk for certain diseases (Negele et al., 2004; Bager et al., 2008). Other important factors could have more effect on the composition of the gut microbiome, as data of study of Bokulich et al. (2016) showed that the mode of delivery had a stronger effect than repeated antibiotic treatments and persisted throughout the first year of life. Some studies showed a persistent decrease in *Bacteroides* populations (Bokulich et al., 2016). Therefore, probiotics should be used for babies that are born by caesarian section and more importantly, in combination with antibiotic exposures.

The focus should be on limiting unnecessary antibiotic exposure, but in certain cases the use of antibiotics is unavoidable. Antibiotics can target and inhibit microorganisms in a variety of ways, which could damage commensal gut bacteria. Differences between particular antibiotics in their effects on the microbiome needs to be understood. The growing knowledge of bacterial genes and genomes should aim on developing narrow-spectrum agents, which would have a decreased effect commensal gut bacteria and therefore on health.

It is important to improve the recognition of dysbiosis microbiota for a better understanding of how antibiotic treatment effects the gut microbiota in children. Beneficial taxa such as *Bifidobacterium* and *Lactobacillus* are known, but the definition of a healthy microbiota is incomplete (Zhang et al., 2015). Other taxa could be beneficial for the developing of the gut microbiome and health in general. Therefore, research to determine those taxa could be done.

Further research is needed to understand how to restore a gut microbiome dysbiosis at the right time and importantly, with the right species. It is not yet clear what strategy is optimal in which bacteria and bacterial products can minimize the deleterious effects of antibiotics on the gut microbiota (West, 2014). The multiple interactions of bacteria with immunity and metabolism have to be characterized to find new targets. If important metabolic pathways for a healthy microbiome are characterized, effective prebiotics and probiotics can be applied. Nevertheless, the differences between the individual gut microbiome and host genetic differences make problematic to optimize therapeutic strategies.

Most of the studies that attended to characterize the gut microbiome in children, were with cross-sectional studies, for example the birth mode (Penders et al., 2006; Dominguez-Bello et al., 2010). It is clear, that more longitudinal sampling studies should be done for strain profiling for studying the establishment and response to antibiotic exposures during infancy. The studies of Yassour et al. (2016) and Bokulich et al. (2016) are examples of longitudinal studies that analyzed the developing gut microbiome with antibiotic exposures and gave an analysis at the level of strains and species. The altered gut microbiota were not linked to health-outcomes in these studies. There has been shown that antibiotic treatment corresponded with short-term gut microbial dysbiosis, but more research is needed to give detailed analysis of the gut microbiome and host to understand long-term effects of antibiotic exposure in early-life.

Thus, antibiotic exposure during early-life does have effect on the developing gut microbiome by changes the composition of taxa or reducing the microbiota diversity. The microbiota imbalances caused by antibiotics during early life can negatively affect health in a variety of ways and also for the long-term. When administration is required, strategies are needed to minimize the negative consequences of antibiotic-altered gut microbiota. It is important to focus further research on providing optimal strategies in which bacteria and bacterial products can minimize the deleterious effects of antibiotics. Moreover, longitudinal studies are needed to determine causal roles in diseases associated with an altered early-life gut microbiome.

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