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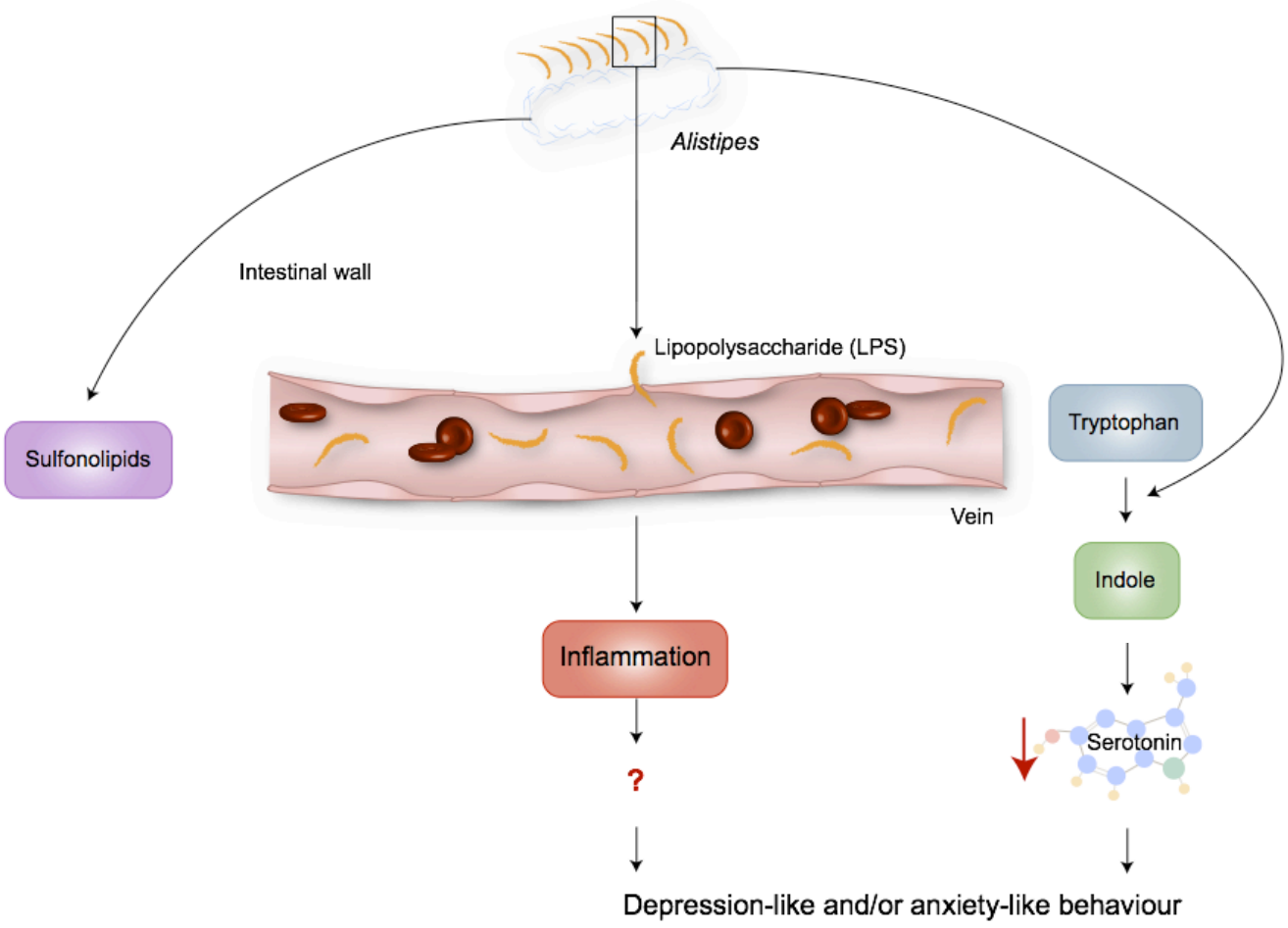

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Alistipes: The influence of a commensal on anxiety and depression

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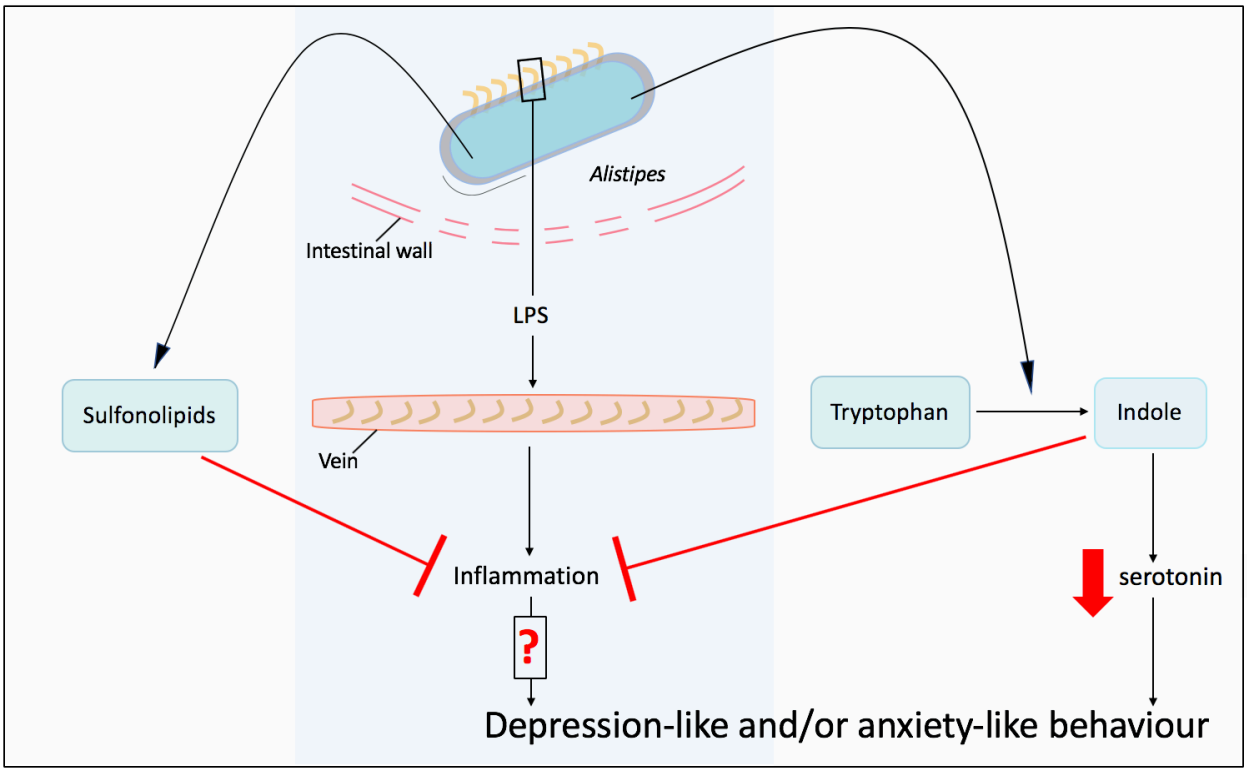

Abstract

The interaction between the gut microbiome and the brain is increasingly recognized as a potential cause for pathophysiology. With a variety of mechanisms for altering host central nervous system (CNS) function, including tryptophan metabolism and releasing modulatory metabolites, the human microbiome is emerging as a target for the development of therapies against disorders such as anxiety and depression. In this review, the gut microbiota and the microbiome-gut-brain axis will be discussed. Then, the mechanisms by which gut microbiota interacts with the CNS with a focus on anxiety and depression will be outlined. Following this, potential mechanisms whereby *Alistipes* may modulate behaviour including the inflammatory, serotonin and secondary metabolites hypotheses are highlighted. Throughout the review conflicting studies involving these pathways are mentioned. Elucidating a mechanism for a clear link between *Alistipes* and anxiety/depression may lead to novel approaches to treat these disorders.

Introduction

The human microbiome constitutes the microbial and gene content of the microorganisms inhabiting our bodies[1]. Although humans only have around 20000 protein coding genes, our microbiota outnumber this by over 100-fold[1,2]. Moreover, most of these microbes live in our gut[3]. Specifically, the gastrointestinal (GI) tract has 10^{13}-10^{14} microorganisms, and over 90% of these microbes belong to the *Bacteroidetes* and *Firmicutes* phyla[1,4]. *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* phyla are present at a lower abundance[4]. These gut microbes are mutualistic and have many important functions such as harvesting nutrients from the diet, synthesizing vitamins, drug metabolism and altering behaviour[1].

Each individual's microbiome is distinct, however there is evidence for a core microbiome shared among individuals for specific body areas[2,3]. Microbiota begins developing shortly after birth and it is dependent on the mode of delivery. For

example, infants delivered vaginally display microbiota similar to their mother's vaginal microbiome and infants born via Caesarean section display microbiota similar to their mother's skin[5]. By approximately 1 year of age, a child's microbiota is comparable to that of an adult and remains stable overtime[2,4]. However, this isn't to say the composition of the microbiome doesn't change at all. Factors such as diet, intake of antibiotics and lifestyle influence the composition of the microbiome[6].

It is also known that changes in the microbiome and its interaction with the body, including the immune and nervous systems, are correlated with disorders such as major depressive disorder (MDD) and generalized anxiety disorder (GAD)[7,8]. For example, *Alistipes* - which is a genus of bacteria under *Bacteroidetes* - is elevated in patients with anxiety and depression[7–9]. This genera of anaerobic, Gram-negative and rod-shaped bacteria are present in a high abundance in the human intestinal tract[9]. Importantly, modifying diet is a low risk option to altering the microbiome that has the potential to function as a personalized therapy for psychiatric disorders[6].

Microbiota-gut-brain axis: how the microbiome interacts with the CNS in anxiety and depression

The bacteria in our GI tract communicate with the central nervous system (CNS) in a complex bidirectional pathway known as the microbiota-gut-brain axis[3,10]. This axis consists of neural, hormonal and immunological branches[3,10]. There are many proposed mechanisms whereby microbiota affect CNS function, and here 7 are discussed in detail.

Mechanism 1: Altering microbial composition

Altered microbial composition can have a wide range of effects on gut-brain signalling. For instance, a recent cross-sectional study comparing the gut microbiota of healthy patients to patients with GAD showed that individuals with GAD have decreased species richness, reduced short-chain fatty acid producing bacteria and an overgrowth of specific bacterial phyla (*Fusobacteria* and *Bacteroidetes*)[8]. Moreover, significant differences in the composition of gut microbes in MDD patients when compared to healthy controls has also been observed[7]. Interestingly, however, Chen *et al.* recently found decreased levels of *Bacteroidetes* in patients with MDD, which opposed the increased abundance of *Bacteroidetes* found in previous studies[7]. This suggests that the relationship between microbiota and disorders is complex. In fact, the relationship may be an interaction of multiple variables such lifestyle and diet - all which should be considered interdependently in future studies. There are many ways to alter microbial composition, some of which can have a beneficial impact on the host such as probiotic and antibiotic administration. Probiotics are organisms that have a beneficial effect on the host and they can alter gut microbial composition through their metabolic by-products, production of toxins and by preventing the colonization of harmful pathogens by competing for dietary substrates and space[4]. For instance, in a study conducted by Bercik *et al.*, it was observed that brain-derived neurotrophic factor (BDNF) levels were reduced in mice infected with *Trichuris muris*, resulting in increased anxiety-like behaviours[11]. However, upon administration of probiotics, BDNF levels were normalized resulting in an effect similar to antidepressant medication on behaviour[11]. Moreover, antibiotics have a drastic effect on microbial composition. Specifically, they alter the richness and diversity of microbiota and it can take up to 4 years post-treatment for the microbial species to return to normal levels[2].

Mechanism 2: Immune activation

Similar to the gut, the immune system also has bidirectional communication with the CNS which allows it to communicate bacterial effects to the nervous system[4]. For example, it is known that treatment with probiotics can dampen anxiety-like and depressive behaviour[12]. However, Ohland *et al.* showed that the treatment of mice lacking interleukin-10 (IL-10, an anti-inflammatory cytokine which normally acts to reduce the immune response) with the probiotic bacteria *Lactobacillus helveticus* did not reduce anxiety-like behaviour when the mice were assessed in the Barnes maze[13]. Contrastingly, when control mice possessing IL-10 were treated with *L. helveticus* they showed decreased anxiety-like behaviour in the Barnes maze[13]. Thus, demonstrating the significance of the immune system in modulating behaviour via the gut-brain

axis. Even more, microbiota can trigger immune activation by stimulating circulating cytokines. This process is amplified with microbial dysbiosis introduced by external factors like antibiotics and probiotics[3,14]. Immune activation is often implicated in psychiatric disorders like depression, as is the case with an increased abundance of proinflammatory cytokines like interleukin-12 and interferon-γ[3]. It is hypothesized that gut microbiota may interact with the immune system in a way that alters gut barrier function which may cause increased gut sensation resulting in anxiety-like and depressive behaviours[14].

Mechanism 3: Vagus nerve

The vagus nerve, cranial nerve 10, has afferent and efferent branches[4]. Vagal afferent neurons relay sensory information from peripheral organs like the gut to the CNS and it is known that this input affects cognition, emotion and behaviour[14]. For example, *Campylobacter jejuni* (a GI pathogen associated with inflammatory bowel disease) acts through vagal pathways to alter behaviour in mice[14]. It is also thought that stimulating vagal pathways reduces anxiety and depression[15]. This comes from a study conducted by Krahl *et al.*, whereby mice exposed to daily vagus nerve stimulation showed increased mobility and antidepressant activity during forced swim testing when compared to control mice[16]. Currently, vagal stimulation is a controversial method used to treat depression that is unresponsive to the available medications[15].

Mechanism 4: Hypothalamic-pituitary-adrenal axis

Microbial colonization affects hypothalamic-pituitary-adrenal axis (HPA) development and responsiveness[17]. The HPA is the endocrine control of the stress system and it mediates the release of corticotropin-releasing factor from the hypothalamus, adrenocorticotropic hormone (ACTH) from the anterior pituitary and cortisol from the adrenal glands during the stress response[4]. Although it is known that microbiota influence HPA development and its response to stress, the reverse relationship is also seen. In fact, cortisol release from the adrenal glands during stressful situations alters gut permeability which can change microbial composition[4]. So, it may be possible to decrease anxiety in patients with GAD by altering microbial composition to alter the HPA. Moreover, the link between microbiota and the HPA was first established when germ-free mice demonstrated increased corticosterone and ACTH levels when compared to specific-pathogen-free mice exposed to stress[17]. Since then it has been shown that the HPA is moulded by early life events. For example, adult animals with maternal deprivation at a young age have an increased HPA response during stressful events when compared to adult animals without maternal deprivation at a young age[17].

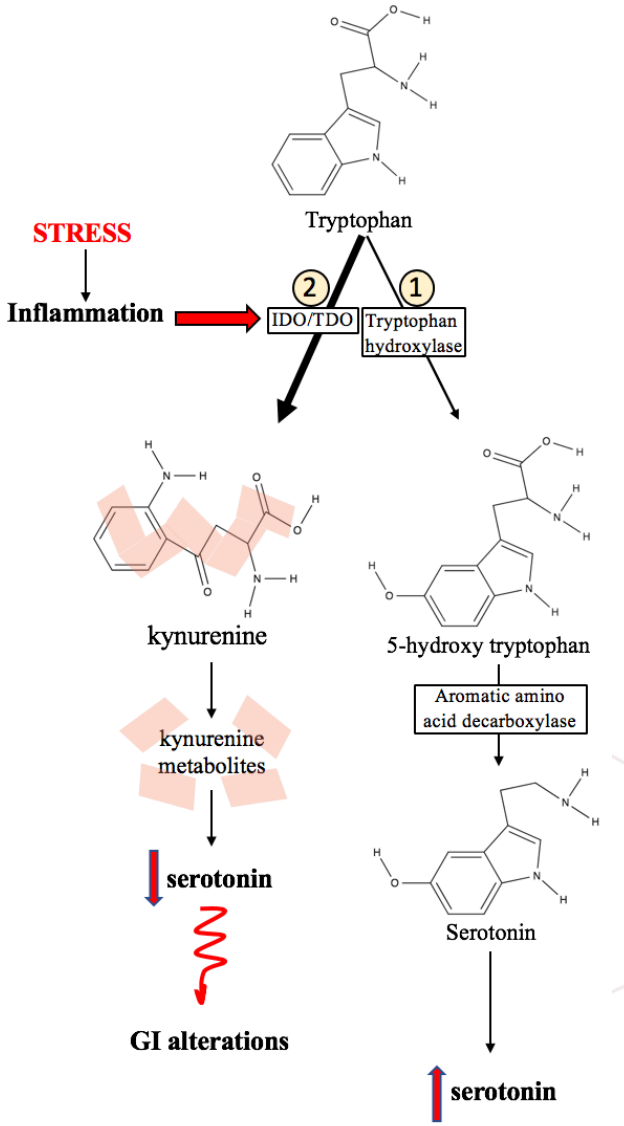

Figure 1. *The two arms of tryptophan metabolism.* The first arm of tryptophan metabolism results in serotonin production through the mechanism depicted above. Dietary tryptophan is converted to 5-hydroxytryptophan by tryptophan hydroxylase, which is then converted to serotonin via aromatic amino acid decarboxylase[10]. The second arm of tryptophan metabolism is the kynurenine arm and it is characterized by the production of

kynurenine (simplified in light orange)[10]. The production of kynurenine and resulting metabolites (simplified as light orange rhombuses) reduces the amount of serotonin available for the host which causes GI alterations.

Mechanism 5: Tryptophan metabolism

Tryptophan (Trp) is an essential amino acid that is obtained from dietary sources[10]. Trp is absorbed through the gut and can enter the circulatory system to cross the blood brain barrier and participate in serotonin synthesis in the CNS[10]. However, over 90% of serotonin is found in the gut where enterochromaffin cells (ECs) of the GI tract convert Trp to serotonin[10,18]. Both in the CNS and the gut, Trp is converted to 5-hydroxytryptophan by tryptophan hydroxylase, which is converted to serotonin via aromatic amino acid decarboxylase (Figure 1)[10]. The kynurenine arm of this pathway is the dominant Trp metabolism pathway and it is dysregulated in many brain and GI disorders[4]. In the kynurenine pathway, kynurenine is produced from Trp by tryptophan-2,3-dioxygenase (TDO) or indolamine-2,3-dioxygenase (IDO) (Figure 1)[10]. This pathway reduces the Trp available for serotonin synthesis and increases metabolites produced by kynurenine which are implicated in psychiatric disorders[3,10].

The serotonergic system is not only implicated in modulating physiological processes like mood and aggression, but it is also implicated in physiological development overall[10]. Moreover, the serotonergic system functions at both sides of the gut-brain axis because serotonin is produced by ECs and in the CNS[10]. Since both gut microbiota and the serotonergic system develop simultaneously, it is plausible that both these systems interact to alter host behaviour[10]. For example, it was previously found that stress causes inflammation which activates IDO and TDO, resulting in decreased Trp and increased kynurenine, which alters GI function (Figure 1)[10]. Even more, germ-free mice reproducibly display increased plasma Trp and exhibit decreased anxiety-like behaviours when compared to microbially colonized mice[10]. Lastly, it is known that certain bacterial strains have tryptophanase enzymes which produce indole from Trp, limiting serotonin production in the host (Figure 2)[10]. It is therefore possible that modifying gut composition could alter kynurenine and indole levels to beneficially modify behaviour[3].

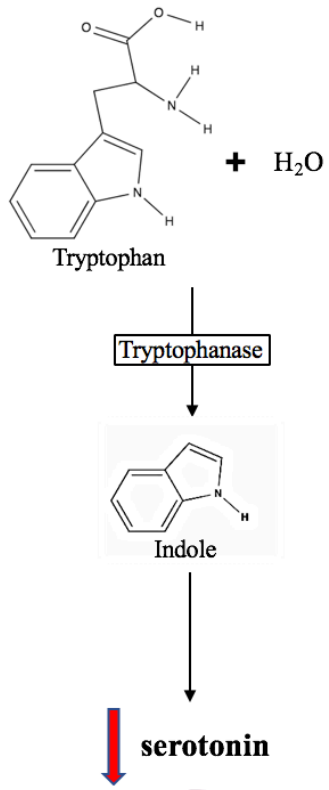

Figure 2. *The production of indole from tryptophan.* Many *Alistipes* species are indole-positive and possess tryptophanase which ultimately disrupts serotonergic balance in the body. It is hypothesized that this property of *Alistipes* may be implicated in anxiety-like and depressive behaviours in individuals[19].

Mechanism 6: Microbial metabolites

Microbial metabolites are the products of the chemical reactions that occur in microbes. There are both primary and secondary metabolites, with the main difference between the two being: primary metabolites are essential for microbial growth whereas secondary metabolites are not[20]. Notably, microbes produce some secondary metabolites that have neuroactive properties during carbohydrate fermentation[4,21]. Such metabolites include: bile acids, choline and short chain fatty acids like L- and D-lactic acid[4,21]. Moreover, it has been shown that increased fermentation results in excess propionate and lactic acid production which causes increased anxiety-like behaviour in mice[21]

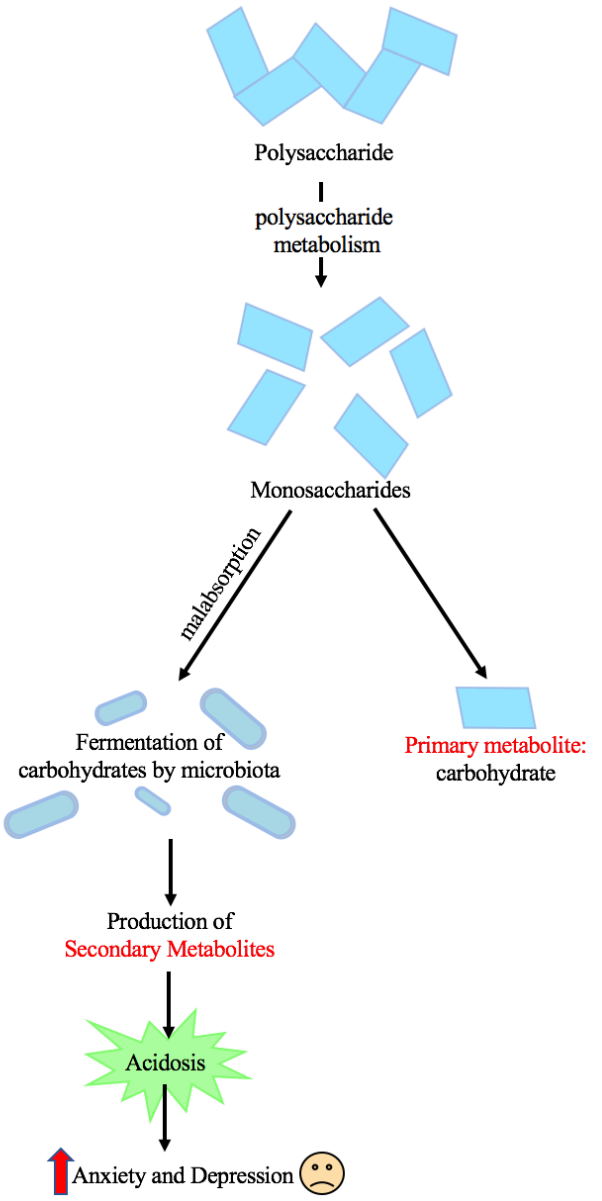

Figure 3. *Depiction of increased anxiety-like and depressive behaviours as a result of carbohydrate malabsorption.* When polysaccharides are broken down to monosaccharides, they are normally absorbed into the gut lumen and stored as primary metabolites. However, in the case of carbohydrate malabsorption, microbiota in the gut (blue rods above) ferment excess carbohydrates and produce secondary metabolites resulting in anxiety-like and depressive behaviour in mice.

Carbohydrate malabsorption also results in a similar effect in humans, whereby malabsorbed carbohydrates are fermented by gut bacteria resulting in high faecal propionic acid and acetic acid concentrations that correlate with negative emotions like anxiety and depression (Figure 3)[22]. This further illustrates how diet can alter conditions like anxiety and depression because a diet higher in carbohydrates will result in more acidosis and therefore increased anxiety-like behaviour[3,21].

Mechanism 7: Microbial neurometabolites

Some microbiota can produce neurotransmitters like GABA, serotonin, catecholamines and histamine[14]. When released, these neurotransmitters interact with epithelial cells in the intestinal lumen to directly alter neural signalling via afferent neurons[4]. These neurometabolites also interact with receptors in the enteric nervous system to affect CNS function[7]. For example, *Lactobacillus* has been shown to produce excess GABA through glutamate metabolism *in vitro* (Figure 4)[23]. This may help reduce anxiety and depression in a manner similar to GABA-like antidepressant and anxiolytic drugs[23].

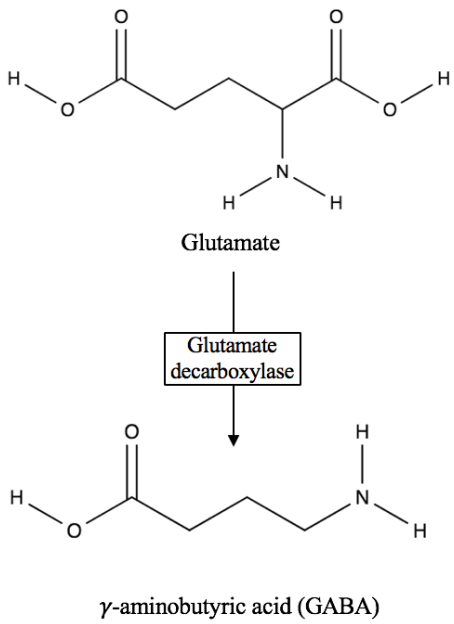

Figure 4. *GABA synthesis from glutamate.* Image adapted from: Production of gaba (γ – Aminobutyric acid) by microorganisms: a review[24]. GABA is a major inhibitory neurotransmitter in the mammalian CNS, and it has hypotensive, tranquilizing effects. It was found that chicken cecal *Alistipes* expresses glutamate decarboxylase which is the enzyme that converts glutamate to GABA as indicated above. It is possible that this may have anti-anxiety and anti-depressive effects on the host due to GABA's role as a potential antidepressant.

Alistipes *and its interaction with anxiety and depression*

It is hypothesized by many different groups that *Alistipes* may cause anxiety-like and depressive behaviours by altering the serotonergic system[25–28]. Although most *Alistipes* test indole-positive and are capable of metabolizing Trp to indole, some species like *A. obesi* and *A. indistinctus* do not produce indole in the presence of Trp[9,29,30]. Therefore, it is possible that not all *Alistipes* species are implicated in anxiety and depression, or there may be multiple mechanisms of action for this interaction. In this review three popular hypothetical mechanisms are discussed in greater detail.

Hypothesis 1: Inflammation hypothesis

Not only are *Alistipes* overexpressed in depressed patients, but they are also associated with the generation of inflammatory molecules that spread into the blood when gut permeability is altered[26,31]. Specifically, *Alistipes* are known to produce lipopolysaccharide (LPS)[32]. LPS is an endotoxin derived from the outer membrane of Gram-negative bacteria[33]. Microbial dysbiosis results in altered intestinal permeability allowing pro-inflammatory molecules like LPS into the bloodstream (Figure 5) [31]. LPS is known to cause systemic and psychiatric changes, otherwise known as sickness behaviour, in mammals[34]. For example, Haba *et al.* demonstrated that LPS injection induces depression-like behaviours 6 and 24 hours after injection in BALB/c mice[34].

Moreover, Qin *et al.* showed that LPS injection in mice increased serum TNF-α which induces pro-inflammatory cytokine production in the brain, serum and liver resulting in prolonged neuroinflammation[33]. The production of inflammatory cytokines in the CNS alters brain activity and modulates the synthesis of neuropeptides, both of which are associated with psychiatric disorders like depression[31]. LPS has also been implicated in increased activity in the emotional centre of the CNS, the amygdala[31]. It has previously been hypothesized that activation of the amygdala for long periods of time underlie depressed exploration in mice[34]. The exact mechanisms by which *Alistipes* participates in increasing inflammation and the resulting depression behaviours observed in mammals is unclear, however the relationship is evident in multiple studies.

Hypothesis 2: Interference with neurotransmitter signalling

Although many different groups, including Inserra *et al.*, found relatively higher levels of *Alistipes* in the gut microbiota of patients experiencing periods of active depression when compared to healthy controls, the role of *Alistipes* in psychiatric disorders is unclear[35]. Many *Alistipes* species are indole-positive and may influence the availability of Trp and therefore disrupt the balance of the serotonergic system[28]. Moreover, it is known that *Alistipes* contain the tryptophanase gene which directly produces indole from Trp (Figure 2), further supporting this hypothesis[19]. However, indole is beneficial and attenuates damage to the GI tract[18]. Contrary to the proposed compromised intestinal permeability and inflammation associated with psychiatric disorders, indole exposure strengthens the mucosal barrier

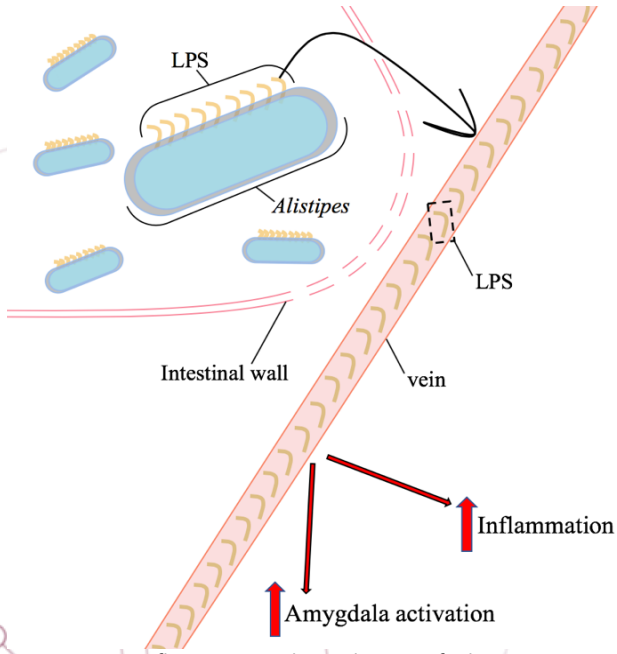

Figure 5. *Inflammation hypothesis of the association between* Alistipes *and depression.* It is hypothesized that when microbial dysbiosis ensues, intestinal permeability is compromised as depicted by the two broken red lines surrounding the *Alistipes*. This altered permeability is thought to allow LPS into circulation and ultimately cause increased inflammation in the liver, CNS and serum[31]. LPS causes prolonged activation of the

amygdala in mice which results in depressed exploration[34].

and suppresses pro-inflammatory cytokine production[18]. Therefore, it is possible that *Alistipes*, although elevated in GAD and MDD, confers a beneficial effect to the host. On the other hand, van Beek *et al*. found that *Alistipes* are associated with IDO overexpression[36]. IDO is an enzyme in the kynurenine pathway which produces Trp metabolites that are implicated in psychiatric disorders (Figure 1)[10,36]. However, van Beek *et al*. stated there is no evidence for a relationship between the abundance of *Alistipes* and altered Trp metabolism[36]. As a result, the intriguing hypothesis of *Alistipes* altering the serotonergic system to cause disorders like anxiety and depression requires further investigation as there is a possibility that this association is either insignificant or beneficial for the host.

Hypothesis 3: Natural products – secondary metabolites

Natural products are produced by organisms and include both primary and secondary metabolites. *Alistipes* are involved in the production of two interesting secondary metabolites. First, Walker *et al*. found that mice fed high-fat diets produce sulfonolipids (SLs) which are a unique type of sphingolipid produced exclusively by *Alistipes* and *Odoribacter*[37]. SLs have previously been described to have anti-inflammatory effects, and in this study sulfobacin B (the specific SL produced by *Alistipes*) suppressed the activation of the proinflammatory cytokine TNF-**α**[37]. Interestingly, LPS is found in the cell wall of *Alistipes* and has the potential to activate TNF-**α** which may cause inflammation[33]. The opposing outcomes of LPS and SLs raise the question of whether *Alistipes* actually contributes to the inflammation associated with psychiatric disorders, or whether it has a beneficial or neutral effect in the host.

Additionally, Polanksy *et al*. found that *Alistipes* expressed glutamate decarboxylase which produces **ɣ**-aminobutyric acid (GABA) from glutamate in chicken cecal microbiota (Figure 4) [38]. Although many studies correlate an increased abundance of *Alistipes* with depression and anxiety, this was a very unusual finding since GABA relieves anxiety[7,8,31,35]. However, it is possible that although *Alistipes* can produce GABA, the neurotransmitter may not be released into circulation[38]. Therefore, further investigation of *Alistipes'* ability to produce GABA and the release of this neurotransmitter in the gut lumen is required to elucidate a potential beneficial effect of *Alistipes* for individuals with anxiety and depression.

Discussion

There is an increasing body of research that supports the role of microbial dysbiosis in psychiatric-related illnesses. However, a clear understanding of the mechanisms of action for over and under expressed microbes in these conditions is required. Specifically, many studies have concluded that there is an increased abundance of *Alistipes* in individuals with anxiety and depression. However, it is unclear whether *Alistipes* have a negative or positive impact on these illnesses because some metabolites that it produces, such as indole, GABA and sulfobacin B may alleviate anxious and depressive behaviours. However other products like LPS and kynurenine metabolites may aggravate these behaviours. Moreover, further work is required to clarify the link between the different *Alistipes* species in both the inflammatory and serotonin pathways.

In the future it is important to work towards developing a comprehensive library of *Alistipes* species. With this library *in vivo* assays using animal models like *Caenorhabditis elegans*, *Drosophila* and *Danio rerio*, all of which show conservation in the genes associated with increased risk of mental illness, can be conducted to elucidate the role of *Alistipes* in psychiatric disorders[39]. Moreover, by conducting whole genome sequencing of all the isolated *Alistipes*, the sequences can be compared to those found in other organisms to identify conserved genes that may be associated with psychiatric disorders. For example, if it is found that human *Alistipes* from the gut of healthy patients all express glutamate decarboxylase as seen in chicken cecal microbiota, it may be possible that *Alistipes* exerts a beneficial effect on these individuals by lowering their risk of developing anxiety-like or depressive behaviours. Lastly, elucidating the

mechanism by which *Alistipes* communicate with the gut-brain axis is pivotal to developing personalized microbiota-based and microbiota-specific therapies to treat anxiety and depression.

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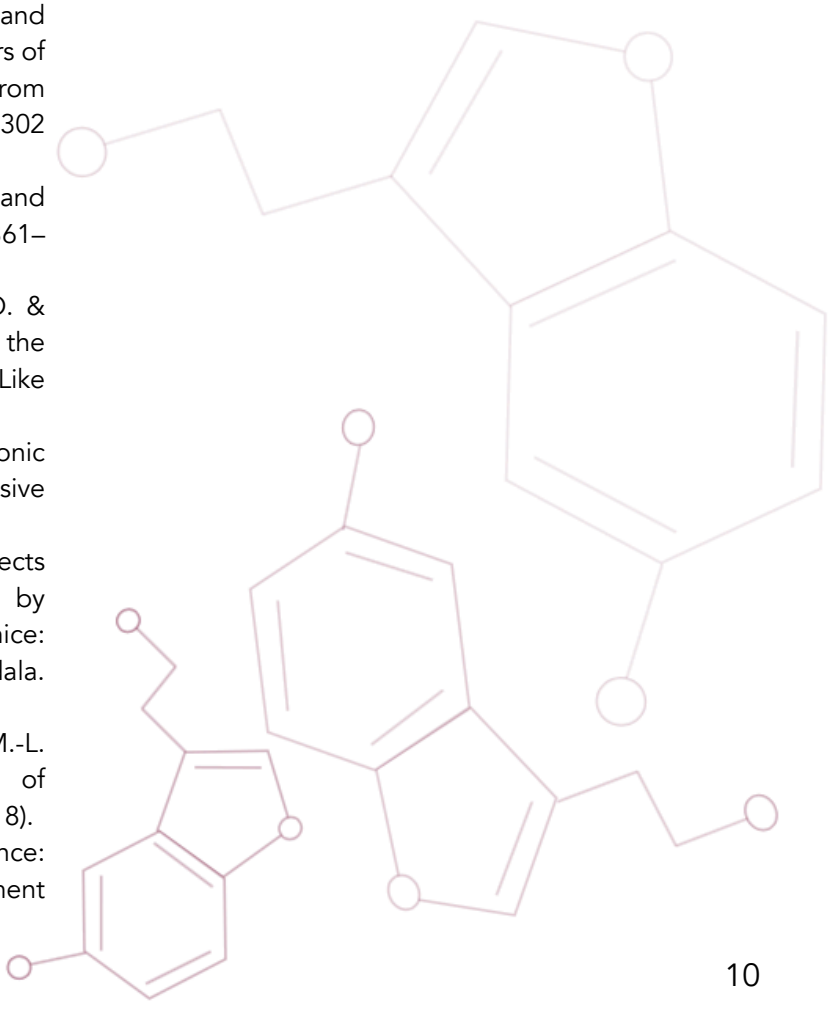

The role of *Faecalibacterium prausnitzii* in health and disease

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Abstract
The human body is host to numerous complex microbial communities that comprise the human microbiome. These microbes and their dynamic interactions with each other and with the host, play critical roles in human development and health. Although mostly considered beneficial, bacteria within the microbiome may contribute to disease as infectious agents, through mediation of antibiotic resistance, and by participation in immune phenomenon as drivers of chronic inflammatory diseases. This review highlights the current research on the gut microbiota, with a particular focus on *Faecalibacterium prausnitzii* and its role in maintaining intestinal health. *F. prausnitzii* is a species of obligate anaerobic bacteria found in the human gastrointestinal tract. This species has been widely associated with human health and is found at lower numbers in a wide variety of human diseases including inflammatory bowel disease (IBD). Based on the current research landscape, however, it is evident that majority of research on *F. prausnitzii* is associative in nature and for this reason, culture-dependent studies are needed to further elucidate the role of this gut bacteria in diseases such as IBD.

Introduction
The human gastrointestinal (GI) tract is home to a diverse group of bacteria, archaea, viruses, and fungi.[1-4] Together this collection of microorganisms is referred to as the 'gut microbiota'.[1-4] Interestingly, majority of these microorganisms are bacteria, approximately equal in number to the amount of human eukaryotic cells in the body.[2,5] In contrast, the term 'gut microbiome' refers to the collective genomes, encoding more than three million genes, of the microorganisms that inhabit the gut. [1-4] In the last 15 years, the role of the gut microbiota in maintaining intestinal health has become increasingly evident.[3] Not only do these microorganisms play a key role in harvesting energy, they also prevent the colonization of pathogens and maintain host immunity.[1,3-4] However, a change in the microbial composition of the gut can lead to the development of disease pathology.[4,6] Dysbiosis or the imbalance between protective commensal bacteria and harmful opportunistic bacteria is proposed to be the underlying cause of several diseases including inflammatory bowel disease (IBD). [1,4,6] IBD refers to a group of disorders characterized by chronic inflammation in the GI tract.[7] With regard to the role of dysbiosis in IBD, research suggests that the depletion of *Faecalibacterium prausnitzii*, a major commensal bacterium, is associated with the disease pathology of IBD.[7-8] The purpose of this review is to summarize the current research on the gut microbiota, discuss the role of *F. prausnitzii* in intestinal health, examine the factors promoting *F. prausnitzii* presence in the gut, and lastly consider the role of *F. prausnitzii* in IBD. Additionally, this review seeks to highlight areas in this field of research that need to be further clarified or addressed.

Composition of the gut microbiota
The human gut microbiota is colonized by at least 1000 different species of bacteria.[1,4] This process of colonization begins right after birth as the infant becomes exposed to the outside environment.[9] Additionally, factors such as the maternal microbiota composition and mode of delivery have been shown to influence early colonization of the gut.[9] Research suggests that two main phyla of bacteria dominate the early infant gut: Actinobacteria and Proteobacteria.[1,9] However, as the infant grows older, the gut microbiota continues to evolve and increase in microbial diversity. [1,9] As the individual reaches adulthood, the composition of the gut microbiota becomes relatively stable, but can still be altered by factors such as diet, lifestyle, antibiotic treatment, illness,

aging, and the environment.[1,9] Although twin studies have suggested a potential role of genetics as a determinant of microbiota composition, the extent and nature of its role remains under debate and further research is needed.[9] Furthermore, due to differences in experience, every individual develops a unique gut bacterial composition.[1,3,9] However, in general, the adult microbiota is composed primarily of two main phyla: Firmicutes and Bacteroidetes.[1,9] Other phyla including Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria, although present, are at a much lower proportion.[4]

Role of the gut microbiota in intestinal health

The gut microbiota plays a critical role in the maintenance of intestinal health, as well as nutrition, immune development, and host defense.[7] One of the primary role of gut bacteria is the production of compounds essential for GI health.[7,10] Not only do commensal bacteria synthesize vitamins, but they also play a critical role in the fermentation of dietary fibers.[7] The phyla Firmicutes and Bacteroidetes have found to be involved in the production of short chain fatty acids (SCFAs) via fermentation.[7,10] These SCFAs are absorbed by the surrounding colonic epithelial cells (CECs) and are able to regulate cellular processes such as altering cell growth and gene expression.[7] The three major SCFAs produced include: butyrate, acetate, and propionate.[7] Among these, butyrate has been shown to be a primary contributor of intestinal health.[1,4,7] Majority of this SCFA is produced by *Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii and Ruminococcus bromii.*[10] By acting as an energy source for CECs, butyrate promotes cell proliferation.[7] Additionally, butyrate has also been shown to be an important regulator of tight-junction proteins (TJPs) in the GI tract.[7,10] Increased levels of butyrate lead to an increased expression of TJPs, thereby promoting intestinal barrier integrity and preventing bacterial translocation across the epithelium.[7,10] Recent evidence suggests that butyrate might also promote *B*-oxidation in CECs, which increases uptake of oxygen, and therefore creates an anaerobic gut environment unfavourable for the colonization of pathogenic facultative anaerobes.[11] Finally, emerging research in this field also suggests anti-inflammatory activity of butyrate in the gut. In contrast to butyrate, both acetate and propionate have shown to play a systemic role in the body.[7,11] Propionate is taken up by the liver, while acetate moves into peripheral organs such as the muscles.[7,10]

The interaction between the gut microbiota and immune system is critical for the maintenance of intestinal health and barrier integrity.[7] In particular, the presence of the gut microbiota influences development of the mucosal immune system.[1,7] Experiments have indicated that germ-free mice (deficient in a gut microbiota) display an underdeveloped and impaired immune system.[7] When the microbiota is reintroduced in these mice, majority of the immune system function is restored.[7] Bacteria in the gut, primarily of the class Clostridia, have been shown to play a role in the differentiation and proliferation of regulatory T cells (Tregs) via butyrate production.[7] Tregs are immune cells responsible for regulating and suppressing other immune cells of the body.[7] These cells play a critical role in maintaining immunological tolerance and preventing autoimmune reactions.[7] Research suggests that individuals with inflammatory gut diseases such as IBD are found to have much lower levels of butyrate-producing bacteria.[7-8] Additionally, species of gut bacteria such as *Escherichia coli* and *Citrobacter rodentium* have been described to contribute to the induction and development of helper T cells such as Th17.[7]

Finally, the gut microbiota is also responsible for protecting the GI tract from pathogenic bacteria. This concept is referred to as 'colonization resistance'.[12] Commensal bacteria are able to protect the intestine from pathogens in two ways: directly and indirectly.[7,12] By consuming essential nutrients or through the production of anti-microbial molecules such as bacteriocins, commensal bacteria are able to directly prevent the colonization of pathogens.[7,12] For example, species of the genus *Bifidobacterium* have been shown to inhibit colonization of intestinal pathogens, such as *Clostridium difficile*, via production of antimicrobial organic acids and peptides.[13] Recent evidence has also demonstrated the role of contact-dependent interbacterial antagonism, notably in the phylum

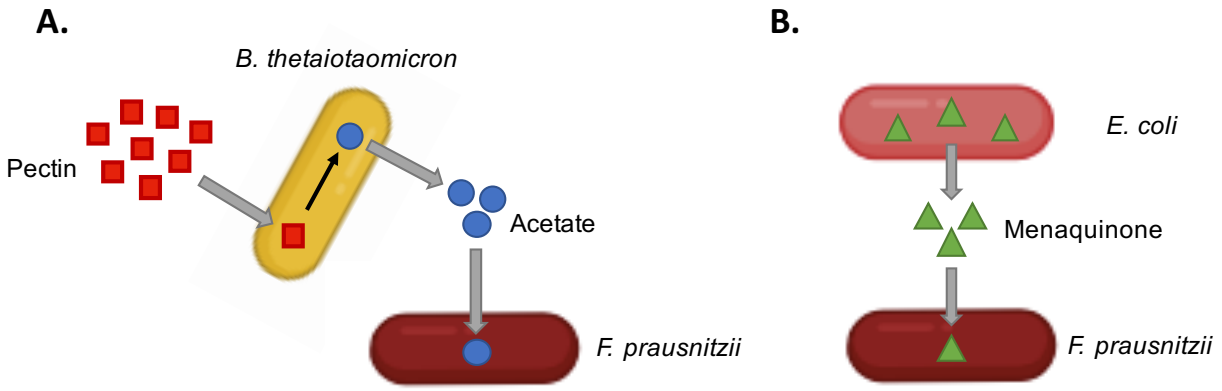

Figure 1. *Examples of syntrophy with* F. prausnitzii. (A) Acetate, which is produced by B. thetaiotaomicron due to pectin fermentation, is rapidly taken up by F. prausnitzii to promote growth. (B) Menaquinone, directly produced by E. coli, is taken up by F. prausnitzii and used in the electron transport chain to facilitate anaerobic respiration.

Bacteroidetes. By injecting toxic effectors into nearby cells, a mechanism known as the type VI secretion system occurs, in which gut commensals are able to prevent the colonization of harmful bacteria and, thereby, maintain a stable gut environment.[14] On the other hand, indirect methods of colonization resistance involve the role of commensal bacteria in the production of pro-inflammatory molecules that prime or enhance the immune response against pathogenic bacteria.[7,12] Together, these mechanisms are critical for preventing GI infections.

It is important to note that a slight disruption in any of the functions mentioned above may lead to the development of disease pathology such as obesity, malnutrition, IBD, neurological disorders, and cancer.[4,6] Dysbiosis or a change in composition of the gut microbiota has been suggested to be one of the primary underlying cause of this disruption.[4,6] It is evident in patients with IBD that there is a decrease in gut bacteria with anti-inflammatory properties and an increase in those that contribute to inflammation.[4,8] Although the levels of many different bacterial species are altered in IBD, the most consistent finding suggests a lower abundance of *F. prausnitzii* in patients with IBD.[15] The role of *F. prausnitzii* in gut health and disease will be further addressed in the following portion of this review.

Role of *F. prausnitzii* in intestinal health

F. prausnitzii is one of the three most abundant bacterial species found in the GI tract, accounting for approximately 6-8% of the gut microbial community in healthy individuals.[15] *F. prausnitzii* is a member of the Firmicutes phylum and the only known species in the genus *Faecalibacterium*.[8,15] It is a Gram-positive, strict anaerobic bacterium.[8,15] Currently, majority of the research on *F. prausnitzii* is based on metagenomic studies of the gut microbiota.[16] These studies have shown altered levels of *F. prausnitzii* in patients with a range of metabolic diseases such as colorectal cancer, obesity, celiac disease, and IBD.[16] However, this data is associative in nature and cannot be used to suggest a causal role of *F. prausnitzii* in disease pathology.[16] Instead, culture dependent studies are required to address the role of *F. prausnitzii* in health and disease.[16] Unfortunately, due to the difficulty in growing this extremely oxygen sensitive (EOS) bacterium, a limited number of studies have assessed the function and underlying biology of *F. prausnitzii*.[16]

Factors promoting *F. prausnitzii* in the gut

When considering isolation strategies for *F. prausnitzii*, it is important to consider the bacteria's growth requirements. Current literature provides insight regarding the role of specific carbon sources, gut physiological conditions, and various bacteria in the growth of *F. prausnitzii*. However, the effects of each of these factors has been evaluated

using a limited number of *F. prausnitzii* strains. One study, in particular, showed that simple carbohydrates including fructose, glucose, cellobiose, and maltose were fermented by 90-100% of *F. prausnitzii* strains.[17] Additionally, diet-derived apple pectin has been shown to promote *F. prausnitzii* growth in culture.[17] Both *in vitro* and *in vivo* studies have demonstrated an increase in *Firmicutes* abundance after introduction of pectin as an energy source.[17-18] Lastly, host-derived carbon sources including glucosamine HCl and N-acetylglucosamine have also been shown to be fermented by *F. prausnitzii* and thereby promote growth.[17] This data suggests a use of any of the above mentioned carbon sources as prebiotics for individuals suffering from IBD.[19] In other words, these carbons sources can be administered to restore *F. prausnitzii* levels in the diseased gut.[19] Furthermore, the aforementioned carbon sources may also be added as supplements to media in order to better improve culture-dependent isolation strategies for *F. prausnitzii*. The success of such strategy should be addressed and evaluated in the future. With regard to mucin, glycoproteins that make up the mucus covering on epithelial cells, little is known about its effect on *F. prausnitzii*.[20-21] Conflicting research about the ability of *F. prausnitzii* to utilize mucin exists in current literature and future studies should focus on addressing this issue.[20]

Tolerance to different physiological conditions in the gut is a key determinant of the ability for bacteria to colonize the GI tract.[17] Experiments testing the pH tolerance of various strains of *F. prausnitzii* show that the optimal pH for growth ranges between 5.7 and 6.7.[18] This data is reinforced by the colonization patterns of *F. prausnitzii* in the GI tract. *F. prausnitzii* is found at higher levels in the duodenum, which has a pH range of 5.7-6.4 in healthy subjects.[22] Additionally, evidence shows that *F. prausnitzii* is highly sensitive to bile salts.[17] Although strain-strain variability exists, *F. prausnitzii* growth is compromised when bile salts concentrations reach 0.5% (wt/vol) or above.[17] This data might explain why Crohn's disease (CD) patients, who exhibit higher bile salt concentration in their gut, have decreased levels of *F. prausnitzii*.[15] Furthermore, based on functional metabolic maps of *F. prausnitzii*, it has become evident that certain strains of this bacteria are unable to synthesize molecules such as cysteine, biotin, and riboflavin.[23] These results signify the importance of having such molecules present in the gut environment for use by *F. prausnitzii*.[23] Nonetheless, this data varies among strains and further research using a larger collection of isolates is required.

Syntrophy, otherwise referred to as 'cross-feeding', is the phenomenon where one bacterial species is dependent on the products of another species.[24] Examples of this phenomenon are widely evident when looking at the diverse microbial communities present in the GI tract.[24] Interestingly, *F. prausnitzii* has been shown to rely on other bacterial species in the gut for cross-feeding.[17,25] Past studies have observed this relationship between *F. prausnitzii* and *Bacteroides thetaiotaomicron* – a Gram-negative, strict anaerobe, found to be abundant in the human gut microbiota.[17] Co-culture experiments containing these two bacteria in media supplemented with pectin showed enhanced growth of *F. prausnitzii*.[17] Specifically, acetate, a SCFA produced by *B. thetaiotaomicron* via pectin fermentation, was observed to be rapidly taken up by *F. prausnitzii* (Figure 1A).[17] These results are supported by previous studies showing the importance of acetate in *F. prausnitzii* growth.[23] Furthermore, experiments in germ-free mice have demonstrated that *F. prausnitzii* is unable to colonize the gut individually.[25] However, when co-colonized with another bacterium such as *B. thetaiotaomicron*, successful colonization is observed.[25] Interestingly, a similar cross-feeding relationship has recently become evident between *F. prausnitzii* and *E. coli*.[26] A group of scientists have evaluated the role of *E. coli* as a 'helper' strain for multiple species of bacteria including *F. prausnitzii*.[26] Using co-culture experiments, the researchers demonstrated that *E. coli* induced growth of *F. prausnitzii* (Figure 1B).[26] Additionally, they tested a library of mutant *E. coli* strains to evaluate the underlying mechanism of induction.[26]

The researchers concluded from the experiments that the *E. coli* genes involved in menaquinone biosynthesis were responsible for inducing *F. prausnitzii* growth.[26] Follow-up genome sequencing of *F. prausnitzii* further supported this notion when a lack of genes responsible for the menaquinone biosynthesis pathway were identified.[26] In majority of Gram-positive bacteria including *F. prausnitzii*, menaquinone participates in the electron transport chain in order to facilitate anaerobic respiration.[26] However, in the case of *F. prausnitzii*, menaquinone is not synthesized on its own but is instead acquired from the external environment.[26] For this reason, *E. coli*, a major producer of menaquinone, is able to induce *F. prausnitzii* growth in culture.[26] With regard to improving current *F. prausnitzii* isolation techniques, future efforts should focus on elucidating the role of menaquinone in the cross-feeding relationship between *F. prausnitzii* and *E. coli*.

Role of *F. prausnitzii* in IBD

As previously described, IBD is a group of metabolic diseases characterized by chronic inflammation of the GI tract.[4,7] There are two main forms of IBD: Crohns disease (CD) and ulcerative colitis (UC).[4,7] Although the exact cause of IBD remains unknown, evidence suggests that a combination of genetic risk and dysbiosis contribute to disease pathology.[7] A reduction in the diversity of the gut microbiota accompanied by lower levels of Firmicutes bacteria can be seen in IBD patients.[8,15] In particular, lower counts of *F. prausnitzii* in the gut microbiota of patients suffering IBD has been reported in several studies.[8,15] It has been proposed that *F. prausnitzii* displays anti-inflammatory properties that may be responsible for maintaining a healthy gut environment.[8,15] For this reason, scientists are currently evaluating the use of *F. prausnitzii* as a probiotic to counterbalance dysbiosis in the gut.[15] Although the exact mechanism by which *F. prausnitzii* maintains intestinal health remains unclear, researchers have proposed several hypotheses. Recent evidence has shown the role of *F. prausnitzii* in butyrate production, thereby maintaining intestinal health and integrity.[8,10,15] Not only does butyrate act as an energy source for CECs, but it has shown to be involved in preventing inflammation. By inhibiting NF-κB and IFN-ɣ, as well as up regulating PPARɣ, a reduction in intestinal inflammation has been observed.[27] In addition, *F. prausnitzii* also displays novel anti-inflammatory properties that have been shown in a DSS colitis murine model.[28] Administration of the cell-free supernatant of *F. prausnitzii* in colitis induced mice led to a reduction in gut inflammation.[28] Furthermore, researchers were successfully able to identify specific peptides in the supernatant responsible for this inhibitory effect.[29] These peptides derive from a 15 kDa protein produced by *F. prausnitzii*, referred to as microbial anti-inflammatory molecule (MAM).[29] Follow-up experiments have shown that MAM is able to demonstrate immunomodulatory activity by blocking both the NF-κB signaling cascade and production of IL-8, a pro-inflammatory cytokine.[29] Due to a very limited number of studies on MAM, future research should focus on evaluating the potential use of MAM as a therapeutic strategy for IBD.

Conclusion

The human body is host to numerous complex microbial communities that comprise the human microbiota. These microbes and their dynamic interactions with each other and with the host play critical roles in human development and health. Specifically, the gut microbiota contributes to intestinal health through the production of metabolites such as butyrate, the development of the mucosal immune system, and by providing protection from pathogenic bacteria via colonization resistance. Unfortunately, a disruption in any of the mentioned functions can lead to severe disease pathology. This is evident when looking at the association between *F. prausnitzii* and IBD. As described in the literature, *F. prausnitzii* is not extensively characterized and there are a limited number of isolates to date. For this reason, future work should focus on development of a culture dependent methodology for the isolation of a diverse set of *F. prausnitzii* strains. Genomic and phenotypic assays of various isolates will allow researchers to assess strain-strain variability,

providing further insight into the immunomodulatory role of *F. prausnitzii.*

Overall, the study of the human gut microbiota in health and disease is an emerging field of research. However, the majority of current research is associative in nature and for this reason, culture-dependent studies are essential to further elucidate the role of gut microbes in diseases such as IBD.

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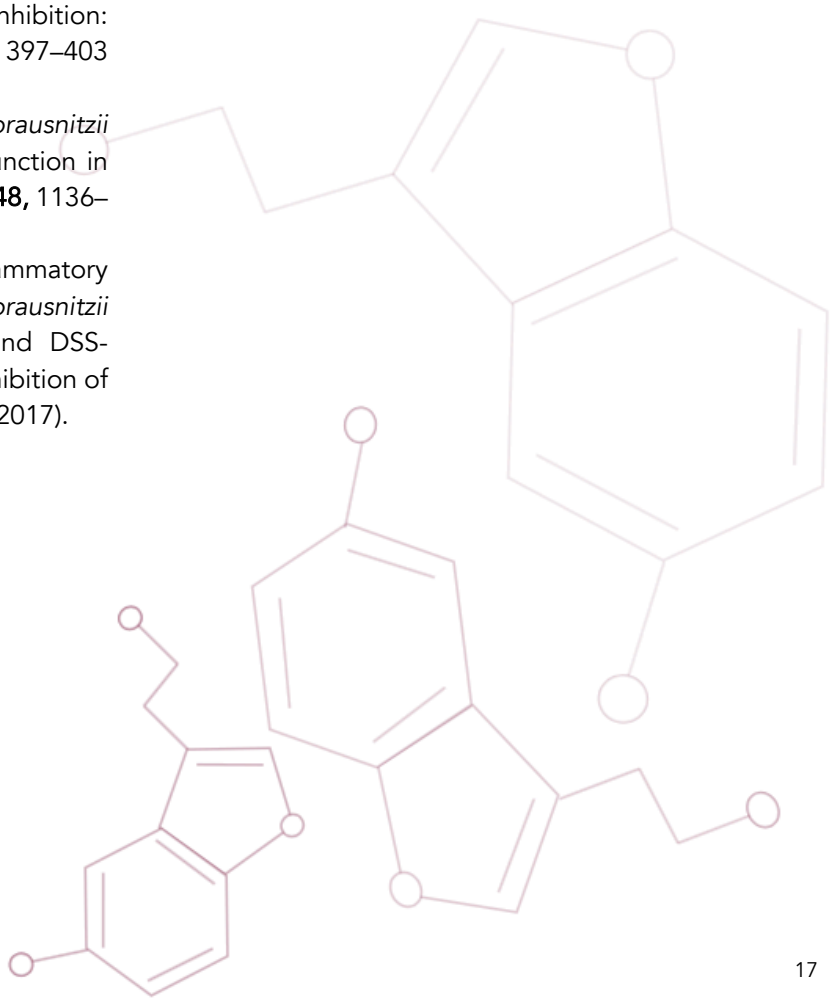

Disinfectants as a double-edged sword: Are disinfectants promoting antimicrobial resistance?

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Abstract
The discovery of antibiotics has been a turning point in modern medicine, having saved countless lives. Antibiotics kill bacteria through different mechanisms; however, shortly after resistance to antibiotics started to emerge. Bacteria are able to acquire resistance to antibiotics via target alteration, efflux pump and enzymatic modification. Disinfectants are used to sterilize an environment and control the spread of dangerous pathogens. Nonetheless, the misuse of disinfectants can promote the rise of resistance via target alteration, impermeability and efflux pumps. These resistant strains may also be co-resistant to antibiotics and this superbug may be untreatable. It is evident that more research needs to be devoted to understanding if and how resistance emerges towards disinfectants and how it can be combatted.

Introduction
The Centre for Disease Control and Prevention (CDC) states that at least 2 million people are infected by resistant bacteria and 23,000 people die from these infections annually[1]. The breakthrough discovery of antibiotics is one of the turning points for modern medicine. Discovered in 1928, penicillin saved numerous lives however, only a few years later resistant strains started to emerge[2]. Resistance occurs when some bacteria are unsusceptible to antibiotics and are able proliferate in the presence of the antibiotic, leading to a rise in a resistant population (Figure 1).

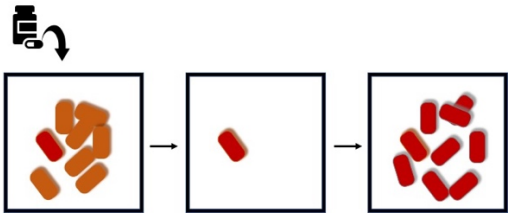

Figure 1. *How antibiotic resistance arises.* The antibiotic is given to a population of bacteria with a few that has resistance. The susceptible population is killed while the resistant one is unaffected. The resistant population is now able to proliferate.

Antibiotic resistance arises via many different mechanisms including target alteration, efflux pumps and enzymatic modification. Sterilization using disinfectants is of utmost importance to mitigate the spread of disease. However, when improperly used, this double-edged sword can lead to resistant strains. Bacteria may also confer resistance to disinfectants through different mechanisms including target alteration, impermeability and efflux pumps. Moreover, bacteria are able to produce biofilms which prevents the disinfectant from eradicating the microorganisms. The resistance that emerges due to the improper use of disinfectants may transfer as cross-resistance to antibiotics, making it a bigger concern[3]. These superbugs that do not respond to disinfectants which have many targets on the microorganism, may not respond to antibiotics with few targets. This harsh reality makes prior cleaning a necessity for appropriate sterilization as well as following the correct procedures when using the disinfectants. Time and money must be invested into research for new and improved antibiotics as well as for researching whether disinfectants are a large concern for the development of resistance.

Antibiotic Resistance
A report by de Kraker *et al.* (2016) states that antimicrobial resistance can kill up to 10 million people and cost $100 trillion annually by the year 2050 (Figure 2)[4].

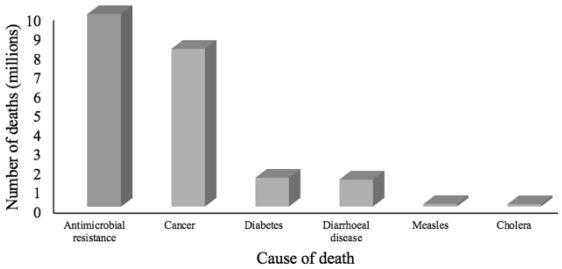

Figure 2. *The estimated number of deaths by 2050 caused by different diseases.* It is estimated that by the year 2050, antimicrobial resistant pathogens are going to be the main cause of death, killing 10 million annually. Following this is cancer at 8.2 million annually[4]. Adapted from Review on Antimicrobial Resistance.

Antimicrobials are compounds that inhibit the growth of or destroy harmful microorganisms without damaging the host. They are used to treat infections during complex surgeries such as organ transplants[5]. In addition, antibiotics are used in agriculture to promote growth and prevent infections in animals[5]. From their discovery, antibiotics have made significant contributions to modern medicine and have saved countless lives[5]. However, resistance to antibiotics is emerging at an alarming rate and is a source of growing burden on the healthcare system. Microorganisms continue to evolve and become unresponsive towards current interventions, through a variety of different mechanisms (Figure 3).

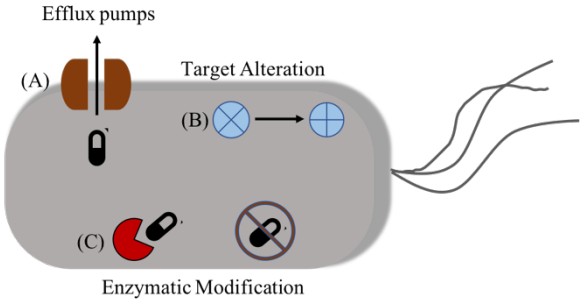

Figure 3. *The different mechanisms of resistance to antibiotics.* (A) Bacteria can pump the antibiotic to the external environment via efflux pumps making antibiotics ineffective. (B) They can have an altered target resulting in the antibiotic not being able to exert its effect on the target. (C) Bacteria possess enzymes that are capable of inactivating the antibiotic.

Additionally, there is a shortage of novel antibiotics entering the market and there are several reasons for this. Due to the fact that antibiotics are taken for about two weeks and are relatively cheap compared to other drugs, pharmaceutical companies have little incentive to research and develop new antibiotics. Their return on investment is simply too low[6]. Furthermore, there is an initiative to decrease the amount of antibiotics prescribed since its overuse may lead to resistance. This impacts sales and consequently research towards finding new antibiotics decreases as companies focus on more profitable drugs[6]. In addition to creating new antibiotics, molecular modifications of current antibiotics could also be useful. For example, Dale Boger and his team at The Scripps Research Institute were able to chemically modify vancomycin and improve its antimicrobial potency against resistant strains[7]. Nonetheless, the startling rise in resistant microorganisms cannot be ignored and makes research towards finding new and improved antimicrobials vital.

Mechanisms of Antibiotic Resistance

Antibiotics are able to induce cell death via four main targets: DNA, RNA, the cell wall and proteins[8]. Target alteration is a mechanism that bacteria use to develop resistance through alteration of an area normally targeted by the antibiotic. For example, bacteria resistant to fluoroquinolones have been reported to have altered DNA gyrase and topoisomerases that the antibiotic cannot target, allowing the bacteria to proliferate[9]. Another mechanism that they use are efflux pumps that transport toxic molecules out of the bacteria[10]. Efflux pumps are able to transport antibiotics to the external environment, protecting the bacteria from death and they confer medium to high level resistance to tetracyclines, macrolides and fluoroquinolones[11]. Finally, enzymatic modification is another mechanism of antibiotic resistance and occurs via two main pathways; hydrolysis or group transfer[12]. Many of these drugs possess bonds that are essential to their activity and bacteria

have evolved enzymes that can destroy these bonds and consequently halt its activity. Hydrolysis is a main resistance pathway against β-lactams due to β-lactamases which are enzymes that hydrolyze the β-lactam ring making them ineffective at destroying microorganisms[13]. Furthermore, group transferases are enzymes that covalently alter the antibiotic and weaken its activity[12]. Acetyltransferases modify hydroxyl or amine groups found on antibiotics and a well classified acetyltransferase is chloramphenicol acetyltransferases[12]. These enzymes possess active sites that deprotonate the nucleophilic hydroxyl group on the antibiotic, inactivating it[12]. It is also important to note that resistance can develop as a result of a combination of the above mechanisms.

Biocide Resistance

Biocides are compounds that include disinfectants, antiseptics and preservatives and its main purpose is to sterilize the area of concern and prevent microbial growth[14]. This review will focus mostly on disinfectants and how misuse and overuse could possibly result in selective pressure and consequently resistance. Disinfectants are classified into three levels: high, intermediate and low-level[2]. High level disinfectants are able to kill all microorganisms except a high number of bacterial spores and examples include hydrogen peroxide and glutaraldehyde. Intermediate level is effective against vegetative bacteria, mycobacteria, most viruses and fungi but not bacterial spores and examples include alcohol and hypochlorite. Finally, low-level disinfectants such as phenolics cannot destroy mycobacteria or spores[2]. Disinfectants are often advertised as an essential safety measure for homes but there is no research to support these claims[14]. In addition, these misleading advertisements encourages the overuse of disinfectants resulting in selective pressure and possible resistance. Sterilizing instruments in healthcare environments are an essential component of ensuring that infections do not spread which is why disinfectants are of utmost importance. However, when these areas are not properly sterilized, or the microorganisms confer resistance by other means, resistant bacteria can spread at a very fast rate. It is clear that disinfectants are crucial for stopping the spread of diseases however, while doing so, we may be selecting for superbugs and the spread of these superbugs are more concerning. Researching the means of resistance against disinfectants can help better understand their mechanism of action and how resistance can be combatted.

Mechanisms of Biocide Resistance

Target Alteration

Disinfectants target multiple aspects of a microorganism making the rise of resistance less common when compared to antibiotics which have one or few targets[15]. However, several outbreaks of resistant bacteria have been reported related to disinfectant use. Target alteration is a mechanism that bacteria use to confer resistance to biocides and occurs when the normal target is distorted, resulting in the biocide being unable to exert its full effect. For example, triclosan is an antimicrobial chemical found in many household items such as soaps and lotions[16] Triclosan exerts its effect by blocking the active site of enoyl reductase, an essential enzyme involved in fatty acid synthesis. Bacteria undergo fatty acid synthesis via the type II fatty acid biosynthetic system[17]. Without fatty acids, the bacteria are unable to build its cell membrane or reproduce leading to cell death. Since this enzyme is absent in humans, it makes it an attractive target for antimicrobial agents. An example of resistance to triclosan has been reported in *Escherichia coli (E. coli)* and emerges due to a mutation in the *fabI* gene. The gene *fabI* encodes for enoyl reductase and a point mutation at codon 93 substitutes a glycine to a valine. Enoyl reductase catalyzes the last step in each cycle of elongation of the type II fatty acid biosynthetic system. Glycine at position 93 is a part of the binding groove and a valine substitution alters the binding groove making triclosan unable to bind[17,18]. This mutation makes the bacteria 300-fold more resistant to triclosan

than wild-type bacteria[19].

Impermeability

Bacteria reduce the ability of the biocide to collect inside by preventing the entry and this is another mechanism of resistance. Gram-negative bacteria have a higher level of resistance than Gram-positive bacteria due to its outer membrane composition. The outer membrane contains liposaccharides which makes it more impermeable to biocides and accordingly, destroying the outer membrane makes it more susceptible[15,20]. For example, as a result of its outer membrane composition, *P. aeruginosa* is less susceptible to quaternary ammonium compounds (QACs) and chlorhexidine diacetate (CHA)[21]. The outer membrane of *P. aeruginosa* is a significant barrier for large molecules and it slows the rate of entry of small hydrophilic molecules making *P. aeruginosa* less susceptible to antibiotics such as β-lactams and quinolones. These antibiotics cross the cell membrane via porins, mainly oprF, that usually form trimers[22]. Loss of porins has been associated with antibiotic resistance and it is hypothesized that it will also lead to biocide resistance since it may prevent or slow the entry of biocides[22]. Addtionally, the outer membrane hydrophobicity is important, as the mycobacterial cell wall is hydrophobic and highly complex which makes it unsusceptible to many biocides[20].

Efflux Pumps

Transporting the biocide out of the microorganism is another mode of conferring resistance and is accomplished by efflux pumps. Efflux pumps, as opposed to the other mechanisms of resistance, is an active method of inactivating the biocide and do so by pumping the biocide out of the cell. While some are specific, others possess a wide range of substrate specificity meaning that they can affect a wide range of biocides. These are called multidrug transporters and are divided into two categories: secondary multidrug transporters and ATP-binding cassette (ABC)[23]. Secondary multidrug transporters use the gradient of protons or sodium ions to pump the toxic molecule out. While protons or sodium ions enter, the toxic molecule exits. Likewise, ABCs use ATP hydrolysis to drive the extrusion of the biocide[23]. For example, methicillin-resistant *S. aureus* is reported to have less susceptibility to the disinfectant chlorhexidine gluconate due to the efflux pumps encoded by *qacA, qacB and qacC* from the major facilitator superfamily[24]. The pump encoded by *qacA* confers resistance to biguanides such as chlorhexidine and diamidine such as pentamidine[25]. The pump encoded by *qacB* is different from *qacA* only by one amino acid at position 323 and has less resistance to biguanides and diamidine[25]. Finally, the pump encoded by *qacC* is accountable for resistance to quaternary ammonium compounds[25].

Biofilms

Biofilms may also promote the development of resistance. Biofilm is a layer of protein and polysaccharide produced by the bacteria and is a film that acts as shelter. The National Institute of Health states that about 65% of all infections are caused by biofilm formation[26]. They are commonly found on medical devices and tissue and are a major source of concern for diseases such as periodontitis, osteomyelitis and cystic fibrosis[27]. However, we will direct our attention to biofilms found on medical devices since this review is focused on disinfectants. For example, biofilms can form on central venous catheters either on the external surface or the lumen where Gram-negative bacteria can grow in intravenous fluids[27]. It has been shown that bacteria in biofilms are 10 to 100 fold more resistant to disinfectants than suspended bacteria[28]. There are a couple of mechanisms to explain resistance due to biofilm formation. First, due to this physiological protection, the biocide may be unable to penetrate the layers and reach the bacteria[29,30]. It was previously shown that chlorine was unable to reach more than 20% of a mixture of *Klebsiella pneumoniae* and *P. aeruginosa* biofilm, making it an ineffective disinfectant for biofilms[31]. Furthermore, when bacteria are starved, they enter a slow growth phase which is

associated with increased resistance. Biofilms are composed of slow growing bacteria and this may be the reason for higher resistance to biocides[31]. Additionally, biofilm environment is different for every strain since the surroundings change for each cell. This is known as heterogeneity which can result in varying responses to disinfectants and the rise of possible resistance[31]. Finally, bacteria in biofilms are in a high-density environment which activates the general stress response. RpoS is a specialized sigma factor that is expressed when the cell is undergoing stress. It was shown that RpoS is expressed by bacteria in cystic fibrosis patients with chronic *P. aeruginosa* biofilm infections which may be contributing to the increased resistance. RpoS activates genes required for the cell to sustain growth during the stationary phase and since they have also been observed in biofilms, it may be mediating protection against biocides[31,32]. It is evident that new measures need to be taken in order to eradicate the formation of biofilms on medical devices. If left untreated, these bacteria can proliferate and cause epidemics while being resistant to current interventions.

Possible Cross-Resistance

The misuse of disinfectants in households and in healthcare settings raises the question of whether this resistance will translate over to antibiotic resistance. It is an alarming reality that if a microorganism develops insusceptibility or resistance to a certain disinfectant, it may also be unresponsive towards antibiotics[3]. Cross-resistance occurs when the biocides induce cell death via the same pathway or target and since disinfectants have many targets, occurrence of cross-resistance to antibiotics is possible[3]. Moken *et al.* (1997) observed that pine oil, which is used as a disinfectant, may be selecting for *E. coli* that overexpresses the MarA protein and confers resistance prompted by certain antibiotics[33,34]. The MarA protein is a transcriptional activator of antibiotic and superoxide resistance promoters and provides *E. coli* with resistance to some antibiotics as well as superoxide-generating reagents[35,36]. In addition, they were able to show that low levels of cross-resistance does indeed occur[20]. Another example of cross resistance occurs when bacteria are exposed to hydrogen peroxide and hypochlorous acid. They undergo oxidative stress and turn on its oxyR radical defense systems. OxyR is involved in the expression of efflux pumps and detoxifying enzymes. The outcome is bacteria that are resistant to both hydrogen peroxide and hypochlorous acid as well as some antibiotics[3]. Furthermore, the inappropriate use of disinfectants may be promoting cross-resistance. The concentration of disinfectant, the contact time as well as the frequency of application is very important for proper sterilization. If sub-inhibitory concentrations are used, it will exert a selective pressure on the microorganism leading to the activation of stress responses. The end result of this would be a change in gene expression which will lead to superbugs that are resistant to disinfectants, while being unresponsive to antibiotics[37]. Finally, the biofilms that form as a result of improper disinfectant use may be creating bacteria that cannot be treated via antibiotics if infection in humans does occur[37].

A Step in Avoiding Resistance: Prior-Cleaning

A critical barrier that affects the efficiency of disinfectants is biofilm formation and other dirty materials because it hinders the biocide's ability to reach the microorganism. This fact makes prior cleaning crucial for proper sterilization[2]. Prior cleaning is the mechanical removal of inorganic or organic materials on a surface before the application of a disinfectant. For example, many medical devices such as surgical instruments require proper sterilization to avoid contamination and disease transmission. Surgical instruments are presoaked or rinsed before disinfection as prior cleaning. Disinfectants are unable to exert their full lethal effect when these inorganic or organic compounds are blocking it from reaching the bacteria and when they are exposed, the disinfectants are more effective. Since the disinfectant will be applied at full inhibitory concentration, as opposed to sub-inhibitory concentration, the chances of a

selective pressure driving resistance will also be lower[2]. Other measures that could decreases chances of resistance include using FDA-approved disinfectants at the proper concentration for the right amount of time and following the proper procedure for preparing the solutions[2]. Finally, it is crucial to avoid diluting the disinfectant too much as this may lower its efficacy[2].

Conclusion

Antibiotic resistance and its mechanisms have been extensively studied and there is a plethora of evidence for the alarming rise of resistance. The ability of bacteria to adapt to its changing environment and still sustain growth is the basis of this resistance. It is evident that action must be taken to avoid a disaster. Unfortunately, not enough antibiotics are entering the market and the current ones are becoming ineffective. Furthermore, while disinfectants are crucial for sterilization, they may also be selecting for resistant strains and therefore contributing to the rise of resistant strains. Disinfectants promote resistance in bacteria via target alteration by the microorganism, efflux pumps which pump the disinfectant out of the cell and impermeability of the bacterial cell wall. Biofilms are another concern for the emergence of resistance that should be taken into consideration.

It is evident that more research needs to be devoted to defining resistance to biocides as well as a better understanding of its mechanisms. Additionally, if biocides are properly used, the emergence of resistance can be also be avoided. Before biocides are marketed, a comprehensive study should be completed to determine if and how microorganisms confer resistance so that a better protocol for its use can be developed. There are still many questions left to be answered in determining if biocides are a real danger for our health. For example, can biocide resistance truly transfer as antibiotic resistance and what is the mechanism of this cross resistance? Understanding biocide resistance is key to controlling superbugs before they cause a catastrophe.

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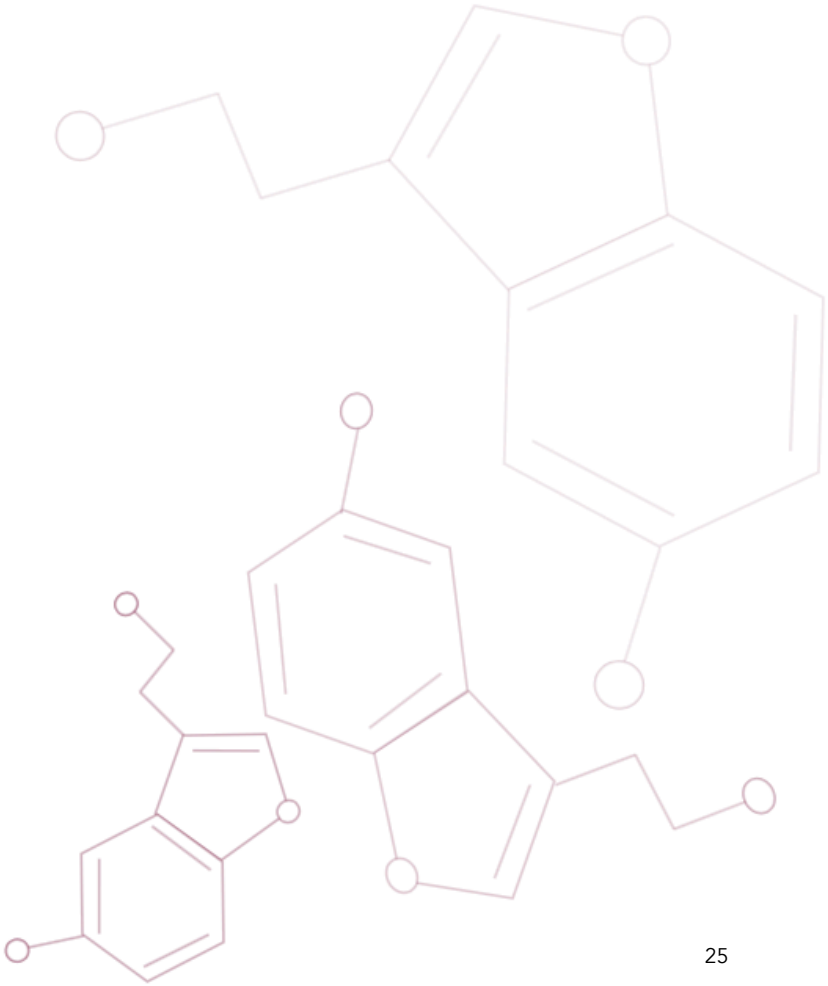

The need for alternatives to DMPA in Sub-Saharan Africa

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Abstract

Depo-Medroxyprogesterone Acetate (DMPA), or depo, is an injectable hormonal contraceptive, which through studies on sex workers in Sub-Saharan Africa has shown to increase the risk of human immunodeficiency virus (HIV)[1,2]. The uptake results in an increase in diversity within the vaginal microbiome[1]. Furthermore, the increase in diversity is responsible for the decrease in *Lactobacilli*, leading to increased inflammation and activation of T cells[1,2]. This is correlated with increased HIV-1 acquisition[1]. Unfortunately, the ease of access to DMPA makes it the most popular contraceptive method in Sub-Saharan Africa[1]. This multimedia submission is meant to educate peers about the issues associated with DMPA in an entertaining way. It also serves the purpose of raising awareness that more research is required along with financial aid in making safer and cheaper alternatives accessible to women of lower social status to protect them from being infected with HIV[3].

Video link: bit.ly/2VeAM5E

A2 GANG – DMPA ft. Dr. Jocelyn Wessels

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