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MICROBIOLOGY

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ملاحظة: ممكن تلاحظوا بأنوا ترتيب المواضيع بالشيت مو نفس الترتيب الي بيشرح عليه الدكتور لكن كل معلومة مذكورة في الشيت الدكتور ذكرها بلحظة معينة بالمحاضرة و انضافت بالمكان المناسب و بسياق مناسب لسهولة ربط المعلومات و الشيت سهل و واضح جداً و ممكن يندرس بدون الرجوع للمحاضرة.

INTRODUCTION

Microbiota is the collection of all microorganisms (including bacteria, fungi, and viruses) that reside within a certain part or organ in our bodies such as our skin and GI tract. **In other words, any group of organisms that live in a specific place in our body are called microbiota. In our studies, we're concerned with the human microbiota**, where the human basically presents the environment for the microorganisms to live and grow.

not considered as normal flora because flora is related to plants.

Throughout thousands of years, human contact with microbes resulted in the presence of a symbiotic relationship in general, in that these microbes were either harmless or even beneficial and important for the development of many systems.

Most bacteria exhibited a mutualistic, symbiotic relationship through which:

- Humans benefit from bacterial enzymes which production nutrients that are essential but not produced by us.
- Simultaneously, bacteria benefit from the nutrients provided by the human body.

"Not all bacteria are bad, some are even beneficial"

**You might agree upon the previous statement's meaning, but it's not worded properly! The sentence indicates that most bacteria are bad and only some are beneficial. But what's true is that, only very few minorities are bad, and the majority are either harmless or beneficial (being part of the microbiota). which depends on several factors to be either beneficial or harmful such as:

- Host factors (immune factors).
- Microbe factors.
- Environmental factors.

****So>** microbiota is an "ecological community of commensal, symbiotic and pathogenic microorganisms" found in and on all multicellular organisms studied to date from plants to animals.

**Sometimes the term microbiome is used to describe microbiota, since the major and most recent technique to study microbiota is through genetics. The microbiome is the collective genome of microbiota (which reflects on microbial species).

most of the genetic material in the human body is viral and not mammalian. Likewise, most of the human body cells are prokaryotic (bacterial) rather than eukaryotic (mammalian). So, you're basically a mixture of all that!

It was said to be that the ratio of bacterial to human cells is 10:1 (10 bacterial cells per human cell). But it was recently calculated that the ratio is 1.3:1; still indicating that bacterial cells outnumber mammalian cells.

**Only during the past 20 years did our studies and knowledge increase about these microbes that live within our bodies. This development is largely due to revolutionary discoveries in the technical field, like NGS (Next Generation Sequencing) and PCR (Polymerase Chain Reaction).

- Microscopes alone are not enough to identify the microbiota present among 2000 different species; therefore, their contribution to this science is minimal. Same applies to culturing and biochemical testing.

- A few decades ago, we were not aware of the huge diversity of these microorganisms. Identification was mainly based on growing cultures in available media, and sometimes the cultures did not even grow or appear. This doesn't mean that no microorganisms were present, instead the media/agar used could've been nutrient-lacking or inappropriate for the type of bacteria grown. [Remember, media could be differential or selective for certain bacteria] To wrap it up, culture techniques did not reflect the variety of microbiota that existed in us.

DNA-based methods contribute widely to the field of microbiota. For example, DNA extracted from a stool sample can be completely analysed and later correlated to certain species of bacteria. This broadens our perspective and understanding of the variety of microbiota present in the skin, gut and even urogenital tract because we are aware of their genetic information.

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The type of bacteria differs according to location; some bacteria are unique to the **skin, others to the **gut**, and some others to **the urogenital tract**. All these types are important and play different roles in our bodies.

The Human Microbiome Project:

The National Institute of Health launched this project in 2007 to **understand the range of human genetic and physiologic diversity** of the microbiome (among different societies, diseases, and even lifestyles).

Many questions arise when studying the microbiome; therefore, **there's not a single centre, university or lab that can alone correlate the microbiome with diseases**. For that reason, this project was established to include all data collected on the microbiome. The data are obtained from different scientists who correlate specific parts of the microbiome with certain diseases, proposing their papers accordingly to this project.

One of the main objectives of this project is to answer several questions like:

(1) **How stable and resilient** is an individual's microbiota throughout one day and during his or her life span? [note: the microbiota is dynamic and can change within a single day or through maturation].

(2) **How do changes in microbiota occur**, and how do they vary across different **communities** and with different **lifestyles** (including the food you eat, the places you visit, or even the type of exercise you practice)?

(3) How is microbiota **acquired and transmitted**?

Microbiota

Resident Microflora:

Usually present for long periods of time

Transient Microflora:

- Keeps changing, could change with a handshake! - Case study: the microbiome on a person's hand is tested before and after a handshake to prove changes in microbiota [Note: it's not necessary for the microbiota of both individuals -shaking hands-to change

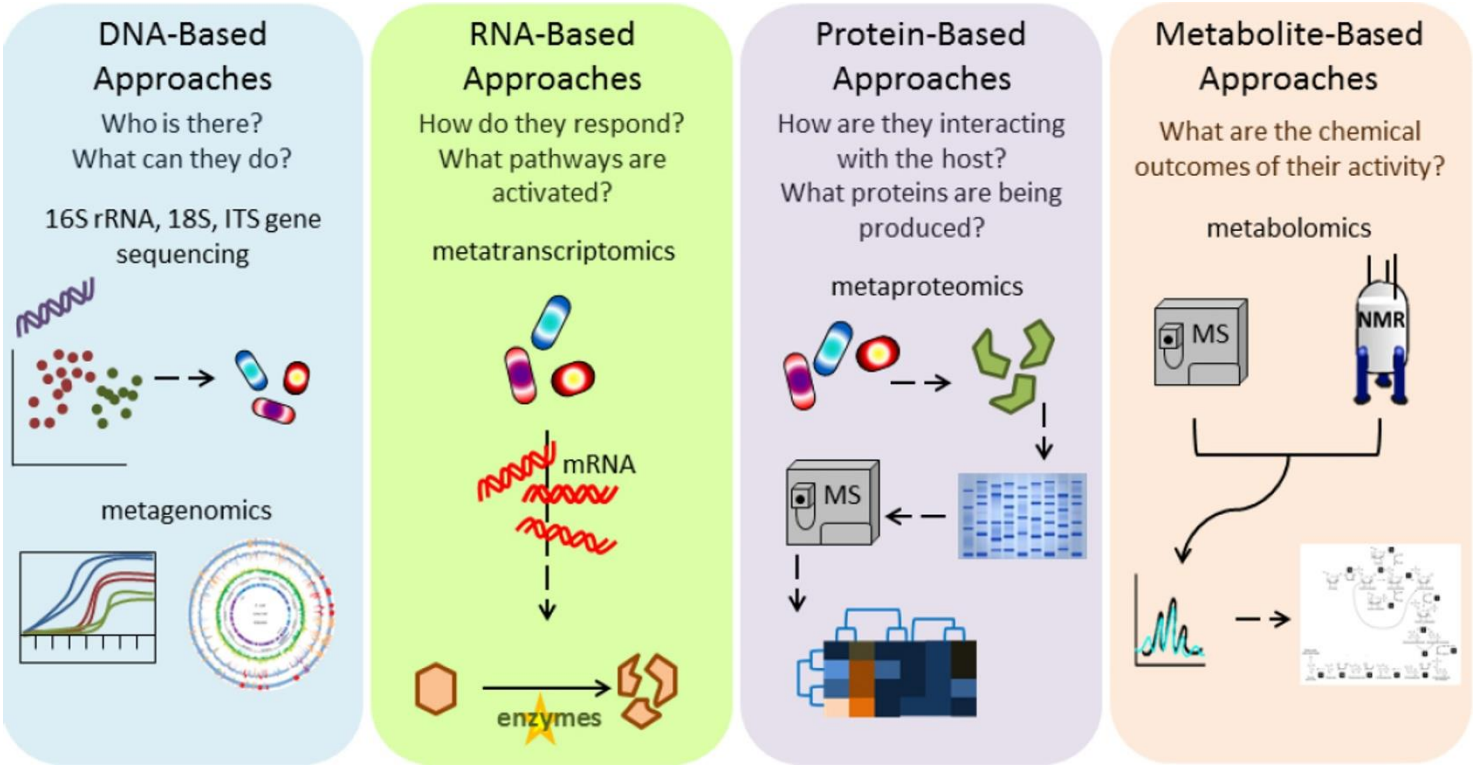
(4) What is the **effect of microbiota on health and disease**?

(5) How can **healthy microbiota** be attained?

What scientists rely on during their study of the microbiome is the **(16s) ribosomal RNA**. They could analyse the whole genetic material of the microbe, but it would consume more time and money. So, what they do is study certain genes.

Bacterial genomics is complicated. During horizontal gene transfer for example, **Staphylococcus aureus** could pick up genes from **Streptococcus pyogenes** (two different genera). Since genes could be easily transmitted between bacteria, it's not beneficial to look at the whole genome.

It was discovered that certain genes are **conserved through time** (unchanged and untransmitted across species) including genes of the small subunit (16s) ribosomal RNA. This gene rarely gets mutated since it's essential for protein synthesis -otherwise bacteria die-.



Hence, it's useless to look at a gene specific for antibiotic resistance because it will be present across different species as well as it will repeatedly get mutated. Same applies for genes that encode features nonessential for bacterial survival, like genes of adhesion molecules.

Note: what is mentioned above the figure correlates to DNA-based methods. Below is a short explanation on each of the approaches shown above.

1)DNA-Based Approaches: the whole DNA is taken and sampled. After that, **metagenomics** is performed. This gives a circular graph that categorizes species based on how closely related they are in terms of genetics. Every species is given a unique colour within the diagram. This approach helps us identify bacteria present in organs and what these bacteria can do (usually by studying 16s rRNA and correlating the microbiome with a disease status).

> Example: If you observe that patients with disease (X) have a certain group of microbes (Y), you can conclude that bacteria (Y) cause disease (X).

Time is still needed to better understand the role bacteria play in health and disease.

2)RNA-Based Approaches: analysing mRNA of bacteria, and by that you look at what pathways are activated. This is called **Metatranscriptomics** because you're examining the transcriptome (all transcripts).

3)Protein-Based Approaches: this approach studies the type of proteins produced and how they interact with the host. In **metaproteomics**, Mass Spectrometry is used. It gives spectrums for different peptides. [some bacteria have signature peptides that can help in identifying the type of bacteria].

4)Metabolite-Based Approaches: through which the chemical metabolitesare examined, and that's called metabolomics.

***The most used methods are the DNA-Based Approach (metagenomics) and protein-based approach(metaproteomics); therefore, The Human Microbiome Project depends on it.

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Skin Microbiota:

Since the skin is in continuous contact with the environment, it's very likely that it exhibits a microbiota despite the harsh aspects of the skin such as: dryness and desiccation/ nutrient-poorness /acidic environment /presence of lysozyme and antimicrobial peptides.

Certain bacteria still manage to colonize the skin and compose a microbiota despite all above-mentioned conditions **[Remember, this microbiota could be either transient (could change simply due to a handshake) or resident (found commonly among most people)].** The microbiome is part of the epithelial barrier. It helps in protecting against incoming pathogens by taking up certain space and nutrients, as well as secreting certain AMPs (antimicrobial peptides)

Even within the skin, bacteria differ according to location. For instance, bacteria found within the nares differ from those in the armpit or at the soles of the feet. There are certain types that are predominant residents of the skin, such as:

***Coagulase-Negative Staphylococci: most common type is Staphylococcus epidermidis

- **Staphylococcus aureus (coagulase-positive)** is pathogenic, although it can colonize the skin without causing problems. But then again, it all falls back into the host-pathogen interactions [no disease/ colonization/ disease]
- **Staphylococcus aureus** (even the pathogenic) could colonize especially the anterior nares and hands of medical professionals. This provides a threat of skin infection on the professional and most importantly the patients (if aseptic techniques were not applied).

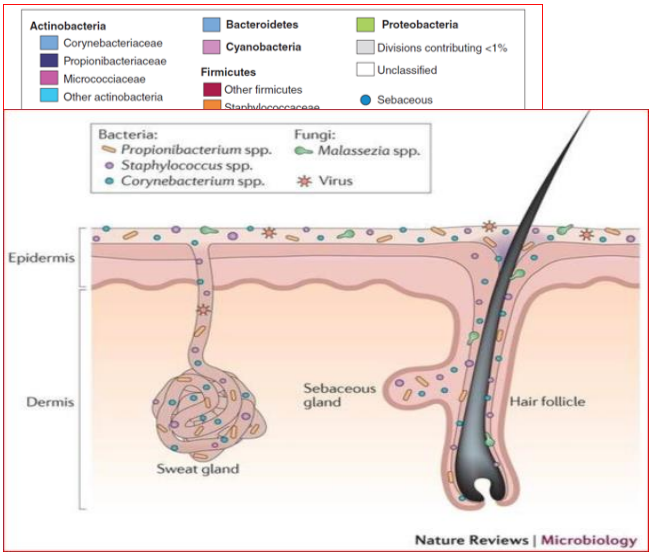
***Propionibacterium: normal commensal type of bacteria that could be associated with pathologic conditions like acne (acne vulgaris).

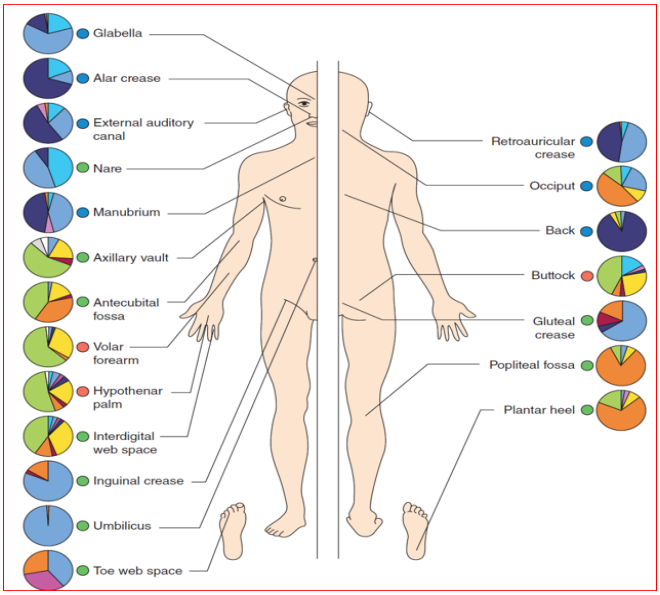
***Corynebacterium.

→Keep in mind that the term "microbiota" could be associated with diseases.

→ For instance, *Staphylococcus epidermidis* <u>is usually part of the normal flora, but still could be pathogenic</u> if it's environment changes (from the surface of the skin to any other media; for example, if it's transmitted into the blood, or even into a deeper layer of tissue).

→Transmission of Staphylococcus epidermidis into the blood stream is the major cause of Sepsis, especially in patients with catheters.





The Gut Microbiota:

Among the nonsterile cavities, the gut has the largest and most complex microbiota, with a strong impact on host homeostasis and immunostasis, being thus essential for maintaining health.

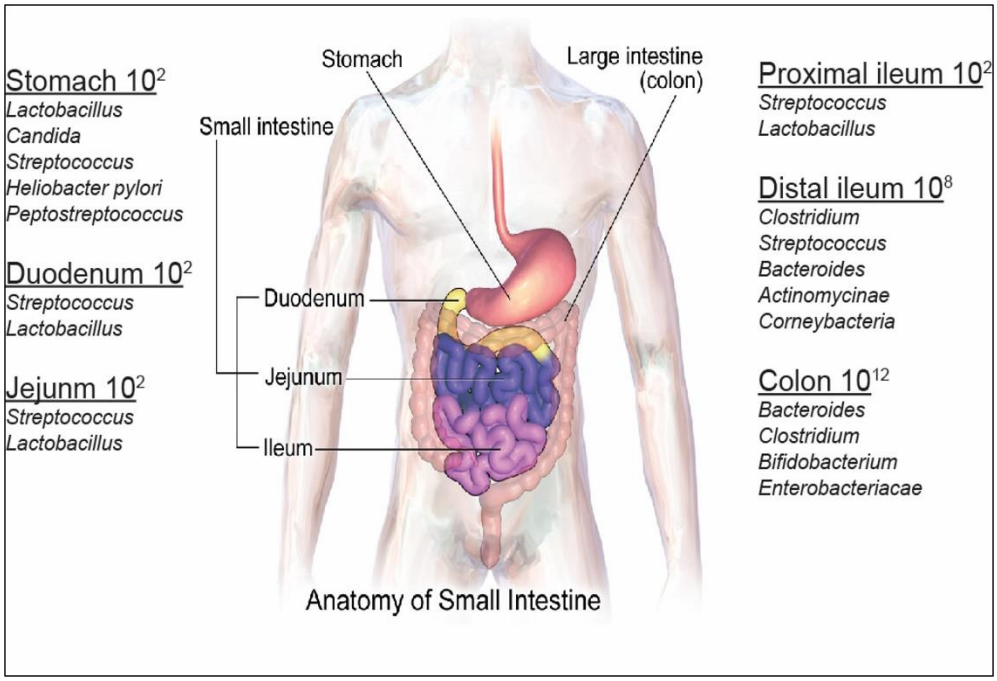
Many factors influence this microbiota, like: ▪ Genetics ▪ Gender ▪ Age ▪ Immune system & health/disease conditions ▪ Geographic & socio-economic factors (urban or rural, sanitary conditions) ▪ Treatments and diet.

[Urban and rural inhabitants exhibit different microbes, due to the different lifestyles]

- The GI tract contains at least 1014microorganisms belonging to more than 2000 species.

- Most of the bacteria of the GI tract is present within the colon; therefore, faces consist largely of bacterial mass that could be weighed in kilograms!

In the colon, these bacteria are majorly of anaerobic origin (like Bacteroides). **Anaerobes outnumber facultative organisms in the colon by 1000-fold.**



Anatomy of Small Intestine

Anaerobes in general are an important part of the gut microbiota.

Enterobacteriaceae form a large part of fecal material

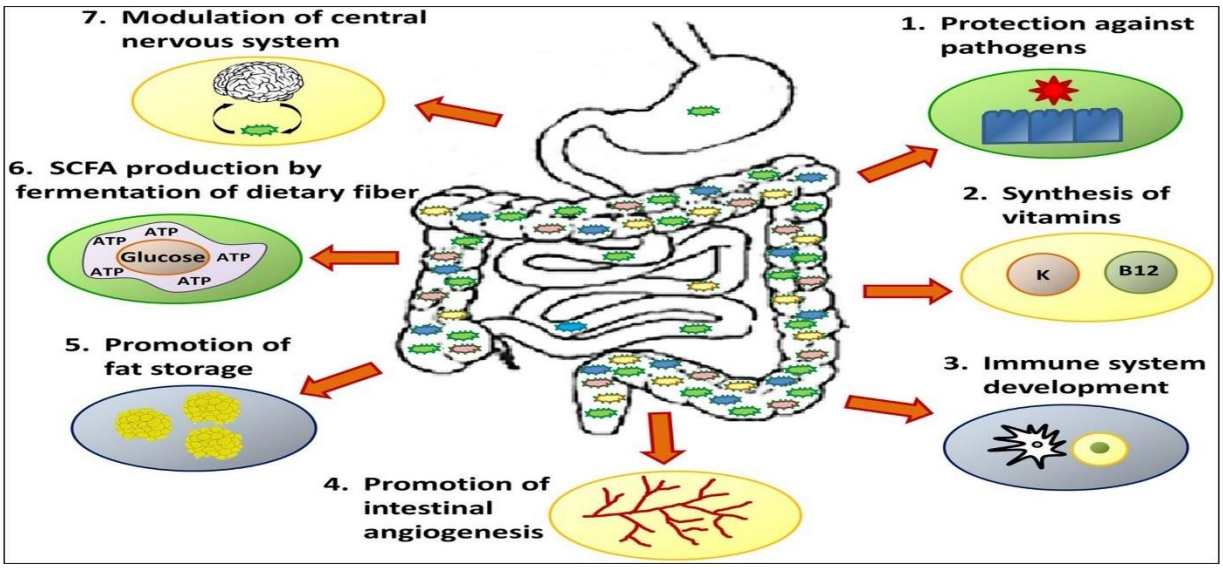
Lactobacilli and Probiotics

▪ Probiotics are basically beneficial bacteria put together in a capsule, for example: 10,000 colony forming units are formulated into a capsule.

▪ The effect of these probiotics is still unconfirmed. However, some physicians prescribe probiotics with antibiotics. (Antibiotics will kill your microbiota and alter homeostasis. In turn, probiotics are expected to compensate the loss of beneficial bacteria).

▪ Probiotics often contain Lactobacilli.

▪ It was recently found that autistic mice (model due to gene knockout) that were given a certain type of lactobacilli, reverted or showed less symptoms of autism.



→ Gut microbiota can **modulate the Central Nervous system** [The Microbiota-Gut-Brain Axis], but we still don't know the degree of modulation.

- Microbiota secrete certain peptides that are sensed by certain neurons which reach the brain. The brain sends chemical signals that affect the epithelial barrier, thereby affecting the microbiota.
- It's true that the type of food you eat affects your mood. That is because of the Gut-Brain Microbiome Axis.

→ Gut microbiota provides **protection against pathogens.**

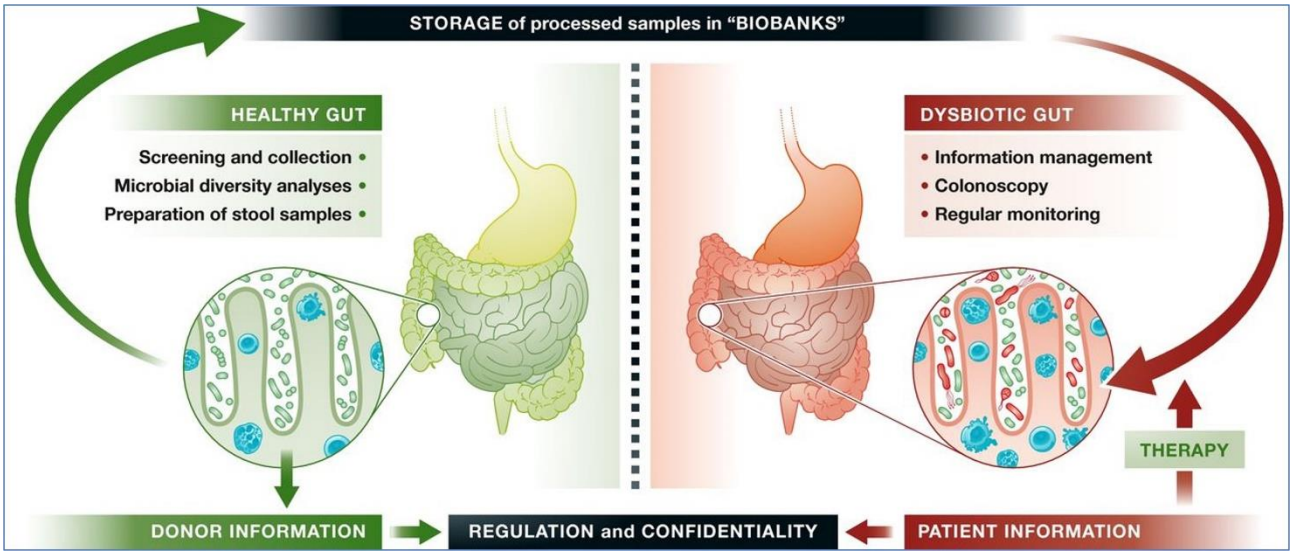
→ GI microbiome can synthesize certain **vitamins like vitamins K & B12**, as well as other nutrients due to their special enzymes.

→ Both, the gut, and skin microbiota play an important role in **immune system development**.

- The immune system continuously samples antigens. Dendritic cells capture antigens from tissues and head towards lymph nodes to present these antigens to lymphocytes. These antigens are important for the proper maturation of the immune system.

- The Hygiene Hypothesis states that one of the major causes of the increase in autoimmune diseases and cancer prevalence lately is the increase in hygiene standards! Thus, the immune system gets exposed to a fewer number of microbes and does not "train" well. This eventually leads to the improper maturation of the immune system due to repeated contact with the very same microbes.

Fecal Matter Transplant (FMT)



▪ A huge mass of the gut microbiome is present in feces.

▪ Many patients take intensive courses of antibiotics for a long time, which kills lots of their microbiome. This gives pathogenic bacteria the space and nutrition to overpopulate in the gut.

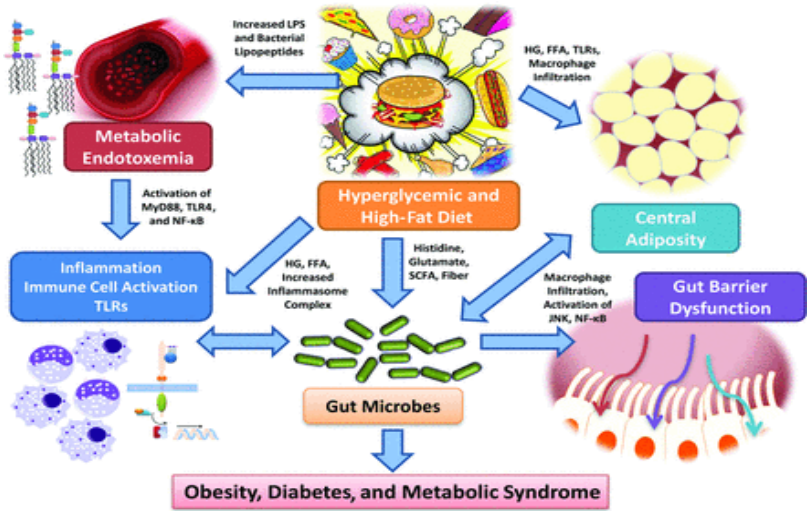
- Clostridium difficile causes pseudomembranous colitis, an inflammation of the colon common in patients who take antibiotics for prolonged periods.

- You cannot treat Clostridium difficile infection with antibiotics since they were the initial cause of infection.

- The US FDA (Food and Drug Administration) approve and recommend this method. It's mainly performed rectally through colonoscopy.

You can store healthy microbiota in biobanks by collecting a healthy stool sample. Upon dysbiosis, (instead of healthy symbiosis of gut microbiota) your healthy microbiome from the sample is analysed to identify which parts of the microbiome had been altered. This is done to help revert into the healthy microbiota. [Of course, we still didn't reach that level yet, but it could be part of prospects.]

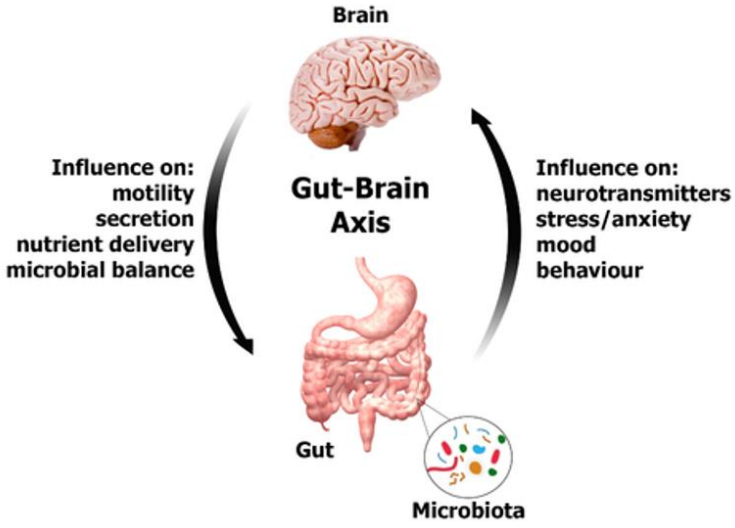
→ **Food can affect the type of microbiome we have:**



- Hyperglycaemic and high fat diets give a certain gut microbiome profile. This profile is highly associated with obesity, diabetes, and metabolic syndromes.

- When you eat, you're also feeding your microbiome, so be aware of the type of microbiome you're growing in your gut. Microbes, for example, love fat due to their ability to store it.

- ❖ The altered bidirectional neurohumoral communication system between gut and brain – known as the gut-brain axis – may cause a series of diseases, such as autoimmune and CNS disorders



The Urogenital Tract Microbiota:

It is part of common knowledge that urine should be sterile. This is however still debatable, because using certain culture techniques, particular types of microbes appeared in urine. [However, we'll just stick to the idea that urine should be sterile] The tip of the urethra can contain certain bacteria that might appear in urine samples.

Soon After Birth: aerobic lactobacilli [acidic pH]

All the Way Until Puberty: mixed flora of cocci &bacilli [neutral pH]

At puberty:

Aerobic and anaerobic lactobacilli

[acidic pH]

Throughout development, different types of microbes appear in the vagina ^

Note: During puberty, Lactobacilli contribute to the maintenance of the acidic pH through the production of acid from carbohydrates.

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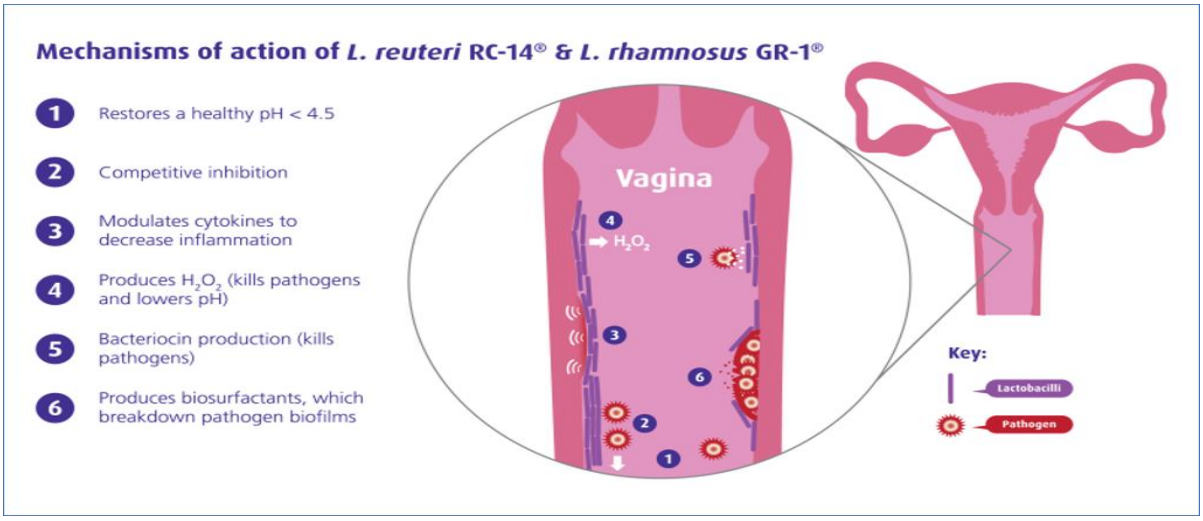
-Lactobacilli are the predominant bacteria in the vagina. If the type of bacteria changes, diseases can occur, such as:

- Bacterial Vaginosis: a syndrome due to a disturbance of the normal flora of the vagina **[from Lactobacilli to Actinobacteria & Bacteroides species].** This disease is associated with abnormal secretions and unpleasant odours.



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As shown in the figure above, some patients are given probiotics containing Lactobacilli to restore a healthy vaginal canal. **[still not confirmed, although some studies support this idea]**.

Mechanisms of Lactobacilli action:

(1) Restores a healthy pH through bacterial metabolism.
(2) Competitive inhibition of pathogenic species (taking up space& nutrients).
(3) Modulates cytokines to decrease inflammation.
(4) Produces hydrogen peroxide (H2O2) (kills pathogens & lowers pH).
(5) Bacteriocin production (bacteriocin is an antimicrobial peptide).
(6) Produces biosurfactants, which breakdown pathogen biofilms.

Vaginally born infants have a microbiota different from that of **caesarean section** babies.

(A) **Vaginally** born babies will have a microbiota containing species derived from the vaginal microbiota of their mothers. (Could be normal like **Lactobacilli,** or pathogenic)

(B) **Caesareans** section infants will have a microbiota like the skin microbiota. Their microbiota is rich in **Propionibacterium & Staphylococcus** species.

[The microbiome is present early on, sometimes even during pregnancy].

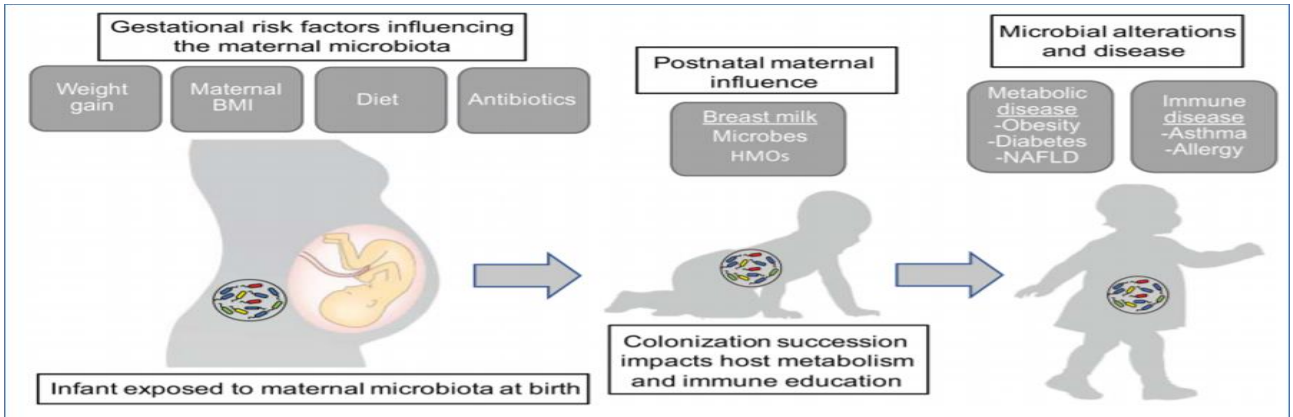

Figure 1
Influential factors on the maternal and infant microbiota. Maternal factors significantly contribute to the initial colonization and succession of the infant gut microbiota. Alterations in this process may have long-term health consequences related to host metabolism and immune education. HMOs, human milk oligosaccharides; NAFLD, nonalcoholic fatty liver disease.

• The mother's microbiota and what she eats can affect her baby.

• Breast milk can also expose the baby to different microbes do not present in other types of milk.

• All these methods expose the developing human being to different microbes, which will have an effect even later in life (like on obesity, diabetes, immune diseases such as asthma & allergies).

• Sometimes if a baby is born by caesarean section, a vaginal swap is taken from the mother, and it's applied over the baby's skin. This is to ensure that the baby gets exposed to the normal bacteria the baby should've acquired while passing through the vaginal canal.

In conclusion, the microbiome has a myriad of effects on us, and most probably with time, our understanding and knowledge of this topic will become broader.

Best wishes.