


Oral Biology
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Lecture - 17
Dysbiosis


Welcome to the lecture cast on Dysbiosis.

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CONTENTS

- Microbiome & their interactions
- Dysbiosis - definition
- Types of dysbiosis
- Origin of dysbiosis
- Immune control of microbial homeostasis
- Impact of dysbiosis on host immune system



In this lecture, we are going to see about the microbiome and their interactions. Dysbiosis definition, types of dysbiosis, origin of dysbiosis, the immune control and microbial homeostasis, impact of dysbiosis on host immune system.

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- Dysbiosis in Systemic diseases
- Dysbiosis in Oral diseases
- Dybiosis in diagnosis
- Targeting dybiosis for therapy
- Future directions
- Conclusion



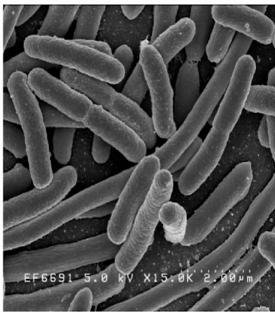


Dysbiosis in various systemic diseases and oral diseases, dysbiosis as a diagnostic tool, targeting dysbiosis for therapy and future directions and finally conclusion.

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Human Microbiome

- Definition - a characteristic microbial community occupying a reasonably well defined habitat with distinct physiochemical properties
- micro-organisms colonise the human body - account for half of total cells on a human body. Includes the collective of genomes from viruses, bacteria, archaea and fungi - composition are individual and site specific.
- Occupies skin, mucous membranes and intestinal tract of the human body - harboured in niches. (Sender et al , 2016).



Microbiome - microbiome is a characteristic microbial community occupying a reasonably well defined habitat with distinct physicochemical properties. Those microorganisms that colonise the human body are called human microbiomes. There are about 10 to 100 trillion microbial cells

associated with the human body. These include a collective of genomes from virus, bacteria, archaea, fungal and protozoal species. The composition of these microorganisms are different from person to person and they are site specific.

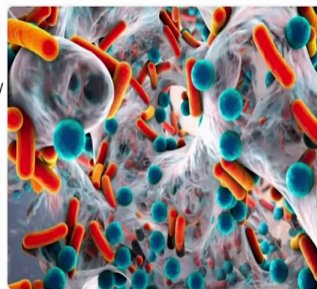
These occupy the skin, mucous membrane and intestinal tract of the human body, which are harbored in various niches. The term microbiota and microbiome is being used interchangeably. while the microbiota represents the type of microorganisms present in a specific environment, Microbiome includes the microorganisms along with the genomes inside a particular ecological niche.

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Human Microbiome



- The diverse microorganisms form a complex ecosystem that thrive in the dynamic body environment in a symbiotic relationship with each other and human host = Eubiosis.
- The healthy microbial community characterised by diversity, stability, resistance and resilience.



Each microbial flora has the characteristic set of microorganisms, the diverse microorganisms form a complex ecosystem that thrive in an ever changing environment. They interact with each other and the human host in a symbiotic or a mutually beneficial relationship. This harmonious state is called Eubiosis.

The healthy microbial community is characterized by diversity that is, more than thousand different species co-exist in the microbial flora, the stability - to withstand the forces against the removal, a resistance - against the pathological microorganisms colonization and resilience

when they are subjected to perturbations or change they strive to gain back the equilibrium or the normal composition.

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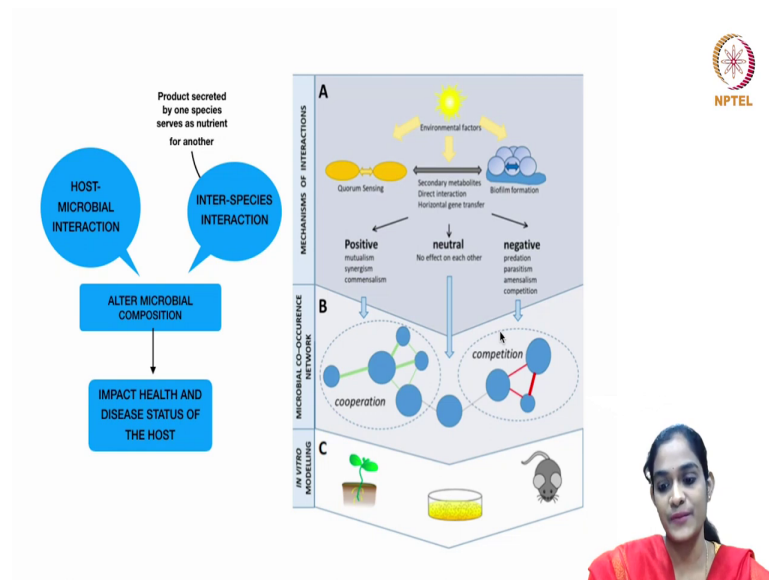
Human Microbiome

- Mucosal microbiome interface - balanced interplay between defence mechanisms of the immune system and symbiotic microbial factors, such as microbial antigens and metabolites.
- the study of metabolomics aims to identify and quantify all the small molecule, microbial-produced metabolites - unravel the dynamic nature of the metabolic function of a microbial community and understand how it influences its human host (Cenit et al, 2014)

The diagram illustrates the human microbiome interface. At the top, 'Immune Exclusion: Neutralization by IgA' is shown. Below, 'Tolerogenic exposure to antigen' leads to 'DC' (Dendritic Cell) activation. 'Oral Microbiome' and 'Oral Excluders' are shown interacting with 'Alpha Secretion' and 'Secretion of IgA to plasma cells'. 'Lactate Permea' is also indicated. The bottom section shows 'Synergism' (Cooperation, DNA transfer, Metabolite exchange), 'Signaling' (Cytokines, At-2), and 'Antagonism' (Low pH, H⁺, Bacteriocins, H₂O₂).

The Mucosal microbiome interface is always a balance between the defense mechanisms of the immune cells and the symbiotic microbial factors, such as microbial antigens and metabolites. The study of metabolomics aims to identify and quantify all the small molecules and microbe produce metabolites, which tells us the dynamic nature of the metabolic functions of a particular microbial community and helps us understand how it influences the human host.

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The microbial composition at a particular flora is being determined by the interspecies interaction and the host microbial interaction, which impacts the health and the disease status of the host. The diverse microorganisms co-aggregate into structured communities, which are embedded or enclosed into a polymeric extracellular matrix forming a biofilm. The molecules present in the biofilm inherit the group behavior called the quorum sensing.

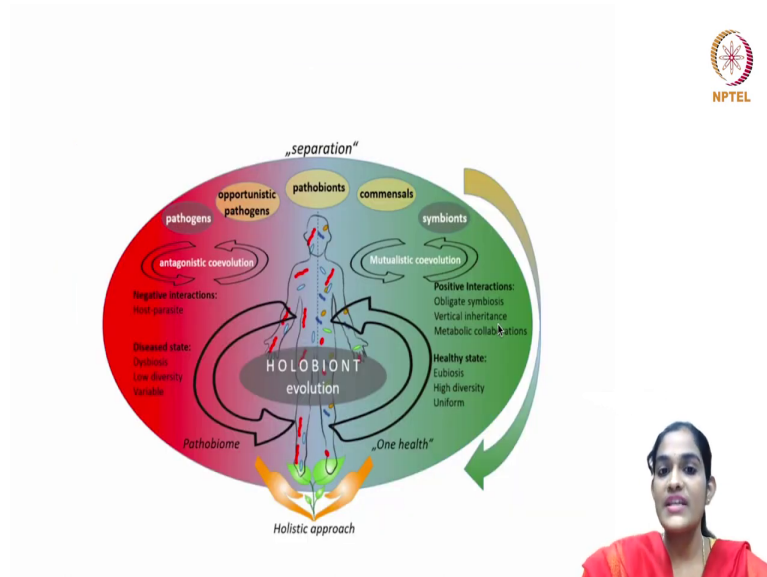
There are three different types of interaction happening in between the microorganisms of different species, genera and family. They include the positive interactions, negative interactions and neutral interactions. The positive interactions are mutualism, synergism, and commensalism.

Mutualism is an interaction happening between two or more species where both the species are benefited whereas, a commensalism is a long term interaction where one of the species is benefited from the other whereas, the other is neither benefited or harmed from the interaction.

In the neutral interaction, there is no effect seen due to the interaction. The negative interaction includes predation, parasitism, amensalism and competition. This is an asymmetric interaction where one of the species predominates and leaves the other weaker or smaller species bereft of the nutrition or resource; they can even get rid of the other species with the help of chemical secretion.

We can see in the picture the positive interactions cause cooperation between the microorganisms leading to a harmonious relationship whereas a negative interaction leads to a competitive behavior causing the survival of only a single species and less of diversity.

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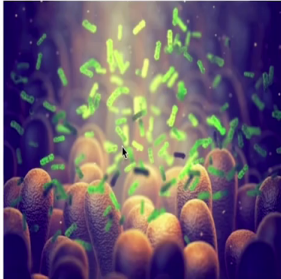


A holistic concept called holobiont has evolved, which treats the host and the microbiome as a single unit. The disease state of the holobiont includes dysbiosis along with low diversity and variability of the associated microbiota, whereas the health state includes the eubiosis with high diversity of the microorganism.

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Dysbiosis

- Compositional and functional alteration in the microbiota driven by set of environmental and host-related factors that perturb the microbial ecosystem to an extent that exceeds its resistance and resilience capabilities.
- dysbiosis is defined as “any change to the components of resident commensal communities relative to the community found in healthy individuals”



Perturbations or the changes that disturb the equilibrium of the ecosystem can cause the outgrowth of species with pathological potential, this deranged state is called Dysbiosis. Dysbiosis is defined as the compositional and functional alteration in the microbiota driven by a set of environmental and host related factors that perturb the microbial ecosystem to an extent that exceeds its resilience and resistance capabilities. It is the change you see in the components of resident my commensal communities in relation to what you see in a normal healthy flora.

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Types of dysbiosis



Moving on to the categories of dysbiosis.

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Bloom of pathobionts

- Commensal microbiota that have the potential to cause pathology - pathobionts.
- low relative abundances but proliferate when aberrations occur in the intestinal ecosystem.
- Increase with genetic defects in immune functions.
- Eg : polymorphism in TLRs - Outgrowth of Enterobacteriaceae seen in IBD




The first category is the bloom or expansion of pathobionts. Pathobionts are the commensal microbiota that have the potential to cause pathology; usually these organisms are less abundant in the microflora, but they proliferate when there is abrasion occurring in the intestinal ecosystem as in case of an infection or an inflammation.


These also increase with the genetic defects in the immune function. for example, if there is a polymorphism in toll like receptor, which senses the bacterial organisms present across the mucosa - there is an outgrowth of pathological proteolytic bacteria, such as Enterobacteriaceae, which leads to colitis as you see in inflammatory bowel diseases.


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Loss of commensals

- Reduction or complete loss of commensals - consequence of microbial killing or diminished bacterial proliferation.
- Diminished development of maturation of immune homeostasis.
- Altered protection against pathogen & tolerance to* commensal organism & self antigens.







The second category of dysbiosis includes the loss of beneficial microorganisms. The reduction or complete loss of commensals can be the consequence of microbial killing or diminished bacterial proliferation. These commensals are not passive bystanders, they are actively involved in the functions of the host like vitamin synthesis, metabolism, maintenance of the circadian rhythms. The important role played by these good bacterias are the development and maturation of the immune homeostasis.


For example, you have the bacteroides fragilis species and the clostridium species, which are important in development and maturation of the regulatory cells, which prevents the development of inappropriate inflammatory reactions against the body's own antigens and their own commensal bacteria.

The organisms such as lactobacillus acidophilus have the direct anti-inflammatory action by neutralizing the inflammatory cytokines. The absence of these commensals can lead to the


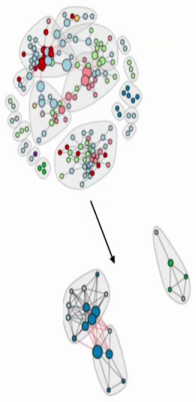
alteration or the loss of balance in the protection against the pathogen and tolerance to commensal organism and self antigen.

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Loss of diversity



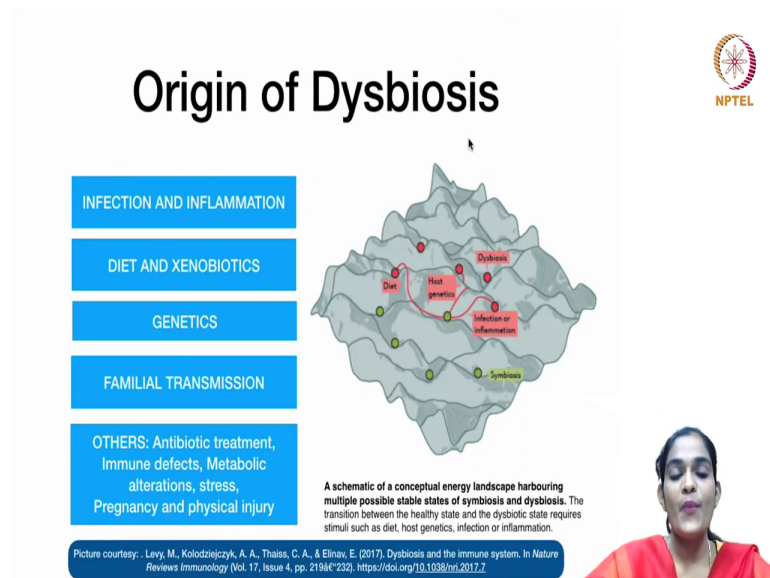
- Reduction in microbial diversity - a recurrent characteristic of disease-associated dysbiosis.
- Provides overlapping protection by multiple organisms in prevention of Non-communicable diseases
- Eg: 30 species of clostridium spp. More effective in stimulating T reg than single species



The third category of dysbiosis is the loss of diversity. Usually, the microorganisms exist as a poly microbial community; the reduction in the microbial diversity is a recurrent characteristic of disease associated dysbiosis. It is also evident in various diet associated dysbiosis along with the various autoimmune disorders like type 1 diabetes.

The microbial diversity provides the overlapping protection by the multiple organisms in prevention of non-communicable diseases. For example, 30 different species of clostridia are more effective in stimulating T regulatory cells than single species of clostridia.

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Moving on to the origin of dysbiosis - there are various causative factors implicated in dysbiosis they are infection, inflammation, diet, xenobiotics, genetics, familial transmission, antibiotic treatment, immune defects, metabolic alterations, stress, pregnancy and physical injury.

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Infection & Inflammation

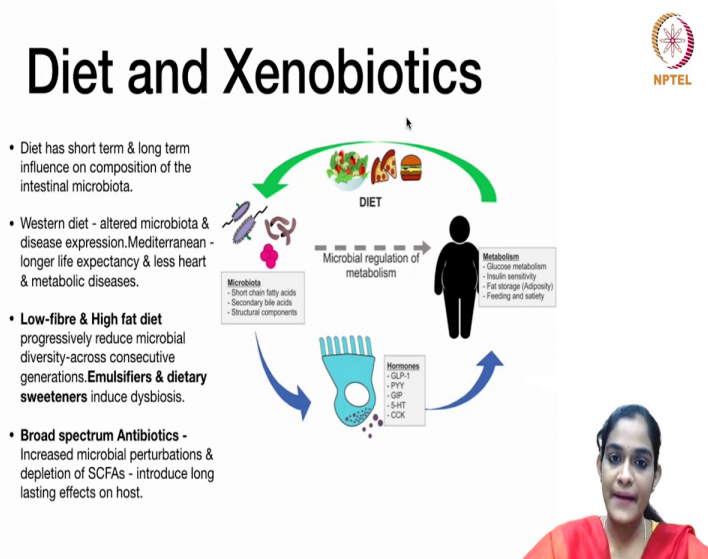
- Infection with Inflammation - compromise the microbiota's ability to provide colonisation resistance
- Pathobionts like enterobacteriaceae establish.
- Molecular mechanisms including release of nutrients, use of metal ions, inter microbial competition and horizontal gene transfer , exploitation of AMP.

Infection and inflammation is a very important causative factor for dysbiosis. Infection with inflammation can compromise the microbiota's ability to provide colonial resistance.

As a consequence, pathological microorganisms like enterobacteriaceae establish themselves, through the use of nutrients, which are released from the dead and dying epithelial cells.

You have the various production and release of chelating agents such as siderophores which attract the iron ions that are necessary for their own colonization. They compete with the other microbial species, release the bacteriocin, which is responsible for getting rid of phylogenetically related organisms. They use the horizontal gene transfer to acquire various virulence factors to thrive in an inflamed gut. they exploit and neutralize the anti microbial peptides produced by the host.

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Diet has a major impact on dysbiosis, it has a short term and a long term influence on the composition of intestinal microbiota. Evidence implicates the western diet with the altered microbiota and disease expression while the mediterranean diet with prolonged life expectancy and less heart and metabolic diseases.


The microbiota when they act on dietary fibers and the protein release certain metabolic waste, such as the short chain fatty acids. These along with the secondary bile acids and the structural components, trigger various hormones, which help in the metabolism of glucose, insulin sensitivity, fat storage, feeding and satiety feeling.

When there is a derangement of these metabolism along with altered dietary functions, you have the deranged or different dysbiotic microbial community occupying the flora. Low fiber and high fat diets progressively reduce the microbial diversity. The diet high in saturated fat produces more of taurocholic acid, which preferentially helps in the growth of organisms, such as *Bilophila wadsworthia*, which is a pathobiont that favors TH 1 type of inflammatory reaction leading to colitis and colorectal cancer.


Dietary emulsifiers and dietary sweeteners also induce dysbiosis. Broad spectrum antibiotics cause a change in the composition and function of the intestinal microbiota; they cause depletion of short-chain fatty acids which also induce the long lasting effect on the host.

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Familial transmission




- Early succession of intestinal microbial colonization after birth is determined by the **maternal microbiota**.
- Mode of delivery & Feed influence microbiota.
- **Environmental transmission** - additional importance, as households feature characteristic microbiome signatures.
- Both familial and environmental microbiome transmission may be of **phenotypic importance** in some disorders.



The early colonization of the human microbiome occurs as soon as a child is being born. The neonatal microbiome is determined by the maternal microbiota, the mode of delivery and the feed the child. The evidence shows that those babies born through the vaginal delivery have good bolus of microbiota whereas, this process has been inhibited or the disturbed by the cesarean section whereas those babies who are born of vaginal delivery have a good bolus of microbiota, which is been interrupted by the cesarean section.

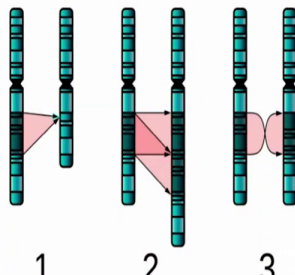
Those babies fed with breast milk have far higher diversity of the microbial flora compared to those who are formula fed. Environmental transmission has an additional importance as households feature characteristic microbial signatures. Both familial and the environmental microbiome transmission can be of phenotypic importance in some disorders; that the transmissible component is being introduced into a non communicable disease through the environmental microbiome transmission.

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Genetics

- microorganisms that colonize the human body is inherited (Frankenfeld et al.2004; Li et al. 2007; Goodrich et al. 2014). **host genetics** are involved in shaping the composition of the intestinal microbiota
- Genome wide association studies have linked several genetic loci with microbial taxa and functional pathways.
- Eg: Association of *Bifidobacterium* genus and the human gene locus that encodes lactase.
- Locus encoding human vitamin D receptor, and other human loci involved in immune and metabolic functions, were highlighted as potential drivers of microbial control through host genetics.
- Genetic influence on microbial composition may be involved in the **manifestation of certain phenotypes**, as demonstrated for *Christensenellaceae* and low body mass index.



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Microorganisms that colonize the host body are inherited . Host genetics are involved in shaping the composition of the intestinal microbiota. The genomewide association studies have linked many loci with the microbial taxa and the functional pathways; the loci related to the human vitamin D receptor and various other immune and metabolic functions are highlighted as the potential drivers of microbial control through host genetics.

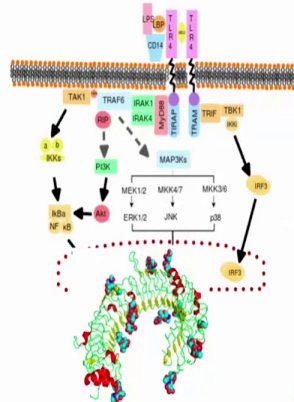
The genetic influence on the microbial composition may be involved in the manifestation of certain phenotypes. Have you all wondered why certain people take less of a food, but are obese while certain other people who take a lot of food do not put on weight. The type of microbiota inherited by these people is the major cause. For example, those inherited with the Christensenellaceae family are associated with low body mass index.

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Immune control of microbial homeostasis



- Host shapes the configuration of normal and dysbiotic microbiome by means of the immune system. Also the gut microbial composition affects local and systemic immunity
- Genetic defects in **recognition and response pathways** to identify microbes - altered microbial colonisation or **misrecognition** of normal microbiota - lead to disease, e.g.: pattern recognition receptors
- Mutations in gene involved in **immune regulatory mechanisms or pro-inflammatory pathways** - cause unrestrained inflammation.
- **Cytokine**/**polymorphism** select and favour growth of specific component in biofilm.



Host shapes the configuration of normal and dysbiotic microbiome by means of the immune system in turn the gut microbial composition affects the local and systemic immunity. Genetic defects in the recognition and response pathways to identify the various microbes can alter the microbial colonization or misrecognize the normal microbiota leading to disease.

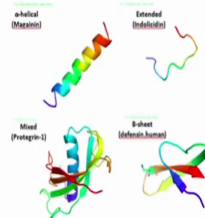
The defect in pattern recognition receptors can lead to increased mucosal associated bacteria leading to colorectal cancer and prolonged inflammatory conditions. Mutations in the gene involved in immune regulatory mechanisms or pro inflammatory pathways cause unrestrained inflammation. The cytokine polymorphism selects and favors the growth of specific components in the biofilm.

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Immune control of microbial homeostasis



- Paneth cells express **AMP** alpha-defensins - essential regulator of intestinal ecology.
- Reduction of IL-22 - Reduced AMP - expansion of SFB populations & systemic colonisation with pathogenic commensals.
- Commensal microbiota derived metabolites - stimulate NLRP6 associated **inflammasome**- secrete mucous and AMP- regulate microbial composition.



The paneth cells express antimicrobial peptides, alpha defensins which is an essential regulator of intestinal ecology. When there is reduction in Interleukin-22, these antimicrobial peptides are produced less leading to expansion of segmented filamentous bacterial population and systemic colonization with pathogenic commensals.

The commensal on their own help produce metabolites, which stimulate the NLRP6 associated inflammasome, that secrete the mucus and other antimicrobial peptides to regulate the microbial composition. When these commensals are destroyed or disturbed, you have the altered immune response in the mucosa.

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Adaptive Immune system in regulation of microbial composition



- **slgA** - specific to bacteria and their functions such as flagella production. In their absence, serum LPS higher, expansion of SFB & anaerobic colonisation.
- **T follicular helper cells** express inhibitory receptor PD1 (programmed cell death protein 1) - deficiency leads to altered microbial composition - ↓ *Bifidobacterium*
↑ *Enterobacteriaceae*.
- **Invariant natural killer T (iNKT) cells** - respond to wide range of microbial glycolipids.
- Others: MHC class 1 like molecule (CD1d) and intraepithelial lymphocytes.



The various adaptive immune responses, which helps in the regulation of microbial composition are secretory IgA, which are seen floating on the mucosal surface. These are specific to the bacteria and their function, such as flagella production; in the absence of these secretory IgA, there is increase in the serum lipopolysaccharide (specific to gram negative bacteria) along with expansion of segmented filamentous bacteria and anaerobic colonization.

The T follicular helper cells express the inhibitory receptor PD1, (programmed cell death protein 1). The deficiency of these cells can lead to altered microbial composition; the less of good bacteria such as *Bifidobacterium* and more of proteolytic bacteria, such as the *Enterobacteriaceae*.

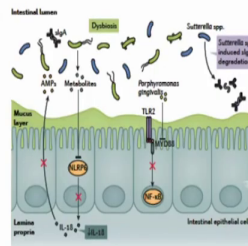
Invariant natural killer T cells respond to a wide range of glycolipids; absence of these cells can again alter the microbial composition. Other mechanisms which help in the processing and presentation of the various microbes and pathogenic stimuli across the mucosa to the immune system, include MHC molecule class 1 and the intraepithelial lymphocytes. Any defect or polymorphism in these proteins and cells can cause altered microbial composition across the mucosa.

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Impact of dysbiosis on host immune system



- Dysbiotic microbial community, once established, substantially **affects both the local mucosal and systemic landscape of immune cells** - important for maintenance of dysbiotic state.
- Microbiota uses various signals and mechanisms to **affect immune activation** including **epigenetic remodelling and altered gene expression**.



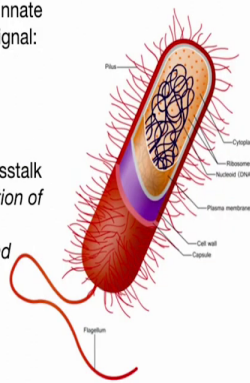
How does dysbiosis impact the host immune system? The dysbiotic microbial community, once established, substantially affects both the local mucosal and systemic landscape of immune cells, which is important for their own survival and also the maintenance of the dysbiotic state. The microbiota uses various signals and mechanisms to affect the immune activation including the epigenetic remodeling and altered gene expression.

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Signalling to Immune system



- Microbiota can influence the host innate immune system via two types of signal: **microbial cell components and metabolites**.
- inhibit antimicrobial response and maintain inflammation through crosstalk
Eg : *P.Gingivalis* promote degradation of MYD88 -
Sutterella species- degrade IgA and associated stabilising peptide
Interaction between TLR2 and complement receptor c5aR.



The microbiota can influence the host innate immune system via two types of signals - their own microbial cell components and the metabolites, which inhibit the antimicrobial response and maintain the inflammatory state through their crosstalk. Example, the *P. Gingivalis* promotes the degradation of MYD88 protein, which is an adapter protein associated with the toll-like receptor.

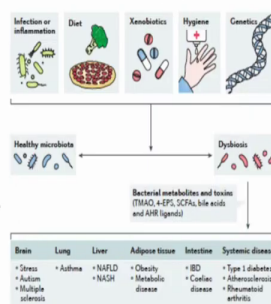
When these proteins are being degraded there is absence of bacterial sensing which can lead to the colonization of pathological microorganisms. The *Suterella* species degrade IgA and associated stabilizing peptides; when there is absence of these antibodies on the surface, the dysbiotic state is prolonged. The interaction between toll-like receptor 2 and the complement receptor c5aR can lead to altered phagocytic and microbial killing.

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Dysbiosis and disease development



- Dysbiosis associated with immune-mediated diseases and cancer.
- Causal contribution- demonstrated by prospective cohorts, interventional trials (109 trials), pre-clinical studies.
- Eg: *P. gingivalis* with RA
EBV with SLE and SS.

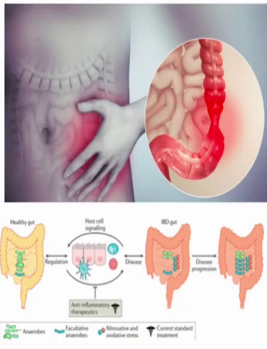


The aberrant interactions of the microorganisms with the host immune system in a genetically predisposed individual can lead to a plethora of immune-mediated diseases and cancer. So, the causal contribution is being demonstrated by the prospective cohorts, interventional trials; almost 109 trials have been registered and the various pre-clinical studies in the experimental animals and the human models. For Example, *P. gingivalis* have been causally associated with rheumatoid arthritis and the Epstein-Barr virus associated with systemic Lupus erythematosus and systemic sclerosis.



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Dysbiosis in Inflammatory Bowel Disease (IBD)

- Chronic relapsing inflammation of intestinal mucosa and extra-intestinal organs.
- Dysbiosis in IBD involves decrease in frequency of **butyrate producing bacteria with increased sulfate reduction** - ↑ epithelial permeability ↑ bacterial translocation.
- Decreased **Firmicutes** and bacteroids - Increased intestinal inflammation



Picture courtesy : Schirmer, M., Garner, A., Vlamakis, H. et al. Microbial genes and pathways in inflammatory bowel disease. *Nat Rev Microbiol* 17, 497–511 (2019). <https://doi.org/10.1038/s41579-019-0458-8>



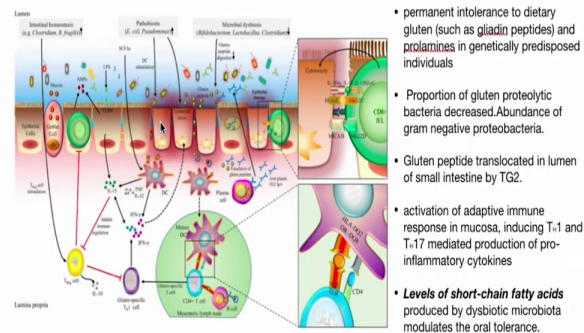
Moving on to the dysbiosis in various systemic diseases... First we will see the association in inflammatory bowel disease. Inflammatory bowel disease has chronic relapsing inflammation of intestinal mucosa and extra-intestinal organs. Dysbiosis in IBD involves decrease in the frequency of butyrate producing bacteria with increase in the sulfate reducing bacteria.

The butyrate is an important energy source for the epithelial cells to protect the epithelial barrier against the entry of the pathogen. So, when the butyrate is being produced less, epithelial permeability is more leading to increased translocation of bacteria with pathological potential.

There is the sulfate reducing bacteria, which converts the sulfate into hydrogen sulfide, which is a toxic molecule that reduces the phagocytosis and killing of pathological organisms. In the IBD, you have decreased Firmicutes and Bacteroides configuration. This altered ratio produces compromised conjugation of bile acids; this destroys the bile acid mediated reduction in the intestinal inflammation leading to an increase in colitis.

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Dysbiosis in Coeliac disease



Picture courtesy: Akobeng, A.K., Singh, P., Kumar, M. et al. Role of the gut microbiota in the pathogenesis of coeliac disease and potential therapeutic implications. *Eur J Nutr* 59, 3369–3390



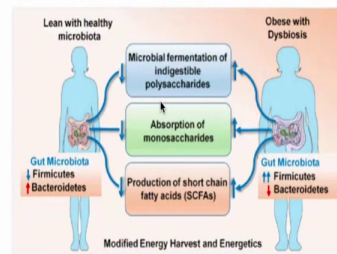
Coeliac disease is the autoimmune disease triggered by ingestion of dietary gluten. There is a permanent intolerance to dietary gluten such as gliadin peptide and prolamines in genetically predisposed individuals. The proportion of gluten specific proteolytic bacteria is decreased in these individuals with abundance of gram negative proteobacteria.

These undigested gluten are transported through the lumen inside the lamina propria with the help of a protein called transglutaminase 2; dendritic cells in the lamina propria sense these gluten, activate or stimulate the adaptive immune response in the mucosa inducing the T_H 1 and T_H 17 mediated production of pro-inflammatory cytokines.

The levels of short-chain fatty acids are also reduced in these individuals, because of which formation of T regulatory cells are disturbed. Overall, there is an increased inflammatory condition. The prolonged inflammatory condition can lead to the destruction of epithelial cells and contribute to the chronicity of the disease.

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Dysbiosis in obesity



- ↓ Butyrate producing bacteria ↑ Firmicutes
- ↓ SCFA - Decreased satiety and inhibit accumulation of fat in adipose tissue
- Obese microbiota - increased bacterial antigen translocation - chronic inflammation & impaired metabolic functions such as insulin resistance.

Picture courtesy 'Microbial Alterations and Risk Factors of Breast Cancer: Connections and Mechanistic Insights, Cells 2020, 9, 1091; doi:10.3390/

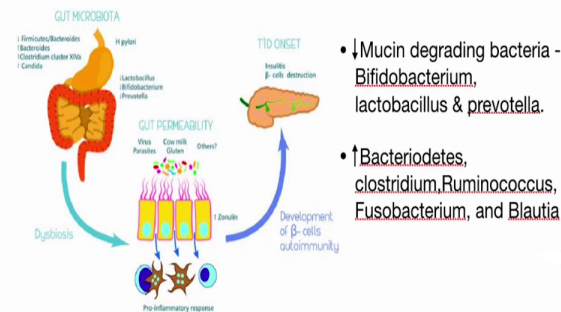


Obesity is a metabolic disorder that is on rise worldwide. These individuals have less of the butyrate producing bacteria, which is important in the breakdown and fermentation of dietary polysaccharides. There is decreased absorption of monosaccharides in these individuals and consequently followed by lack of fermentation and production of short-chain fatty acids.

Short-chain fatty acids, under normal conditions act through the fatty acid receptor and stimulate certain hormones. They stimulate the satiety feeling in the brain; when these are at loss, you have decreased satiety. The short-chain fatty acids also lead to the increased oxidation of fatty acids in the adipose tissue; thus they would not let the fat get accumulated in the adipose tissue. The obese microbiota can cause increased bacterial antigen translocation leading to chronic inflammation and impaired metabolic functions, such as the insulin resistance.

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Dysbiosis in Diabetes mellitus



Picture courtesy : Stefano Bibbò, Maria Pina Dore, Giovanni Mario Pes, Giuseppe Deltala & Alessandro P. Deltala (2017) Is there a role for gut microbiota in type 1 diabetes pathogenesis?



Diabetes is a very common rising chronic disorder. There are two types of diabetes : type 1, which is an autoimmune disorder due to reduced insulin production. Type 2, caused by insulin resistance. Dysbiosis is seen in both of these types. You have decreased mucin degrading bacteria, such as Bifidobacterium, Lactobacillus and Prevotella and rise in pathobionts such as Bacteroides, Clostridia Fusobacterium, Blautia, Ruminococcus.



So, these bacteria cause altered epithelial permeability. When there is an altered permeability, various antigens traverse through the lumen causing pro-inflammatory response and development of autoantibodies against the beta cells of pancreas.

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Dysbiosis in Asthma

- Germ-free conditions and early-life antibiotic exposure associated with increased susceptibility to allergy and asthma.
- Microbiota through B cell-intrinsic MYD88 signalling limits serum IgE levels and basophil abundance.
- **Absence of microbiota -preferential isotype switching to IgE** (rather than IgA) - supports allergic inflammation.
- **Treg cells were reduced** in vancomycin treated mice.

Picture courtesy : Salameti et al. The role of gut microbiota in atopic asthma and allergy. Scand J Immunol. 2020;91:e12855.



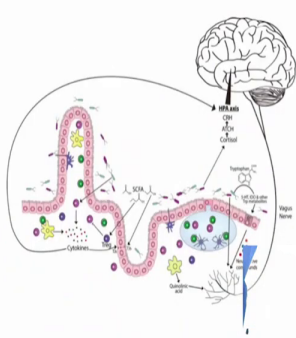
Dysbiosis in asthma - children grown in germ free conditions and those exposed to antibiotics in the early-life are associated with increased susceptibility to allergy and asthma. The commensal microbiota through the B cell-intrinsic of LMYD88 signaling limits the conversion of serum antibodies to IgE type and lessen the basophil abundance, but there is an absence of these microbiota there is a preferential switch over to the IgE class, which supports the allergic inflammation.

Those experimental models, where the mice have been treated with vancomycin - production of T regulatory cells development was shown to be affected . So, these antibiotics kill the microorganisms, which helps in the development and maintenance of T regulatory cells.



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Dysbiosis in Autism

- Gut microbiota and its metabolites affect CNS via gut-brain axis (GBA).
- Synthesis of neurotoxins interfere with neurodevelopment.
- Replenishment of diminished commensal bacteria has proved effective. Eg : Autistic experimental models, replenishment of *Lactobacillus reuteri* and *Bacteroides fragilis* - reduced disease severity.



Picture courtesy : Hughes, H.K., Rose, D. & Ashwood, P. The Gut Microbiota and Dysbiosis in Autism Spectrum Disorders. *Curr Neurol Neurosci Rep* 18, 81 (2018). <https://doi.org/10.1007/s12275-018-9700-0>

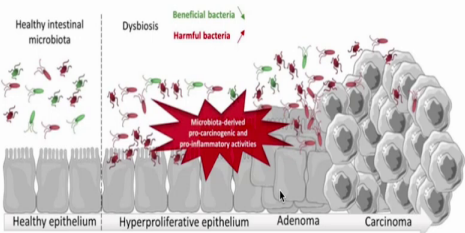


Dysbiosis in autism - the gut microbiota and its metabolites affect the CNS functioning via the gut-brain axis. The characterized microorganisms present in these individuals, produce neurotoxins which cross gut brain barriers and interfere with the normal neural development.


Thus affecting the behavior and brain chemistry in these kids. The replenishment of diminished commensal bacteria has been effective. For example, in the experimental autistic models, replenishment of *Lactobacillus reuteri* and *bacteriologist fragilis* had reduced the disease severity.

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Oncobiosis





- Microbial dysbiosis associated with neoplastic diseases are termed **oncobiosis**. **Oncobiome**- characterised in various cancers.
- **Oncobiomes** from long and short term survivors are different.
- Oncobiosis drives carcinogenesis, metastasis and poor survival - characterised in Pancreatic, ovarian and breast cancers.
- Bacterial metabolites and functional metabolisms have role in microbiome driven pathogenesis of cancers .




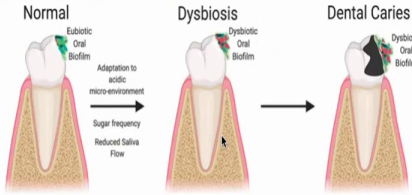
Oncobiosis, dysbiosis in cancer - the microbial dysbiosis associated with neoplastic disease are termed oncobiosis; Oncobiome are the microorganisms that are characterized in various cancers. Oncobiomes from long and short term survivors are different in various experimental animal tumor models and the transfer of oncobiome from the long term survivor has induced prolonged survival in the organism. The oncobiosis drives carcinogenesis, metastasis and the poor survival, which has been well characterized in cancers such as pancreatic ovarian breast and the oral cancers too. The bacterial metabolites and the functional metabolisms have a role in microbiome driven pathogenesis of these cancers.

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Dysbiosis in oral cavity



- Dental caries - infection of specific bacteria present in dental plaque - *S. Mutans* and *Lactobacillus*.
- In susceptible host, Oral microorganisms convert carbohydrates into acids resulting in demineralisation of teeth.
- Mineral loss increases enamel porosity, softening of surface - increased diffusion of acids - invades dentin and pulp.



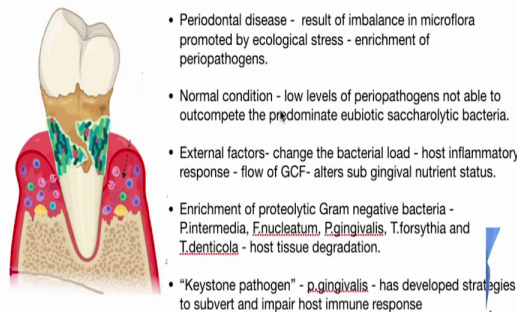
Dysbiosis in the oral cavity - Common oral lesions are associated with dysbiosis. Dental caries is a common poly microbial disease. This is characterized by the infection with specific bacteria, such as the streptococcus mutans and Lactobacillus. In a susceptible host ingesting sugars frequently, streptococcus mutans ferment the carbohydrates into acids (preferentially lactic acid), which causes the demineralization of the teeth.

The mineral loss initially causes the enamel porosity and the softening of the subsurface. So, this increases the diffusion of the acids. Progressively, the microorganisms invade the dentine and the pulp leading to bacteremia. In the picture, you can see the eubiotic or oral biofilm where there is a poly microbial community.

When they are subjected to an acidic microenvironment created by a high sugar fermentation and the co-factor such as reduced salivary flow, there is the development of dysbiotic oral film. This altered ecosystem is rich in organisms, such as streptococcus mutans, lactobacillus acidophilus and actinomyces viscosus. So, these dysbiotic oral films can lead to the progression of the dental caries, affecting the pulp and creating the bacteremia.

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Dysbiosis in oral cavity



Another lesion commonly encountered in the dental setup is periodontal disease which results in imbalance in microflora, promoted by ecological stress enriched with the perio pathogens. Under normal conditions, the levels of perio pathogens are not able to out compete the predominant eubiotic saccharolytic bacteria.



When there is external factors (such as plaque and calculus), change in the bacterial load induces the host inflammatory response. When there is increased flow of the gingival crevicular fluid. This exudate alters the sub gingival nutritional status and causes proliferation of the proteolytic gram negative bacterias since these feed in the anaerobic environment.

The proteins released in the inflammatory environment, have the preferential growth towards the pathogenic organisms such as *Prevotella intermedia*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*. These organisms destroy the periodontal ligament causing the host tissue degradation.


The keystone pathogen theory focuses on a single pathogen, which is important in creating the whole of the dysbiosis. The *P.gingivalis* has been portrayed as the keystone pathogen which has developed strategies to subvert and impair the host immune response along with changing the entire oral biome into a dysbiosis.

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Dysbiosis in oral cavity



- Periodontal disease - result of imbalance in microflora promoted by ecological stress - enrichment of periopathogens.
- Normal condition - low levels of periopathogens not able to outcompete the predominate eubiotic saccharolytic bacteria.
- External factors- change the bacterial load - host inflammatory response - flow of GCF- alters sub gingival nutrient status.
- Enrichment of proteolytic Gram negative bacteria - *P.intermedia*, *F.nucleatum*, *P.gingivalis*, *T.forsythia* and *T.denticola* - host tissue degradation.
- "Keystone pathogen" - *p.gingivalis* - has developed strategies to subvert and impair host immune response



Another lesion commonly encountered in the dental setup is periodontal disease which causes imbalance in microflora, further promoted by ecological stress and enrichment of periopathogens. Under normal conditions, the levels of periopathogens are not outcompeted by the predominant eubiotic saccharolytic bacteria.

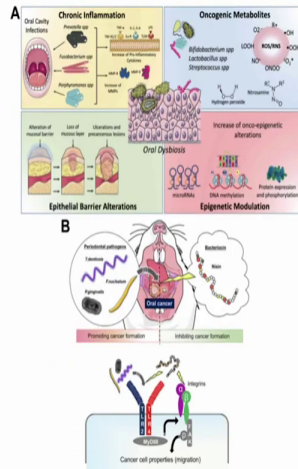
When there is external factors such as plaque calculus along with change in the bacterial load, it induces the host inflammatory response. Increase in flow of the gingival crevicular fluid (an exudate) changes the subgingival nutritional and causes the increased proliferation of proteolytic gram negative bacteria since these feed in the anaerobic environment.

The organisms include the *Prevotella intermedia*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*. These organisms are pathogenic and they can destroy the periodontal ligament causing the host tissue degradation.

The keystone pathogen theory focuses on a single pathogen, which is important in creating the whole of the dysbiosis. The *P.gingivalis* has been portrayed as the keystone pathogen which has developed strategies to subvert and impair the host immune response along with changing the entire oral biome into a dysbiosis.

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Dysbiosis in oral cancer



Dysbiosis in oral cancer has been well associated recently. Species such as prevotella, fusobacterium and porphyromonas can trigger the pro-inflammatory micro environment wherein the inflammatory cytokines and the matrix metalloproteinases are produced, which favors the development and progression of the tumor. The various oncogenic metabolites are produced by species, such as bifidobacterium, lactobacillus and streptococcus.

The reactive oxygen species and nitrogen species produced along with nitrosamines induce the genetic defects causing mutations responsible for induction and progression of cancer. The various other mechanisms include alteration of the epithelial barrier by these organisms, which can cause the occurrence of precancerous lesions not only that they induce various epigenetic alterations such as the increase in the oncogenic microRNA, DNA methylation and protein phosphorylation.

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Oral Dybiosis in systemic diseases



- Dysbiotic oral bacteria associated with dental diseases have access to blood stream - causing bacteraemia.
- Bacteria in circulatory system lead to colonisation of other host tissue - associate with various systemic diseases.
- patients with periodontitis have 25% higher risk of atherosclerotic plaque formation.
- *Campylobacter rectus*, *P.gingivalis*, *P.intermedia*, *P.endodontalis*, *Prevotella nigrescens* - oral pathogens unique to atherosclerotic plaque.
- Mechanism in pathophysiology - bacteraemia from pathogenic oral microbiome invades arterial wall - Promote plaque formation. Release of specific bacterial toxins with proatherogenic effects.



Mainly it is surprising that many of the systemic diseases are evidenced with oral dysbiotic organisms, how is it so? the dysbiotic oral bacteria associated with various dental diseases such as pulpitis or periodontitis gain access to the bloodstream causing bacteremia.

The bacteria in the circulatory system lead to the colonisation of other host tissue thus they associate with various systemic diseases. The common examples are atherosclerosis, alzheimers disease type 2 diabetes and the pregnancy complications. The patients with periodontitis have 25% of higher risk of atherosclerotic block formation.

The five different types of oral pathogens are unique to atherosclerotic plaque; these organisms gain the entry to plaque with the help of bacteremia. They invade the arterial wall and promote plaque formation there. They release various specific bacterial toxins with proatherogenic effects.

Apart from atherosclerosis you have the treponema species, which is more commonly associated in Alzheimer's disease and you have various periodontal pathogens, which are associated with diabetes.

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Dybiosis in diagnosis



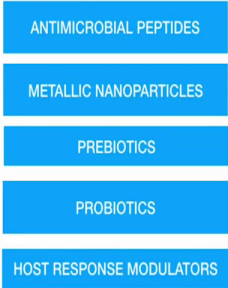

- Information regarding the state and function of microbiota utilised for diagnosis and therapy of human immune-mediated or immune-associated diseases.
- Analytical tools - DNA sequencing - identification of strains and genomes.
- RNA sequencing - determination of microbial gene activity
- Metabolome, metatranscriptome and metagenome analysis - microbial community function.
- Potential as diagnostic tool in Parkinsons disease, Alzheimer disease.



How are dysbiosis being used in diagnosis: information regarding the state and function of the microbiota utilized for diagnosis and therapy of various human immune-mediated diseases. The analytical tools include DNA sequencing, which identifies the strains and genomes. There is RNA sequencing which determines the type of microbial gene activity. The metabolome, metatranscriptome and metagenome analysis helps in finding the microbial community function. These tools are being already tried as a potential tool in Parkinsons disease, Alzheimers.

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Targeting dysbiosis for therapy




ANTIMICROBIAL PEPTIDES

METALLIC NANOPARTICLES

PREBIOTICS

PROBIOTICS

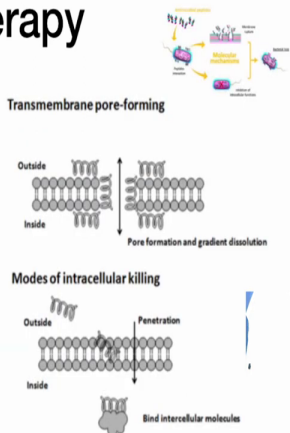

HOST RESPONSE MODULATORS



Next we will move on to therapies that use dysbiosis as the target. The various therapeutic options include antimicrobial peptides, metallic nanoparticles, prebiotics, probiotics, synbiotics and host response modulators.

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Targeting dysbiosis for therapy



- Antibiotic usage eliminate both pathogenic & commensal bacteria.
- AMP - broad spectrum antimicrobial activity- modulates dysbiosis.
- Bacteriocin like nisin active against both gram positive and gram negative bacteria.

Transmembrane pore-forming

Outside

Inside

Pore formation and gradient dissolution


Modes of intracellular killing

Outside

Inside

Penetration

Bind intercellular molecules

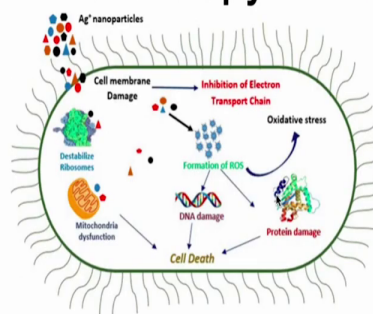


The antibiotic usage eliminates both the pathogenic as well as the commensal bacteria. Thus studies had focused on antimicrobial peptides, which has a broad spectrum antimicrobial activity

that could modulate the dysbiosis, but bacteriocin like nisin are found to be active against both gram positive and gram negative bacteria. These antimicrobial peptides work by inserting themselves to the surface layer of the bacteria, causing pore and release of intracellular components, causing the lysis of the bacteria.

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Targeting dysbiosis for therapy



- **Metallic nanoparticles** - incorporated into polymer matrices as filler particles to control biofilm growth.



The various metallic nanoparticles are being incorporated into a polymeric matrix as a filler particle to control the biofilm growth. These metallic nanoparticles insert and internalize the cell membrane. They cause damage by inhibition of the electron transport chain. Once they gain access to the cell, they form reactive oxygen species, which cause the oxidative stress leading to DNA damage and death. These can also destabilize ribosomes and mitochondrial functions leading to death.

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Targeting dybiosis for therapy



- **Faecal microbiota transplantation (FMT)** - entire intestinal community of patient replaced by microbiota of healthy donor.



The Faecal microbiota transplantation has been used in the treatment of various chronic disorders where the entire intestinal community of the patient is being replaced by the microbiota of a healthy donor.

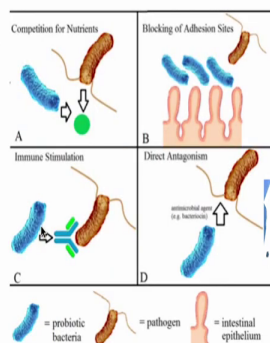
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Targeting dybiosis for therapy



- Intense effort made to engineer or reconstitute microbiota to prevent or treat disease.
- Administration of **Probiotics** such as *Lactobacillus* and *Bifidobacterium* species to support expansion of healthy microbiota.

How Probiotics Work



Intense efforts have been made to engineer or reconstitute the microbiota to prevent or treat diseases. Administration of the probiotics, which are nothing but the healthy microorganisms such as the Lactobacilli and Bifidobacterium species, cause expansion of the healthy microbiota.

They act through various mechanisms - they compete for the resources and nutrition and also they block the various additional sites present to prevent the growth of pathological microorganisms. They directly cause the stimulation of the immune system against the bacteria and other pathogens. They produce the antimicrobial agents, such as bacteriocin to get rid of the pathogens.

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Targeting dybiosis for therapy



- Dietary **Prebiotics** (Natural or synthetic food) aim to modify the composition of intestinal ecosystem through nutritional changes.
- Nitrate & arginine - rapid changes in structure & function of polymicrobial community.
- N-acetyl D Mannosamine - selective growth of commensal




TOP 20 PREBIOTIC FOODS
FOR A HEALTHY GUT





Prebiotics are the dietary natural or synthetic food, which aims to modify the composition of the intestinal ecosystem through nutritional changes. Nitrate and arginine can cause rapid changes in the structure and the function of the poly microbial community, and induce the restoration of the normal microbiome. Certain agents such as the N-acetyl D Mannosamine can lead to the selective growth of commensals.

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Future directions



- Concept of dysbiosis deserves a functional rather than a taxonomic interpretation.
- Microbial functionalities and metabolite profiles associated with particular condition more consistent - higher causal relevance.
- Extent and manifestation of dysbiosis -highly context dependent. Factors like host genotype, environmental microbial repertoire considered for susceptibility to dysbiosis development.
- Understanding of Precise '*dysbiotic*' microbiota derived or microbiota modulated molecules that mediate disease development allow design of metabolite-based interventions.




Future directions - what are we up to? The concept of dysbiosis deserves a functional rather than a taxonomical interpretation. We are more bothered about the metabolites produced by the microorganisms rather than their composition. Even those microorganisms, which are present in a small quantity can have a very big effect. The microbial functionalities and metabolite profiles associated with particular conditions are more consistent and they have a higher causal relevance.



The extent and the manifestation of dysbiosis are highly context dependent. Factors, such as the host genotype, environmental microbial receptor should be considered for susceptibility to dysbiosis development. Understanding the precise dysbiotic microbiota derived or the microbiota modulated molecules that mediate the disease development can allow us to design metabolite based interventions.

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Conclusion




- Primary goal of *Human Microbiome Project* - establish the normality of intestinal composition and function.
- Subsequent efforts aimed to define and understand dysbiotic states associated with human diseases.
- New associations have brought about promising implications for future diagnostic and therapeutic approaches.




To conclude, the primary goal of the Human Microbiome Project, which was initiated in 2007 with 10 projects, was to establish the normality of the intestinal composition and function. Now, the years have rolled out and the subsequent efforts are being aimed to define, understand the dysbiotic states associated with human diseases. The new associations have brought about promising implications for further diagnostic and therapeutic approaches.

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These are the references.

Thank you.