Sequencing in microbiology & the human microbiome

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18.12.2023

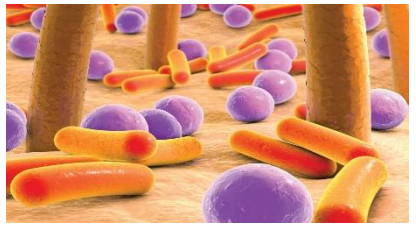
2. LÉKAŘSKÁ FAKULTA
UNIVERZITA KARLOVA

FN MOTOL

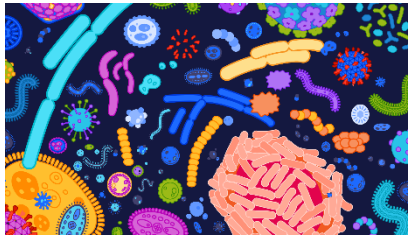
Content of the lecture

- 1) Sequencing in microbiology

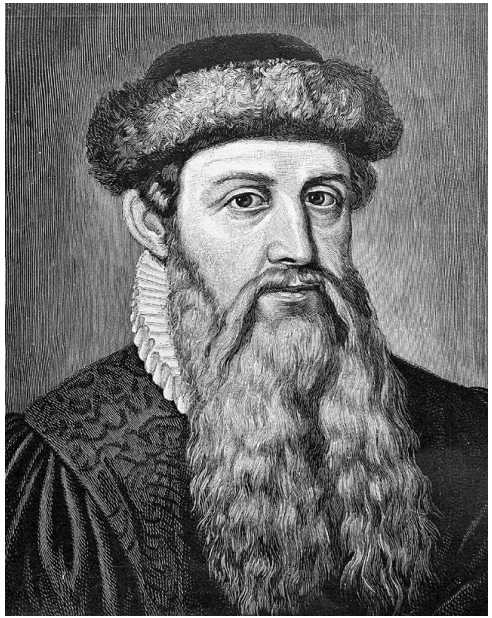
- 2) Physiological microbiota



- 3) Human microbiome



Historical parallels



Johannes Gutenberg (1450)
The invention of the printing press

Making knowledge available to the public (=loss of influence of the Church)

And what about Microbiology?

Human Genome Sequencing
(HGP officially started 1990 and completed 2003)

Next Generation Sequencing (2007)

It took 13 years to sequence the first human genome
And today: the WGS - days; the NGS (specific sections) - only tens of hours!

Making data publicly available:
Massive development of microbiome science

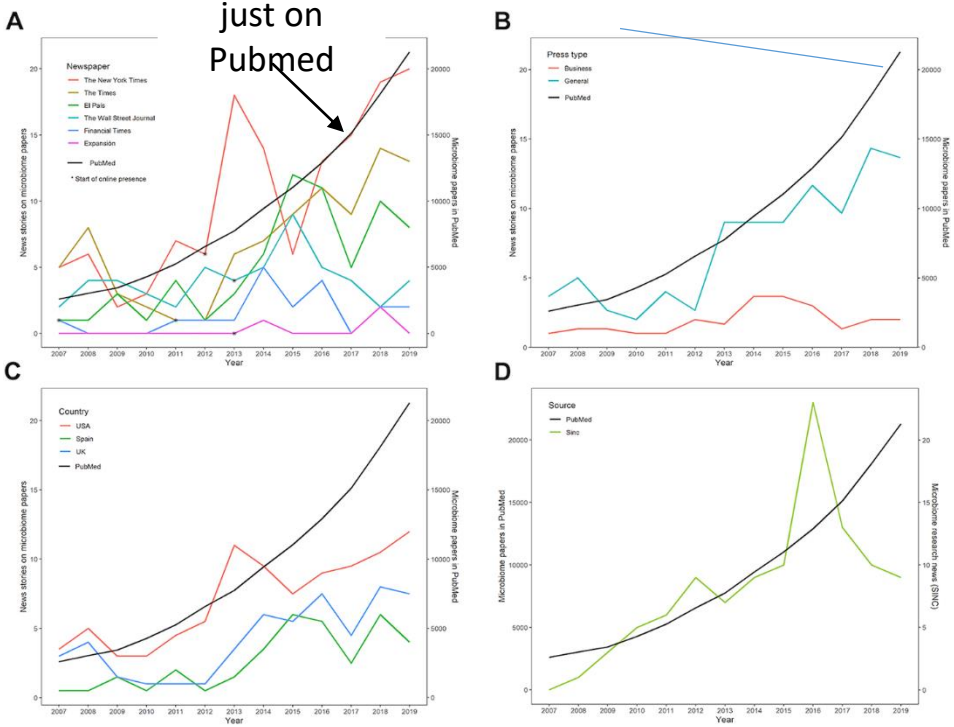
Something scientists are interested in and maybe you should be too

10x more articles on Pubmed in 12 years

	Annual cites from 2007 to 2019	Cites in 2007	Cites in 2019	Average annual percentage change	Correlations with microbiome papers in PubMed[1] (p-value)	Correlations with microbio news published by SINC[1,2] value)
Microbiome papers in PubMed	9297,0 (6063.3)	2600	21292	19.6%	-	**0.62 (0.023)**
Biomedicine papers in PubMed	1111673,6 (203280.1)	785933	1397557	4.9%	-	-
Microbiome/ biomedicine in PubMed	0.8%	0.4%	1.4%	9.6%	-	-
Microb SINC[2]						-
Biomed SINC[2]						-
Microb biomed						-
Total newspapers	4.6 (4.9)	2.5 (2.2)	7.8 (7.5)	13.9%	**0.88 (<0.001)**	**0.66 (0.014)**
Individual newspapers						
The New York Times	10.3 (6.4)	5	20	16.0%	**0.83 (0.005)**	0.48 (0.095)
The Times	6.8 (4.4)	5	13	14.3%	**0.82 (0.005)**	0.47 (0.102)
El País	5.1 (4.0)	1	8	22.7%	**0.74 (0.004)**	**0.71 (0.006)**
The Wall Street Journal	4.1 (1.8)	2	4	2.9%	0.14 (0.652)	0.35 (0.236)
Financial Times	1.5 (1.6)	1	2	11.8%	0.39 (0.177)	**0.58 (0.038)**
Expansión	0.2 (0.6)	0	0	4.3%	0.41 (0.166)	0.11 (0.713)
Country						
USA	7.2 (5.6)	3.5 (2.1)	12.0 (11.3)	12.0%	**0.85 (0.002)**	**0.57 (0.039)**
UK	4.1 (4.2)	3.0 (2.8)	7.5 (7.8)	14.5%	**0.81 (0.001)**	**0.57 (0.042)**
Spain	2.7 (3.7)	0.5 (0.7)	4.0 (5.7)	23.1%	**0.75 (0.003)**	**0.68 (0.010)**
Newspaper type						
General newspaper	7.4 (5.4)	3.7 (2.3)	13.7 (6.0)	15.7%	**0.91 (<0.001)**	**0.61 (0.024)**
Business newspaper	1.9 (2.1)	1.0 (1.0)	2.0 (2.0)	7.2%	0.39 (0.185)	**0.56 (0.043)**

Mean followed by the standard deviation in parentheses is indicated for microbiome/biomedicine papers in PubMed, microbiome/biomedicine news in SINC and stories on microbiome papers in newspapers.
[1]The numbers showed the Pearson correlation coefficient.
[2]News stories published by SINC were available from 2008 to 2018.
Significant p-values are highlighted in bold.

And not just on Pubmed

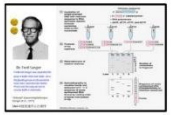


Prados-Bo, Casino, *Plos* One, 2021

1) Sequencing in microbiology

First generation sequencing (Sanger sequencing)
- panbacterial PCR
- typing of some bacteria (spa types of *S.aureus*)

Third generation sequencing
(PacBio, Nanopore)
- Real-time sequencing

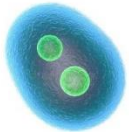


Sanger method

1977

Human Genome Project

1981



Complete eukaryotic genome

1990

Second generation sequencer：454 GS20

1995

Research Human Microbiome Project

2001

Nanospace sequencing

2007

2011

2019

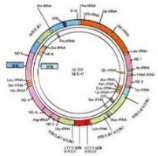
1996

2005

2008

2014

Human mitochondrial genome sequence



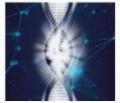
Complete cell genome

Complete the Human Genome Project

Second generation sequencer：Genetic Analyzer 2

Third generation sequencer：PacBio RS

The third stage Human microbiome project



Second generation sequencing (Next-generation sequencing)
= massively parallel sequencing
- Whole genome (WGS) / exons (metagenome) / specific sections only (profiling)
- bacteriomes, viromes, bacterial genomes (resistomes, relatedness for epidemiology)

Thanks to
Dr. Marcela Krutová

Where can it be used?

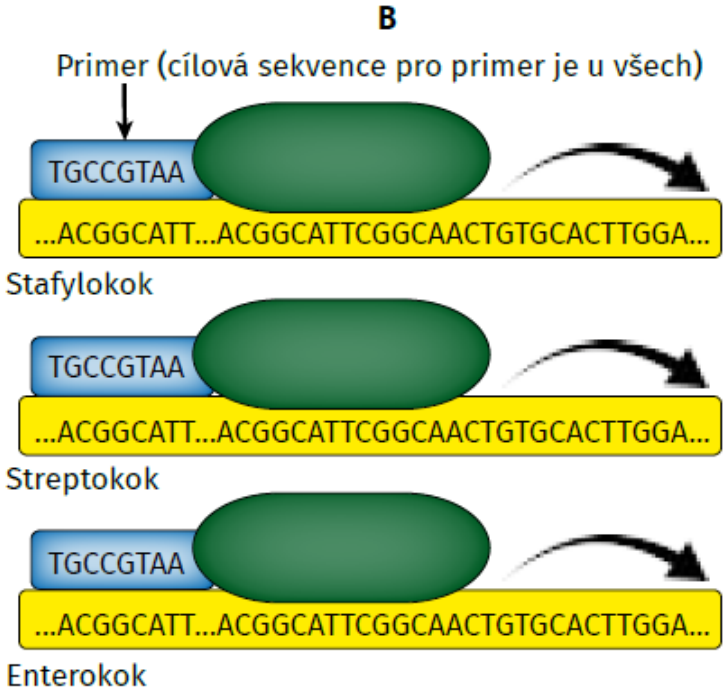
Diagnosis of infections from primarily sterile materials

Analysis of the bacterial genome
- Virulent strains
- Resistance genes (ResFinder)
- Clonal spread (BioNumerics)

Fecal microbiota transplantation
- donor testing
- monitoring of marker bacteria retention

Study of the human microbiome
association with non-infectious diseases: IBD, IBS, T1D, obesity, etc.

Panbacterial PCR

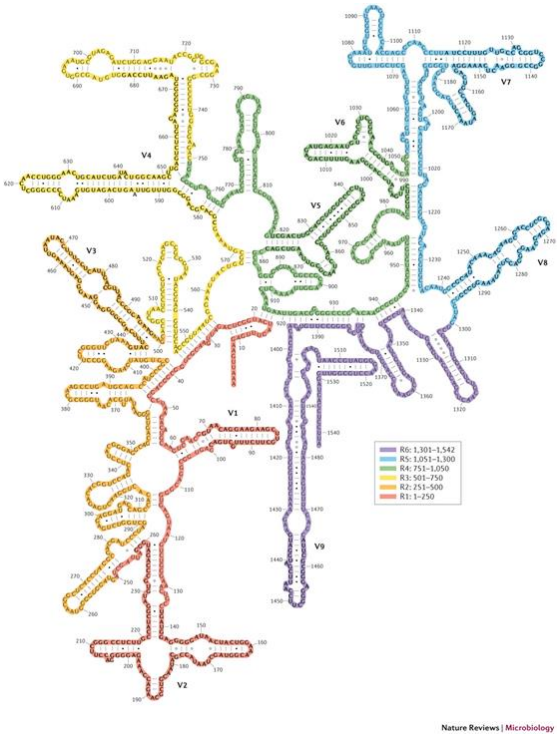


Materials?
Primarily sterile!

- Heart valves and other tissues
- Aspirates (joint, pleuaral etc)
- CSF
- Very rarely: whole blood, BAL
- Even more rarely: cultures

How do you do it?

- Two-round process



Nature Reviews | Microbiology

1. 16S rDNA amplification

2. Sequencing of the 16S rDNA amplicon

16S rDNA is a linear structure - > transcribes into a linear rRNA, and folds.

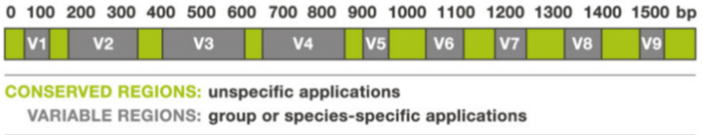


Figure 1: An example of a 16S rRNA gene. The regions in green are conserved in all microorganisms. These are the sites that are targeted by primers for PCR amplification so that all the 16S rRNA genes in a sample are amplified. The grey regions are the species-specific regions that-- when sequenced-- allow for scientists to see which species are present in a community. Image courtesy of: http://www.alimetrics.net/en/index.php/dna-sequence-analysis

Other uses of panbacterial PCR

- It is also tested from NON-STERILE MATERIALS (i.e. often polymicrobial)

For the exam, however, the indications for panbacterial PCR are primarily sterile materials!

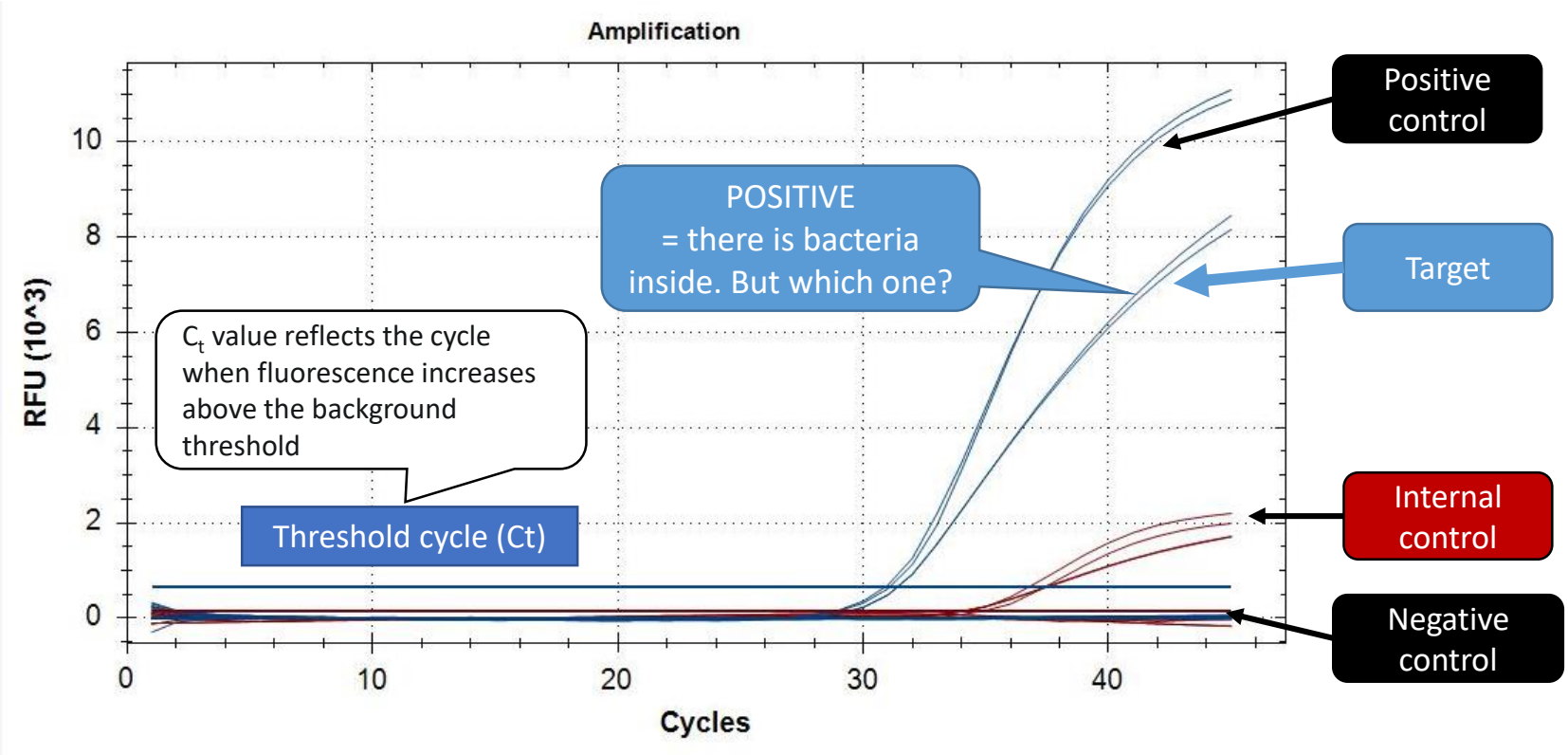
3.3. Suspected polymicrobial samples

Figure 7: Suspected polymicrobial samples with single or no pathogen reported by P

https://static.metagenlab.ch/2023_ESGMD_metagenomics/

1. Evaluation of the PCR curve

Material: culture-negative joint aspirate



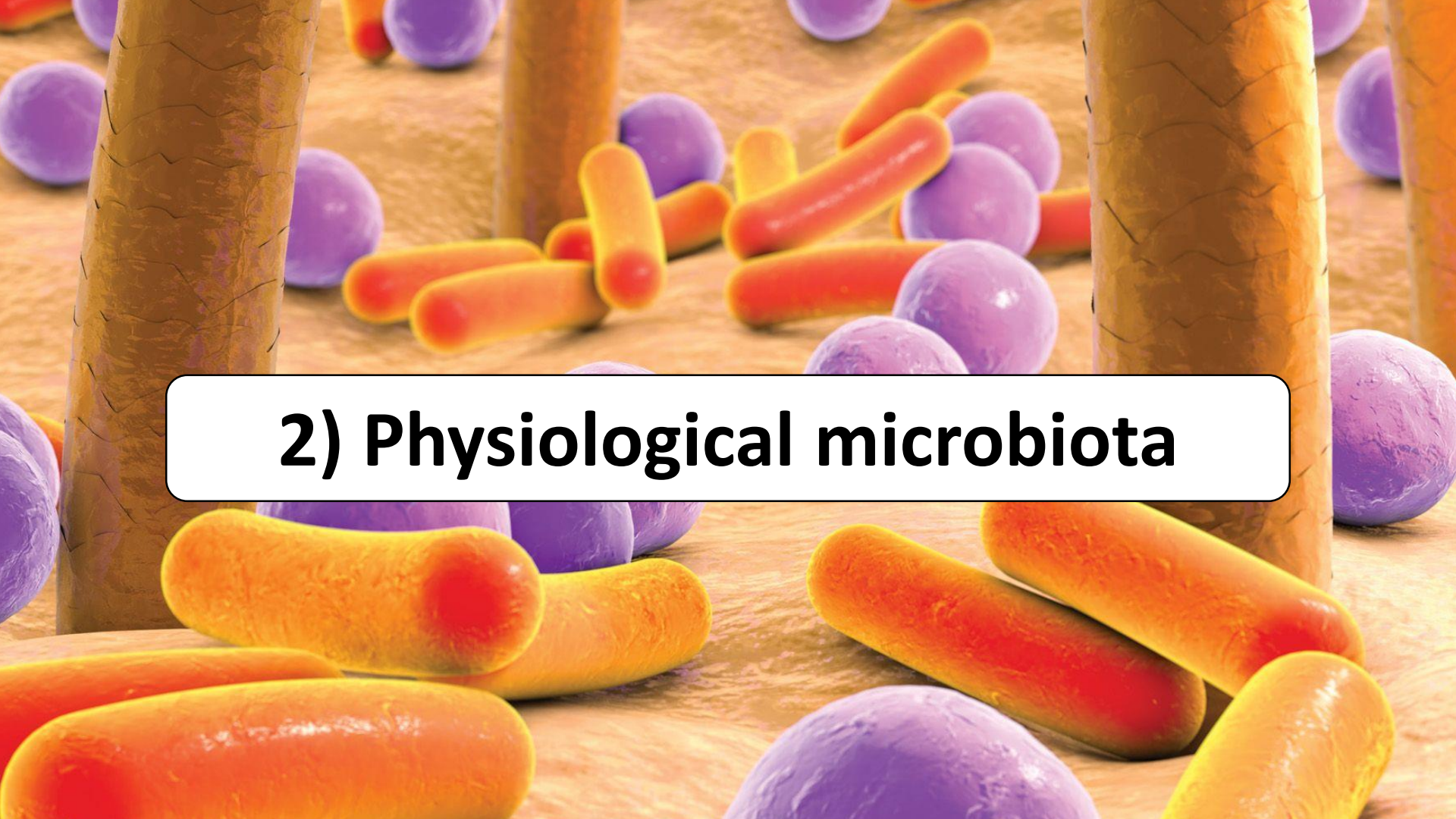
2. Sequence evaluation

Sequence goes to the NCBI BLAST database

Rating:
- Sequence similarity (98-100%)
- Number of hits

Sample: BA-9454

CGCCGCGTGAAGTGAAGGTCTTCGATCGTAAAACTCTGTTATTAGGAAGAACATATGTGTAAGTAACTG
TGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTATCCGGAATTATTATTGGGCGTAAAGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACG
GCTCAACCGTGGAGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGGAAAGTGGAATTCCATGTGTAG
CGGTGAAATGCGCAGAgATATATGGAGGAACACCAGTGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGAT
GTGCGAAAGCGTGGGATCAAACAGGATTAGATACCC



2) Physiological microbiota

A little terminology to start with

Microbiome

= genome

Molecular methods

Microbiota / ~~microflora~~

= living organisms

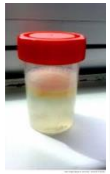
Cultures methods

3% !

PHYSIOLOGICAL MICROBIOTA

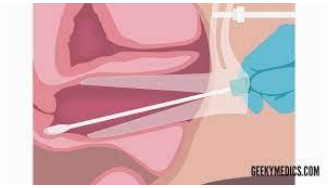
Coagulase-negative staphylococci, diphtheroids

Viridans streptococci, oral neisseria, diphtheroids

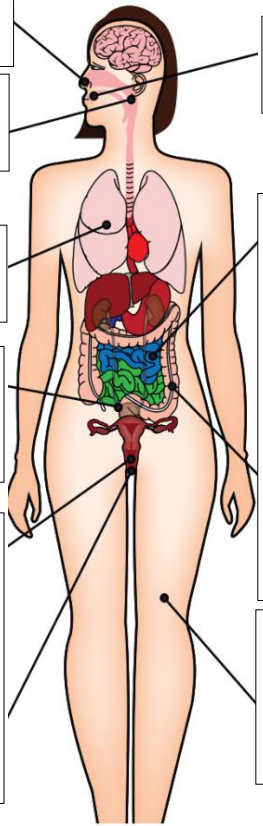


Viridial streptococci (swallowed)

CoN staphylococci, diphtheroids, enterococci (10^3 and less in urine)

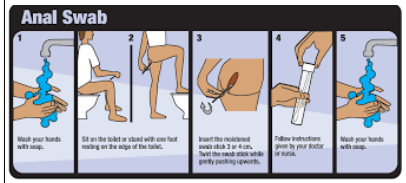


Lactobacilli, diphtheroids CoN staphylococci, viridal streptococci

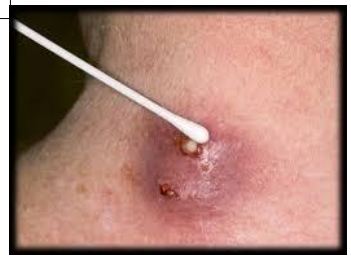


Viridans streptococci, oral neisseria, diphtheroids

Almost everything:
- except GI infections (Campylobacter, Salmonella, Yersinia)
- Toxigenic *C. difficile* and *H. pylori* (in stool)
- Parasites (*Cryptosporidium*, *Entamoeba histolytica* etc.*)* but not *Blastocystis* unless symptoms are present



Coagulase-negative staphylococci, diphtheroids, micrococci (*Cutibacterium acnes*)

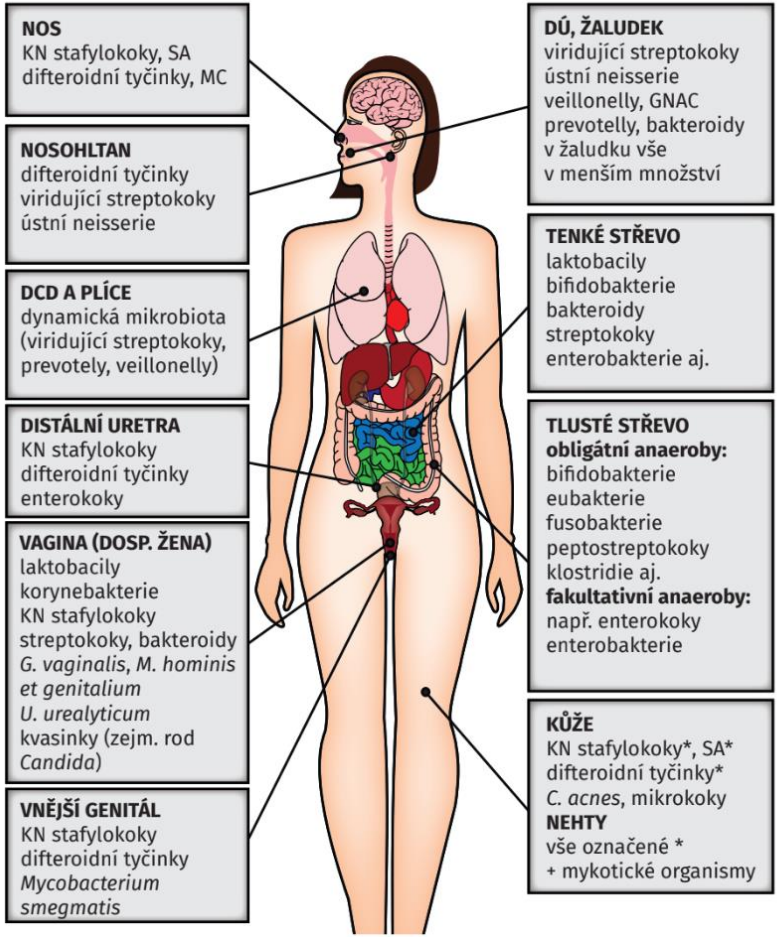


PHYSIOLOGICAL MICROBIOTA

Most common materials with physical microbiota

- Skin abrasion
- Nasal and nasopharyngeal swabs
- Throat swab
- Vaginal swab
- Rectal and stool swab

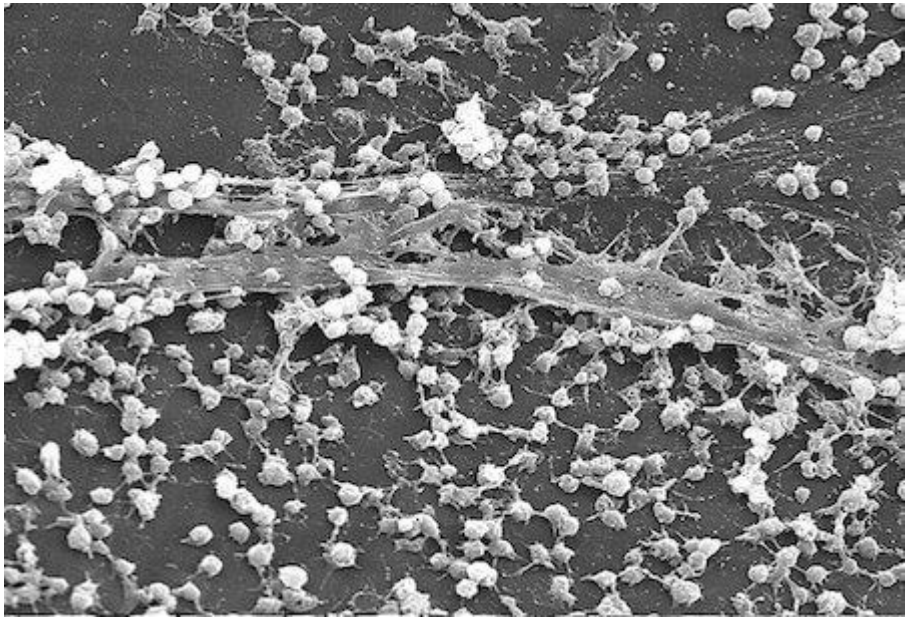
(Sputum and aspirates from DCD - but that's contamination from HCD)



Material	Physiological findings
Skin abrasion	Coagulase negative staphylococci, diphtheroids
Nasal and nasopharyngeal swabs	Skin microbiota, *S. aureus* carriage
Throat swab	Viridans streptococci and neisseria, anaerobes
Sputum and aspirates from DCD	Almost "sterile"
Vaginal swab	Lactobacilli, skin microbiota
Rectal and stool swab	Enterobacteriaceae, enterococci, skin microbiota

Coagulase-negative staphylococci

- Where does it make sense to test them? For all materials at risk of **biofilm** formation:
  - Blood cultures *
  - Catheters with significant quantity*
  - Orthopaedic materials (tissues, aspirates, swabs)
  - Wound swabs in spondylosurgical patients
  - Deep wounds with signs of infection



Staphylococcus aureus biofilm collected from an infected indwelling catheter *(The Role of Bacterial Biofilms in Antimicrobial Resistance*, ASM, 2023)

Name	Haemolysis on blod agar	Pathogenicity	
S. aureus (coagulase-positive)	Yes	+++	Physiologically in the nose (about 20%); Pathogenic potential: - IKMT, orthopaedic, pneumonia (! PVL+), IMC, ICU, - Enterotoxicosis, STSS, SSSS
S. capitis	Yes	+	Physiologically on the skin; Colonisation of catheters, substitutes and valves
S. epidermidis	No	+	
S. hominis	No	+	
S. haemolyticus	Yes	+	
S. lugdunensis	Yes	++	Physiologically on the skin; IKMT, orthopaedic, endocarditis, ICD
S. saphrophticus	No	++	Physiologically on the skin; IMC

CoNS are not only *S. epidermidis and S. saphrophticus*

* IKMT = skin and soft tissue infection; BSI = bloodstream infection; IMC = urinary tract infection; STSS = staphyl. toxic shock syndrome; SSSS = staphyl. scalded skin syndrome; PVL = Panton-valentine leukocidin)

Positive:
2/4 bottles

[redacted]

číslo: [redacted]
mater: **Hemokultivace**
upř+lok: aerobní periferie
odděl: INDM Interna - standard. 7.stanice

no: 16.12.2023-00:12
přijato: 16.12.2023-09:48
uzavřeno:

Výmaz Kopie | F2-rámec F5-oper F6-vyš

P-Půda | I-Izolace | .L-Identif | M-Mikro | A-ATB | K-kvantita | D-Maldi | C-ceka | H-Dohřáti | O-opak | E-Klin | V-Iden.Koky | Z-Zamrazit | S-ser | X-mimo | F-DUPLIKÁT

Kult	Dat	Operace	Výsledek ([F10] - vstup do editoru, [Ins] - tisk, [Ctrl /Ins] - kopie operace , [Alt/Ins] - kopie větve, [Shift/Ins] - vložit kopii)	T	U
		MIKROSKOPICKY	Uzavřít		
	17.12-05:41	Preparát z klinického materiálu:	g+koky ve shlucích	☑	☑
		Kultivace	Uzavřít		
1	16.12-09:48	krevní agar (Columbia) - hemokultura	dtto	☐	
	18.12-07:16	Maldi - koky	Staphylococcus epidermidis	☑	☑
	17.12-07:55	citl zóny Stafylokoky (3 řady)	OXA+ PEN? COT+ ERY+ KLI+ TET+ RIF+ OFL+ VAN- TEI- GEN+ LNZ+ TGC+ CPT+	☑	☑
	17.12-00:52	Doba do pozitivity	~~1d 0h 41m~~	☐	
	17.12-05:41	MacConkey půda HK		☑	
		Mikroaerofilní kultivace	Uzavřít		
	17.12-05:41	čokoládový agar		☑	☑

Materiál: **Hemokultivace** aerobní periferie
Vyšetření: hemokultivace, hemokultivace pozitivní , hemokultivace vyočkování

MIKROSKOPICKY

Preparát z klinického materiálu: g+koky ve shlucích

Kultivace

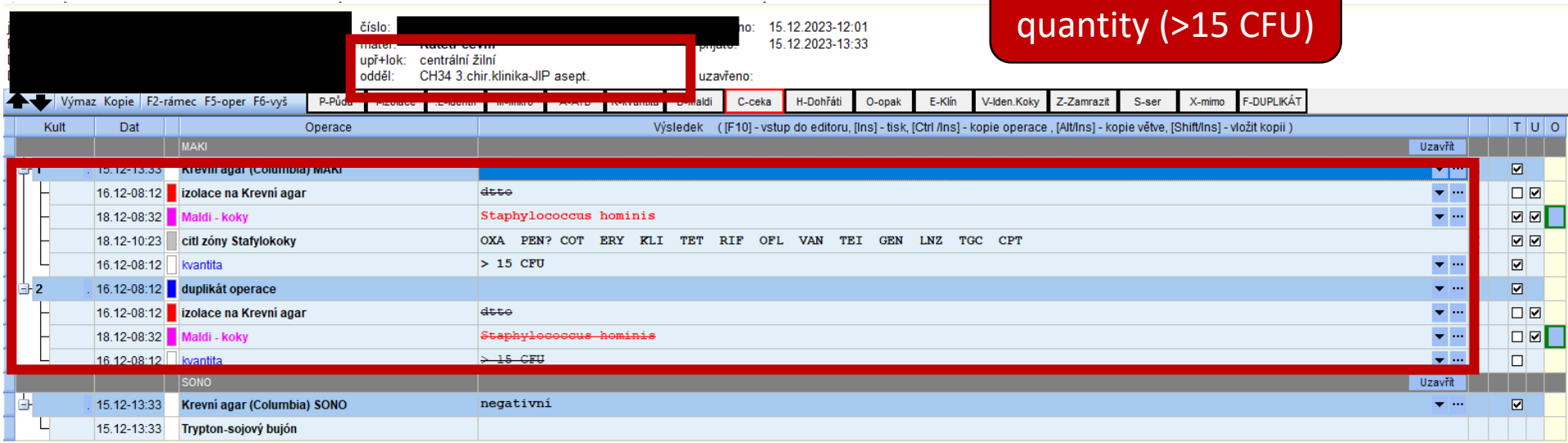
Nález 1: **Staphylococcus epidermidis**

ANTIBIOGRAM (disková difusní metoda)

oxacilin.....	C	ofloxacin.....	C
kotrimoxazol.....	C	gentamicin.....	C
erythromycin.....	C	linezolid.....	C
klindamycin.....	C	tigecyklin.....	C
tetracyklin.....	C	ceftaroline.....	C
rifampicin.....	C		

Zkratky: C = citlivý, R = rezistentní, I = intermediální, * = výsledek k dispozici po konzultaci s ATB střediskem

Significant quantity (>15 CFU)



Materiál: **Katetr cévní** centrální žilní
Vyšetření: cévní katetr - vyšetření

MAKI

Nález 1: **Staphylococcus hominis** > 15 CFU

SONO

Nález: **negativní**

[redacted]

číslo: [redacted]

mater: Tkáň
upř+lok: jiné (nutno uvést lokalizaci) TEP genus I. sin.
odděl: OSPE 1.ortoped.kl.-septické odd.

o: 12.12.2023-10:40
přijato: 12.12.2023-13:54
uzavřeno:

Výmaz Kopie | F2-rámec F5-oper F6-vyš

P-Půda | I-Izolace | .L-Identif | M-Mikro | A-ATB | K-kvantita | D-Maldi | C-ceka | H-Dohřáti | O-opak | E-Klín | V-Iden.Koky | Z-Zamrazit | S-ser | X-mimo | F-DUPLIKÁT

Kult	Dat	Operace	Výsledek ([F10] - vstup do editoru, [Ins] - tisk, [Ctrl /Ins] - kopie operace , [Alt/Ins] - kopie větve, [Shift/Ins] - vložit kopii)	T	U	O
		MIKROSKOPICKY	Uzavřít			
	12.12-13:54	Preparát z klinického materiálu:	buněčná drť, leukocyty masivně, bez mikrobů	☑	☑	
		PRIMOKULTIVACE	Uzavřít			
	12.12-13:54	krevní agar se stafyl.čárou (Columbia)	negativní	☑		
	13.12-06:50	dohřáti	~~dtto~~	☐		
	12.12-13:54	MacConkey agar		☑		
		POMNOŽENÍ	Uzavřít			
	12.12-13:54	bujón thioglykolátový		X		
2	12.12-13:54	krevní agar (Columbia) - pomnožení	~~dtto~~	☐		
	14.12-08:18	Maldi - koky	Staphylococcus epidermidis	☑	☑	
	14.12-08:18	citl zóny Stafylokoky (3 řady)	OXA- PEN? COT+ ERY- KLI- TET+ RIF+ OFL- VAN+ TEI+ GEN+ LNZ+ TGC+ CPT+	☑	☑	
	12.12-13:54	MacConkey agar - pomnožení		☑		
	12.12-13:54	Schaedler - 1.čtení	~~negativní~~	☐		
	12.12-13:54	Schaedler VL	Výsledek prodloužené kultivace sdělíme dodatečně.	☑		
	12.12-13:54	Bujón pro anaerobní kultivaci - thioglykolátový		☐		
	19.12-00:00	Schaedler - vyočkování po 5. dnech		☐	☑	
1	12.12-13:54	Schaedlerův agar - primokultivace	~~dtto~~	☐	X	
	14.12-07:05	Maldi - anaerobi	~~Staphylococcus epidermidis~~	☐	☑	
	14.12-08:33	kvantita	~~zcela ojediněle~~	☐		

Materiál: **Stěr z rány, defektu, píštěle, eflorescence...** hluboká operační 1.vzorek (Odběr)
Vyšetření: hluboká rána - kultivace vč. anaerobů

PRIMOKULTIVACE

Nález 1: **Staphylococcus pseudintermedius**

ANTIBIOGRAM (disková difusní metoda)

oxacilin.....	C	vankomycin.....	C
kotrimoxazol.....	C	teikoplanin.....	C
erythromycin.....	C	gentamicin.....	C
klindamycin.....	C	linezolid.....	C
tetracyklin.....	C	tigecyklin.....	C
rifampicin.....	C	ceftaroline.....	C
ofloxacin.....	C		

Nález 2: **Corynebacterium sp**

ANTIBIOGRAM (disková difusní metoda)

penicilin.....	R	cefotaxim.....	C
ampicilin.....	C	rifampicin.....	C
klindamycin.....	R	vankomycin.....	C
kotrimoxazol.....	C	linezolid.....	C
norfloxacin.....	C	tigecyklin.....	C

POMNOŽENÍ

Nález: **dtto**

Anaerobní kultivace

Nález: **negativní**

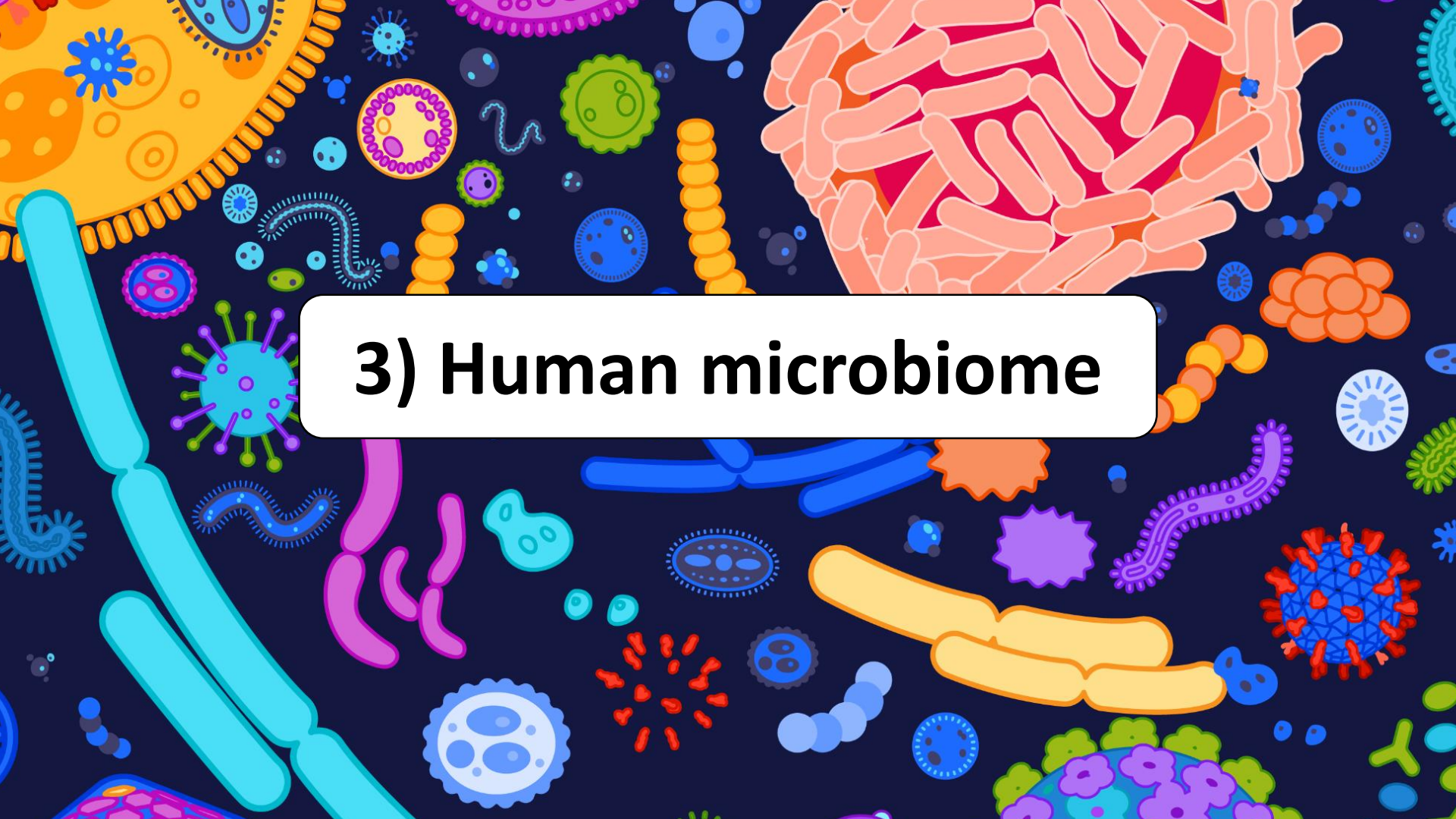
WATCH OUT FOR THESE FINDINGS with physiological microbiota

All in a sterile sample:

- ❑ Tissues
- ❑ Heart valves
- ❑ Blood cultures (except one of several vials where CN staphylococci - susp. contamination)
- ❑ Joint aspirate
- ❑ Cerebrospinal fluid

Among other things:

- ❑ More than 10^3 CFU from suprapubic puncture
- ❑ STD pathogens in children
- ❑ *E.coli* in from stool samples in infants and toddlers


3) Human microbiome

A little terminology to start with

Microbiome

= genome

Molecular methods

Microbiota / ~~microflora~~

= living organisms

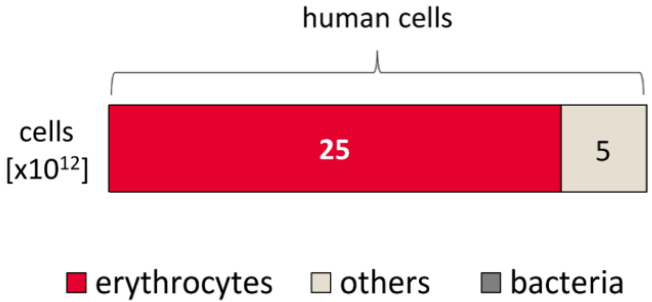
Cultures

3% !

Human super-organism

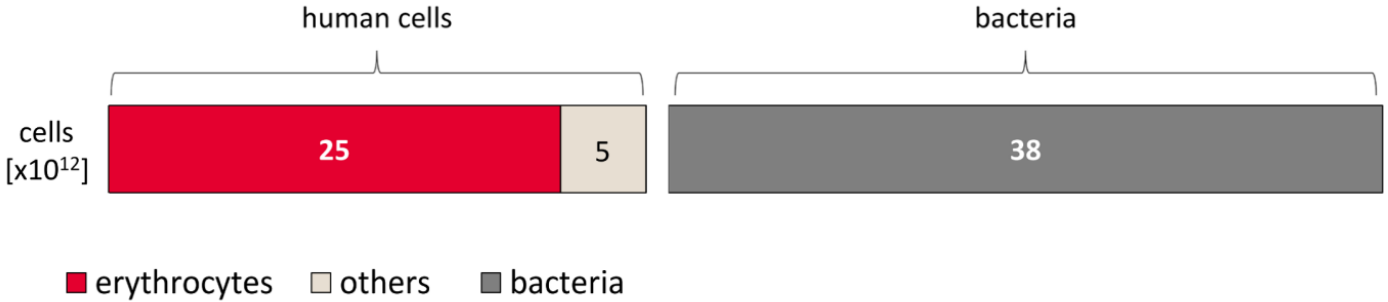
Do they live with us or do we live with them?

Are we more human or more microbes (bacteria)?



Sender et al, PLOS, 2016

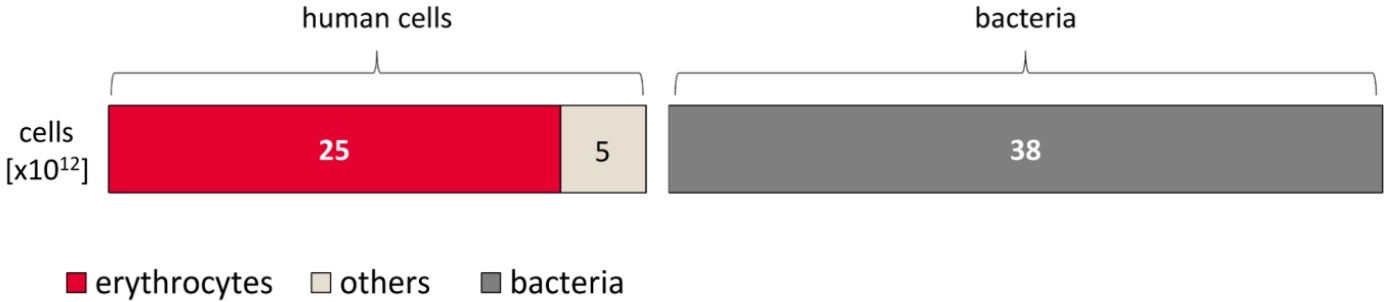
Are we more human or more microbes (bacteria)?



Sender et al, PLOS, 2016

	Number of cells
Human	30 trillion $(3.0 \times 10)^{13}$
Bacteria	38 trillion $(3.8 \times 10)^{13}$
	1.3x more bacterial

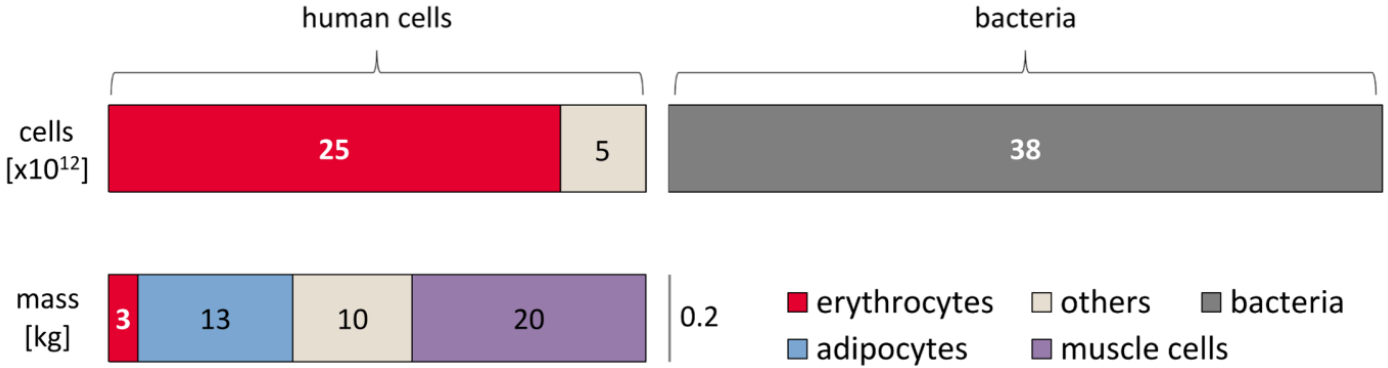
Are we more human or more microbes (bacteria)?



Sender et al, PLOS, 2016

	Number of cells	Number of genes
Human	30 trillion $(3.0 \times 10)^{13}$	20-25 thousand $(2.0 \times 10)^{4}$
Bacteria	38 trillion $(3.8 \times 10)^{13}$	2-20 million $(2.0 \times 10^{6} - 2.0 \times 10)^{7}$
	1.3x more bacterial	**100x more bacterial**

Are we more human or more microbes (bacteria)?

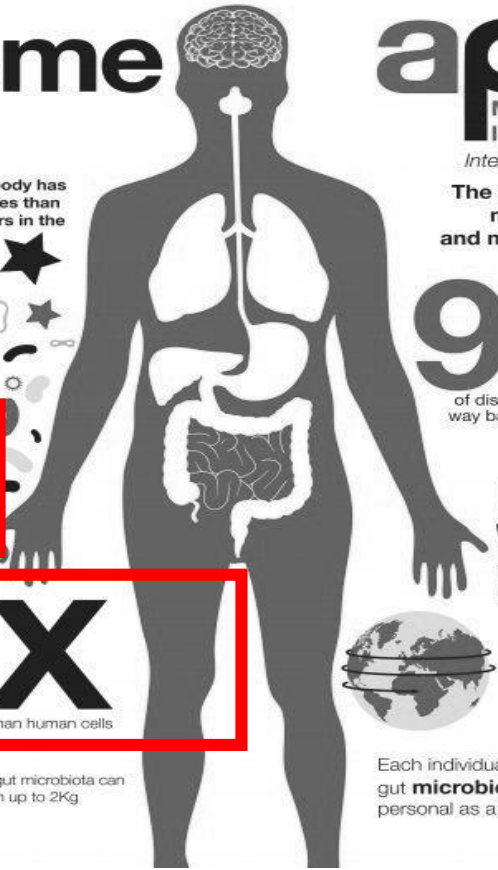


Sender et al, PLOS, 2016

	Number of cells	Number of genes	Matter
Human	30 trillion $(3.0 \times 10)^{13}$	20-25 thousand $(2.0 \times 10)^{4}$	70-100 kg
Microbes	38 trillion $(3.8 \times 10)^{13}$	2-20 million $(2.0 \times 10^{6} - 2.0 \times 10)^{7}$	0.2 kg
	1.3x more bacterial	100x more bacterial	**350-500x more human**

Microbiome

IN NUMBERS



38 trillion

symbiotic microbes live in and on every person and make up the human microbiota

The human body has more microbes than there are stars in the milky way

95%

of our microbiota is located in the GI tract

150:1

The genes in your microbiome outnumber the genes in our genome by about 150 to one

The surface area of the GI tract is the same size as 2 tennis courts

>10,000

Number of different microbial species that researchers have identified living in and on the human body

You have

1.3X

more microbes than human cells

2kg

The gut microbiota can weigh up to 2Kg

Microbiome Ireland

Interfacing Food & Medicine

The microbiome is more medically accessible and manipulable than the human genome

It is thought that

90%

of disease can be linked in some way back to the gut and health of the microbiome

5:1

Viruses:Bacteria in the gut microbiota

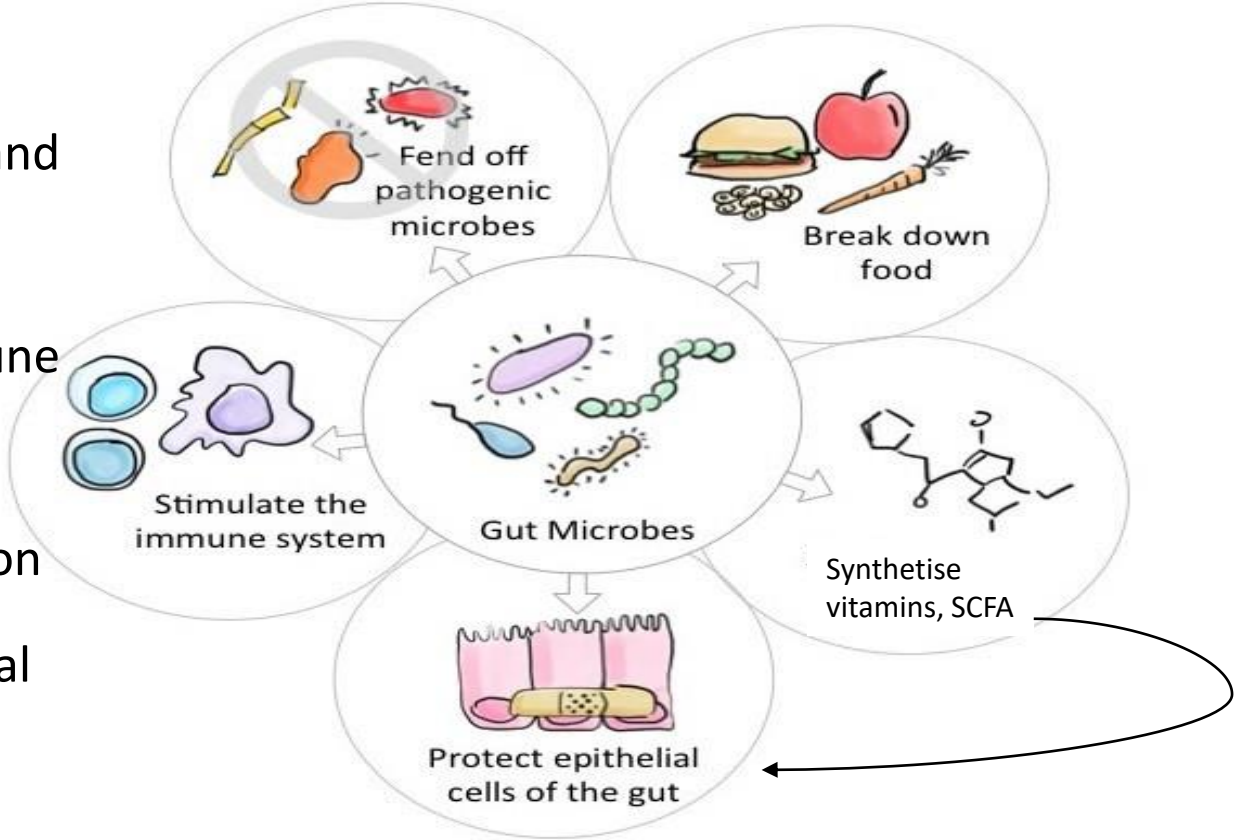
2.5

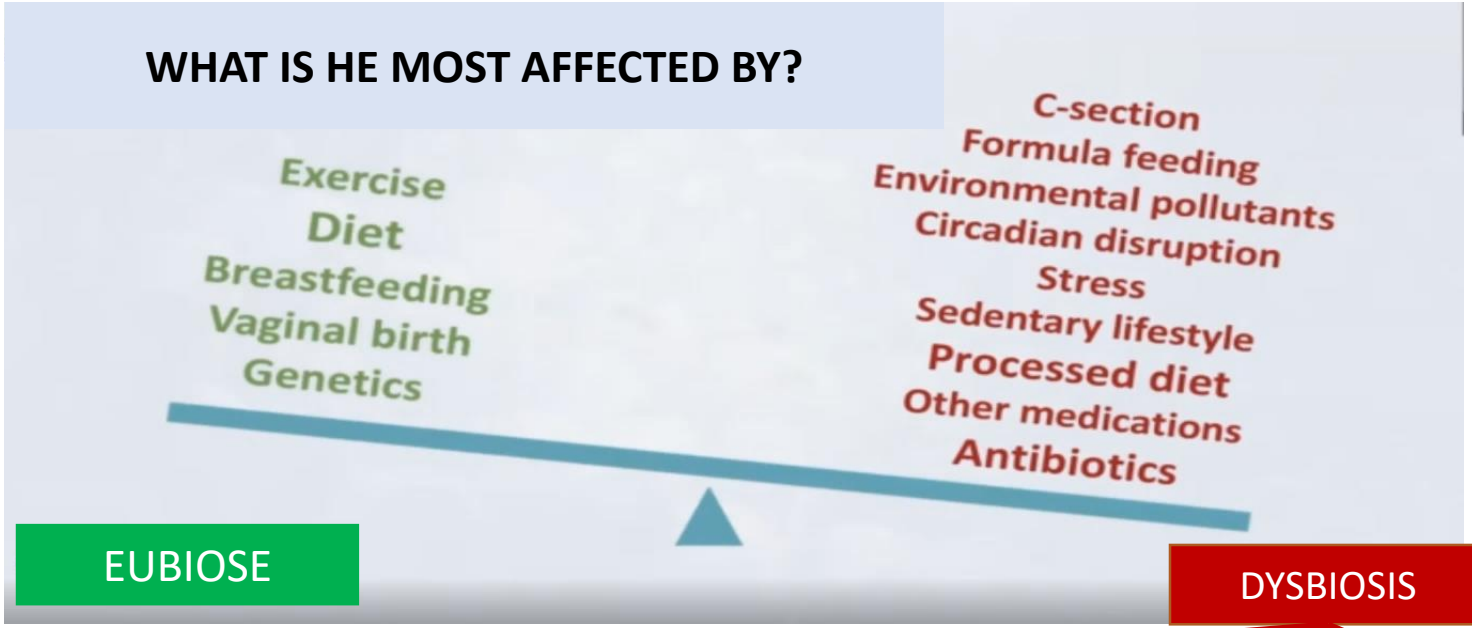
The number of times your body's microbes would circle the earth if positioned end to end

Each individual has a unique gut **microbiota**, as personal as a fingerprint

The physiological role of the microbiome

- Digestion
- Metabolism (vitamins and short-chain fatty acids)
- Regulation of the immune system
- Defence against infection
- Integrity of the intestinal wall





Non-specific intestinal inflammation (IBD)

- Crohn's disease (CD)

- Irritable bowel syndrome **(IBS)**

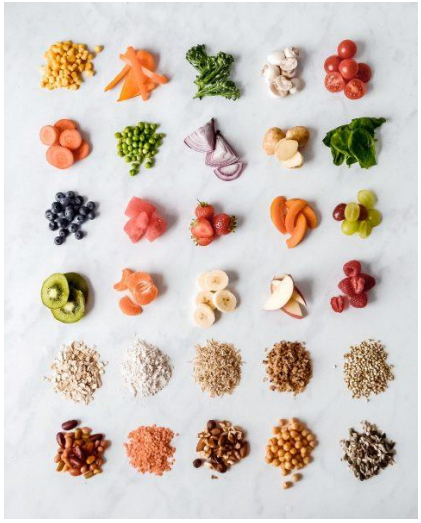
- Allergies, asthma, celiac disease

- Obesity, T2D

- Atherosclerosis and CVD

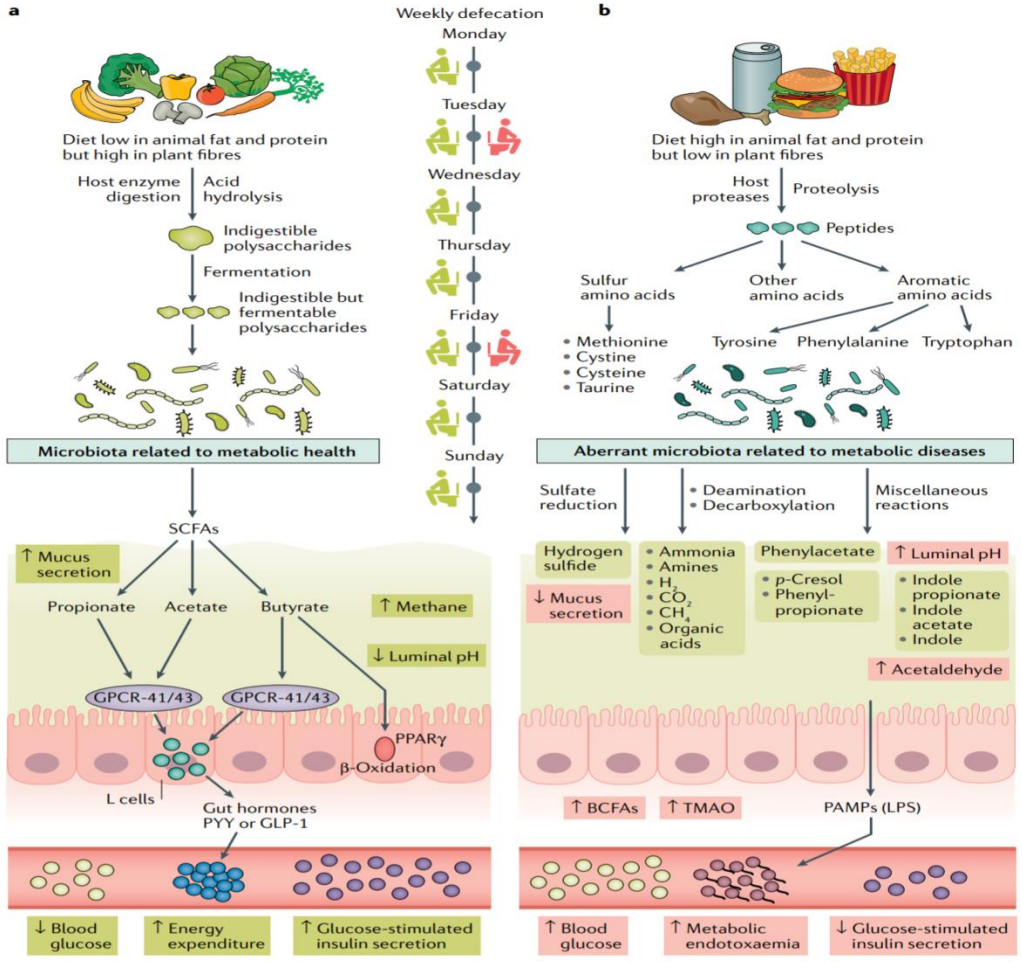
So what is a "good" diet?

(microbiome-wise)



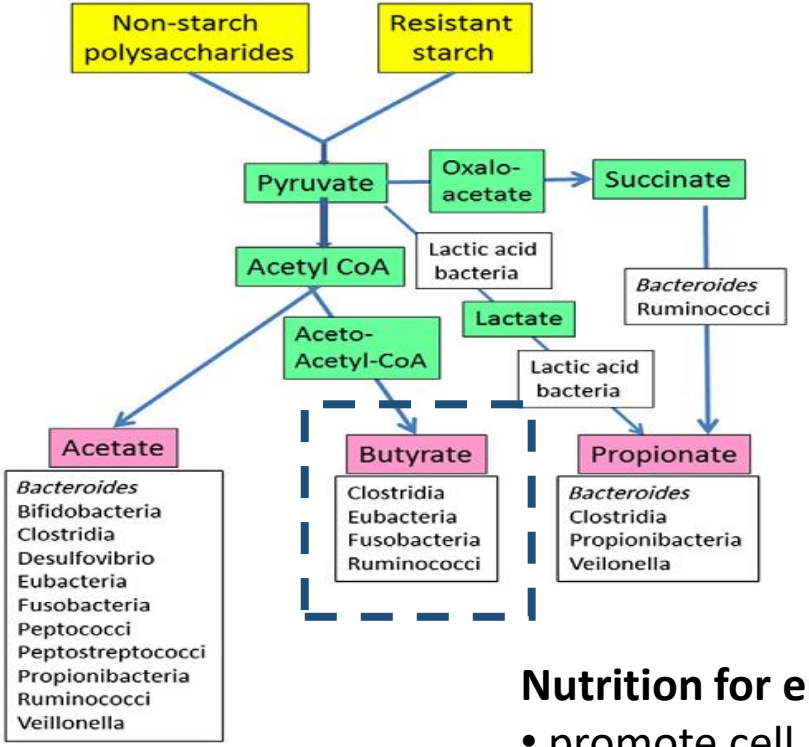
"Thirty different plants per week"

(Knight et al, **American Gut Project**, 2012)



Fan & Pedersen; *Nature Reviews Microbiology,* 2020

Short chain fatty acids (SCFA)

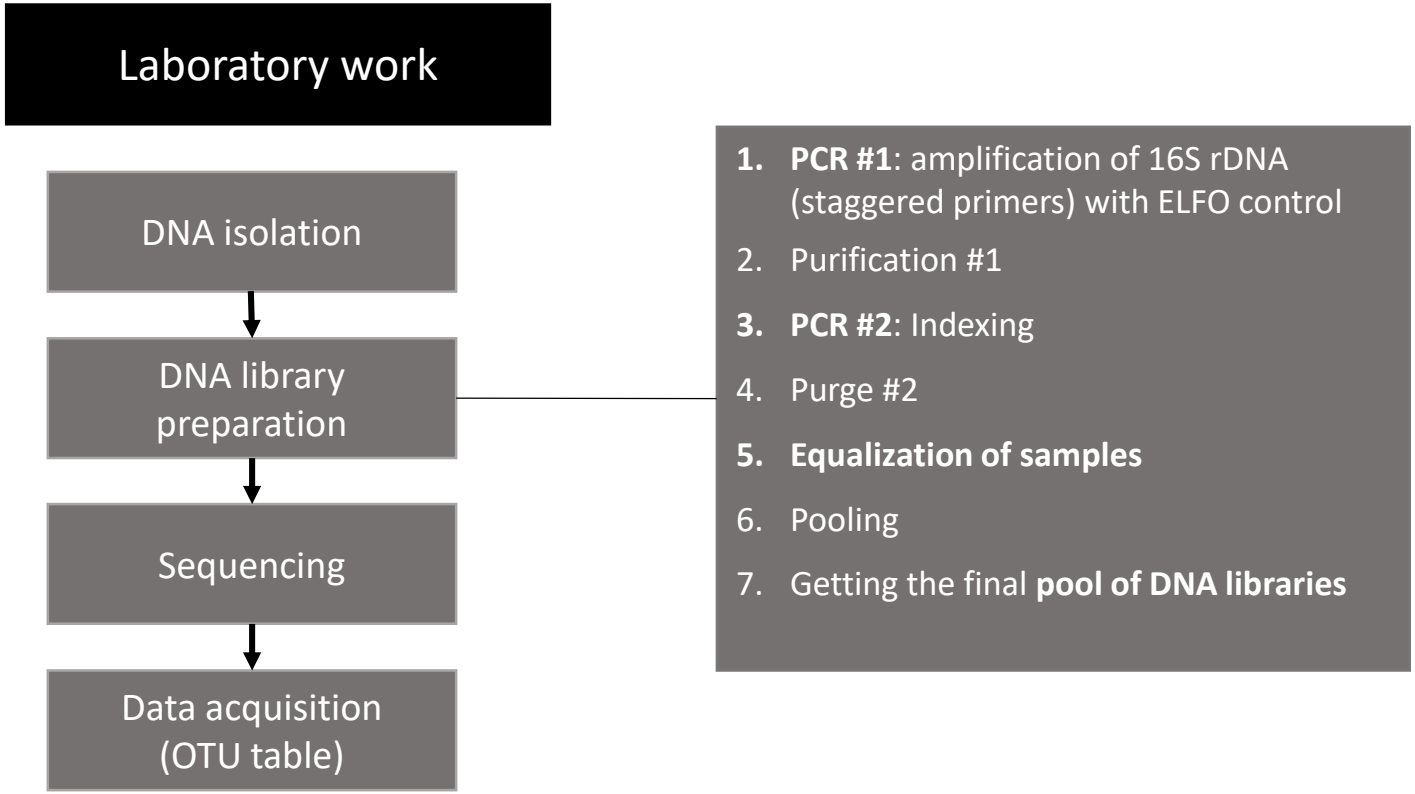


Ramakrishna BS. *J Gastroenterol Hepatol* 2013

Nutrition for enterocytes:

- promote cell proliferation and repair
- promote differentiation
- tighten connections (tight junctions)

How is this analyzed?
Or step by step - from sample to pretty pictures



Stool collection

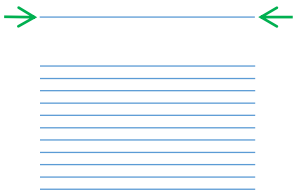
Stored at -80°C

DNA Extraction

DNA stored in -80°C

16S rDNA profiling

PCR for 16S rDNA



Mass sequencing of this gene

Group reads by similarity, count them

>OTU1:
AAGCATATGCTATGATCGATCATCATGACT
>OTU2: CATGATCTGACTATTATTCGCGATTTG
>OTU3: GCGATATTCGATCTATTCGATGCGGAT

Classify taxonomy

>OTU1: Firmicutes; Clostridia; Clostridium piliforme
>OTU2: Firmicutes; Clostridia; Ruminococcus bromii
>OTU3: Firmicutes; Bacilli; Leuconostoc

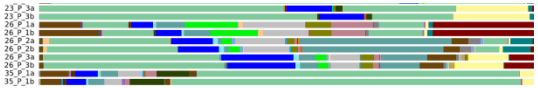
Metagenomic sequencing

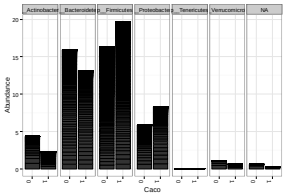
Randomly fragment **total DNA**

Sequence, assemble genes

Data analysis

Analyze composition





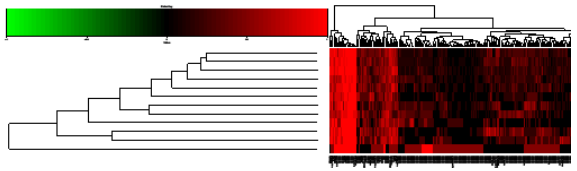
Compare cases with controls

Classify **genes by function**

> glucosidase
> lambda phage capsid
> lactase
> cable pilus
....

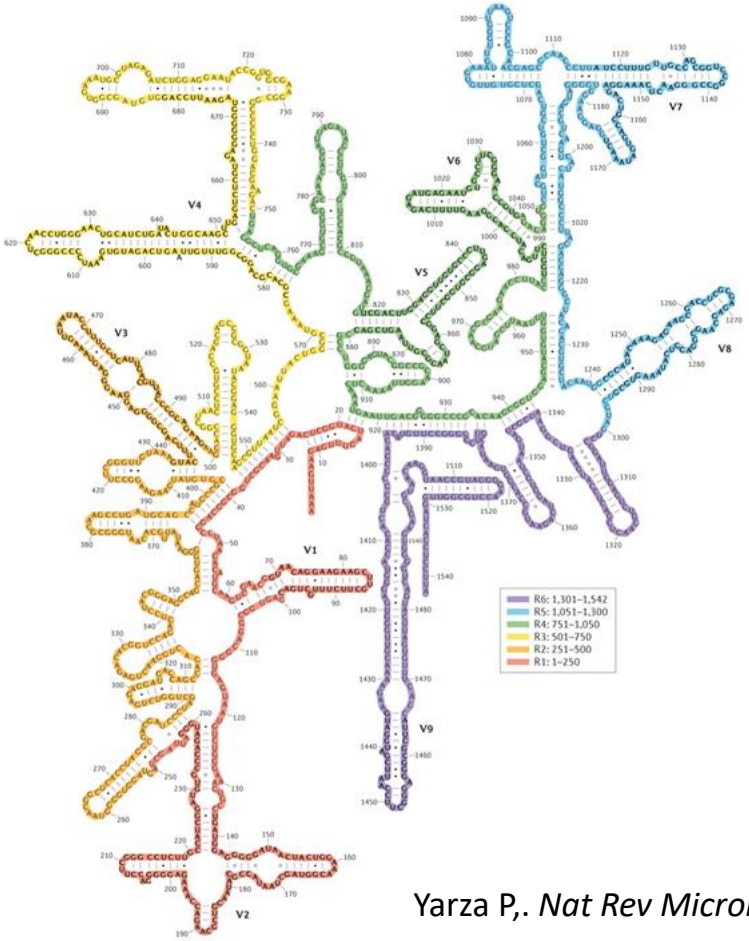
Assess **functional capabilities** of the microbiome

Compare cases with controls



Gene for 16S rRNA

- = part of the bacterial small ribosomal subunit (30S, consisting of 21 proteins and 16S rRNA)
- Size: 1542 bp
- Gene structure:
  - conserved regions (where primers are inserted) and 9 hypervariable regions V1-V9 (this is amplified and then sequenced).
  - The most used area is **V3-V4** (about 440 bp)



Yarza P,. *Nat Rev Microbiol*; 2014

Gene for 16S rRNA

16S rDNA is a linear structure - > transcribes into a linear rRNA, and folds.

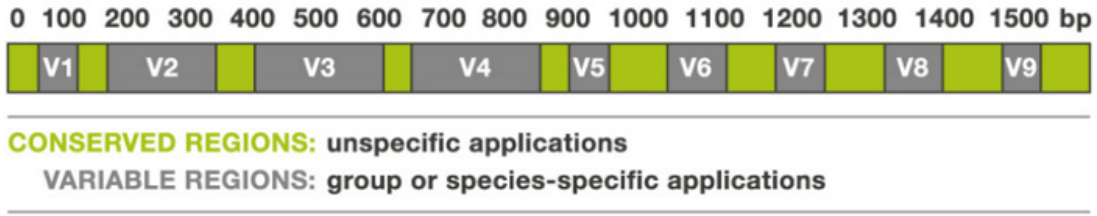
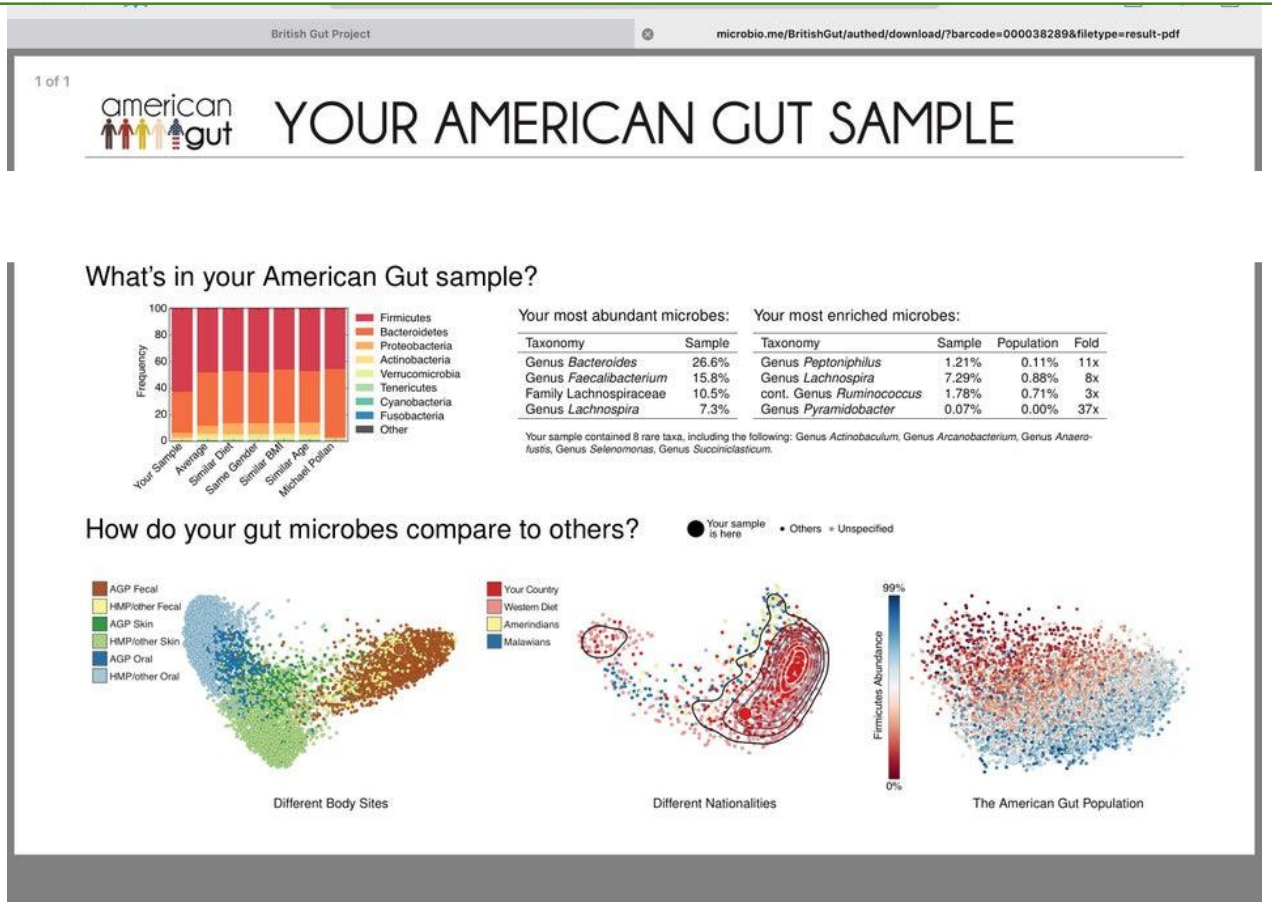


Figure 1: An example of a 16S rRNA gene. The regions in green are conserved in all microorganisms. These are the sites that are targeted by primers for PCR amplification so that all the 16S rRNA genes in a sample are amplified. The grey regions are the species-specific regions that-- when sequenced-- allow for scientists to see which species are present in a community. Image courtesy of: http://www.alimetrics.net/en/index.php/dna-sequence-analysis

<u>Benefits and why it is used:</u>

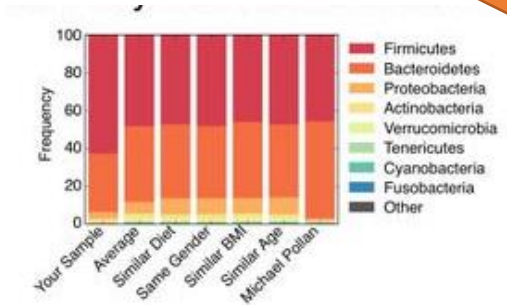
- It is both a highly conserved and ubiquitous sequence
- It's relatively easy and cheap to sequence
- There is a good reference database (Silva, GreenGenes, RDP)

What the result may look like



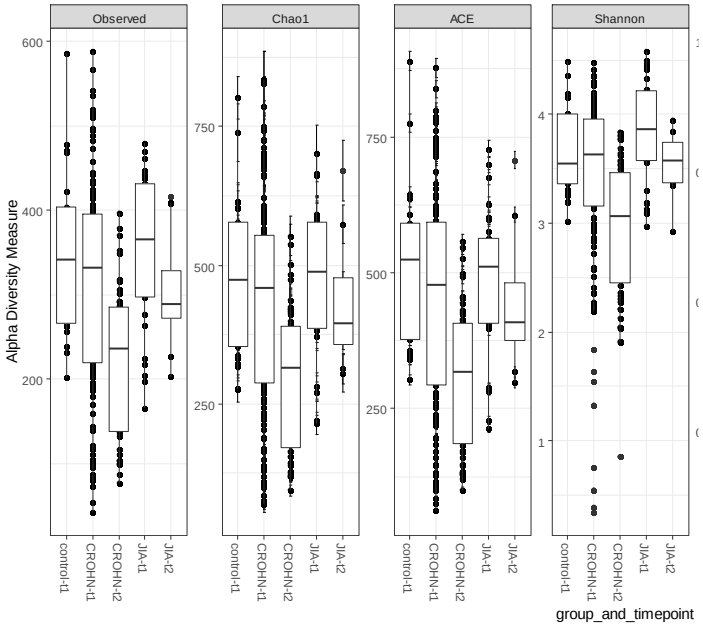
Composition - what lives there?

Main strains	Classes	Examples of genera
Actinobacteria	*Actinobacteria*	*Actinomyces; Bifidobacterium*
Bacteriodetes	*Bacteroidia*	*Bacteroides; Prevotella; Alistipes*
Firmicutes	*Bacilli*	*Bacillus; Staphylococcus*
		Enterococcus; Lactobacillus; Lactococcus; Streptococcus; Leuconostoc
	Clostridia	*Clostridium; Coprococcus; Roseburia; Faecalibacterium; Ruminococcus*
	Negativicutes	*Veillonella*
Proteobacteria	*Epsilonproteobacteria*	*Helicobacter; Campylobacter*
	Gammaproteobacteria	*Citrobacter; Escherichia; Shigella; Klebsiella; Providencia ....*
Verrucomicrobia	*Verrucomicrobiae*	*Akkermansia*

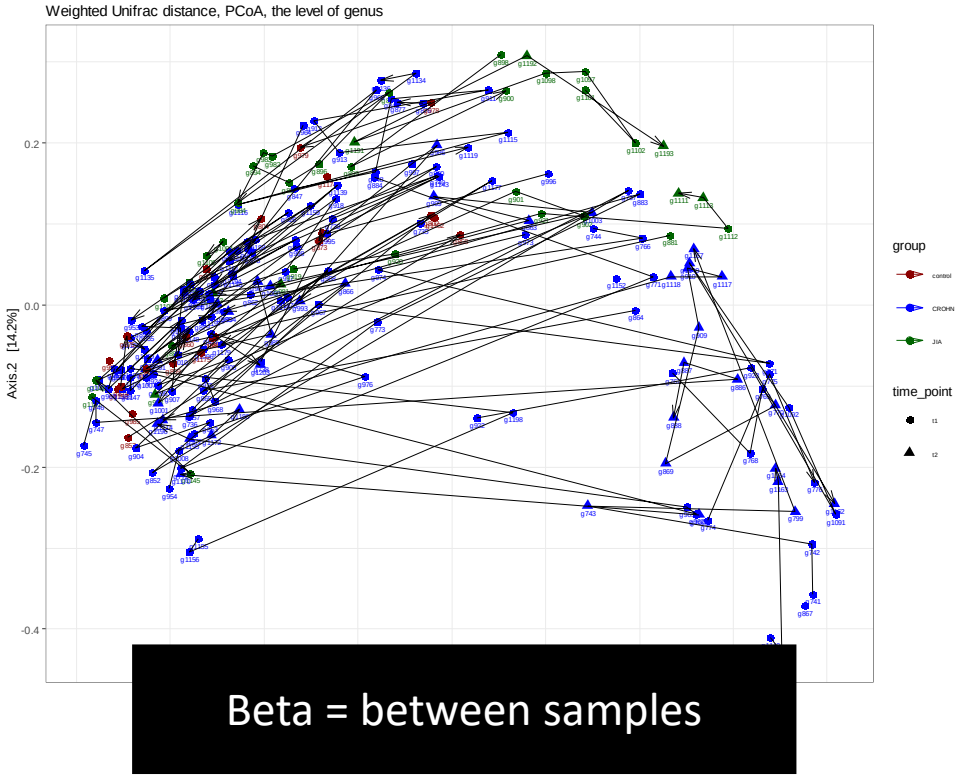


90%

Diversity - alpha and beta



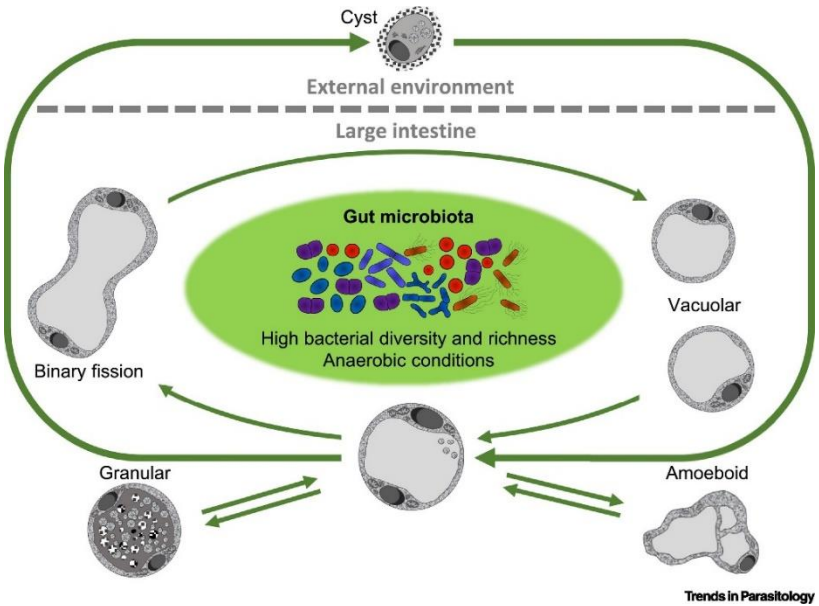
Alpha = in one sample



Beta = between samples

Outside bacteria: *Blastocystis*

- The most abundant eukaryote in the human gut [1-3]
- **Marker of high bacterial diversity** [4,5]
- Prevalence varies
  - Higher in developing countries (40-100%) [6-8]
  - Lower in industrialized countries (7-50%) and intestinal diseases (up to 5%) [9-12]
- It is classified into subtypes (ST1-ST41) [13]
  - Confirmed 37 STs
  - 15 of them in humans (ST1-ST4 represent 90% of all)



Stensvold, Trends in Parasitology, 2020

1)Tito. *Gut.* 2019; 2) Andersen. *FEMS Microbiol Ecol.* 2015; 3) Rostami. *Parasitol Res.* 2017; 4) Clark. *Adv Parasitol.* 2013; 5) Cinek. *Parasite Vectors.* 2021; 6) Poulsen. *Am J Trop Med Hyg*. *2016;* 7) Mohammad. *Asian Pac J Trop Med*. 2017; 8) Oliveira-Arbex; *Infect Genet Evol*. 2018; 9)Wawrzyniak. *Ther Adv Infect Dis.* 2013; 10) Stensvold. *Parasitol Int*. 2016; 10) Bart. BMC Infect Dis. 2013; 11) El Safadi. *BMC Infect Dis*. 2016; 11) *Scanlan. Infect Genet Evol.* 2016; 11) Scanlan. *FEMS Microbiol Ecol*. 2014. 12) Lhotska. *Front Cell Infect Microbiol*. 2020; 13) Hernandez, *J Eukaryot Microbiol*, 2023)

What is a "good and bad" outcome?

GOOD

High alpha diversity (300-1000 species)

Anaerobic environment (e.g. very few *Proteobacteria*)

More SCFA producers

Blastocystis positive

A healthy microbiome?

BAD

Low alpha diversity (less than 100 species)

Many facultative anaerobes (e.g. multiple *Proteobacteria*)

Few SCFA producers

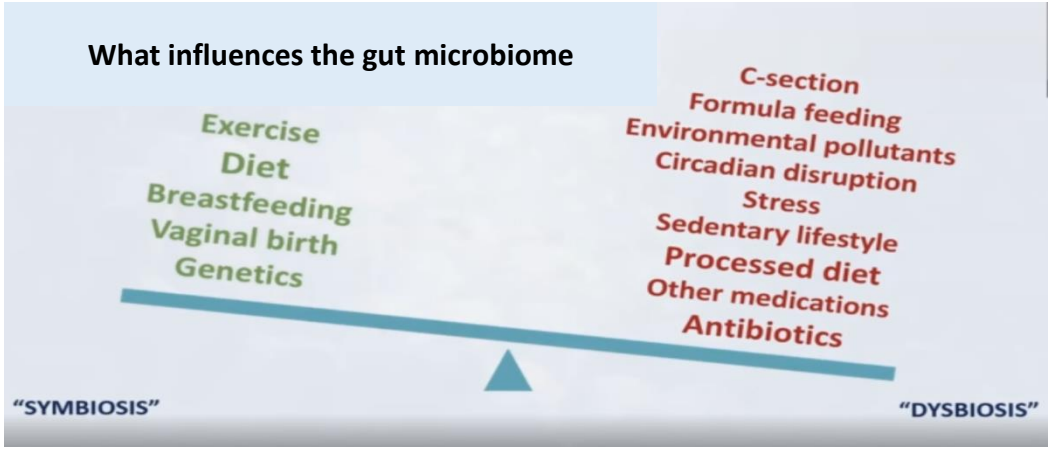
Blastocystis negative

When parents/patients ask about intestinal myrobiomyoma

Tell them to:

- Eat a wide variety of plant foods
- Sleep well, they exercise and they are outdoors

Consultation of results: among others diversity and abundance of anaerobes



What you can do as doctors:

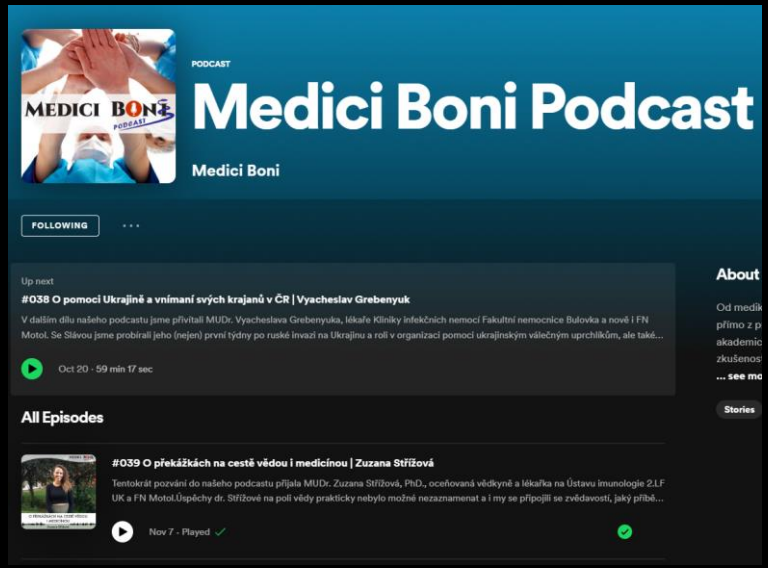
- Tell them the same thing without being asked
- Prescribe ATBs only when necessary - **antibiotics are not candies!**
- Do not treat *Blastocystis* in an asymptomatic patient

Be calm when you see the physiological microbiota

- But feel free to give us a call

Take-home message

1. You are a **superorganism** (cellular: 1.3 times more microbial than human)
2. Fibre-rich foods are the best food for gut microbes that produce SCFAs, which are food for enterocytes and maintain gut integrity, among other things
3. The main strains of bacteria in the gut are *Firmicutes* and *Bacteroides*
4. Tell patients to eat a variety of plant foods, exercise and encourage breastfeeding. Prescribe ATBs only when necessary.
5. Learn what a physiological finding from each sample looks like, so you'll be at ease when you see it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#038 O pomoci Ukrajině a vnímaní svých krajanů v ČR | Vyacheslav Grebenyuk
V dalším dílu našeho podcastu jsme přivítali MUDr. Vyacheslava Grebenyuka, lékaře Kliniky infekčních nemocí Fakultní nemocnice Bulovka a nově i FN Motol. Se Slávou jsme probírali jeho (nejen) první týdny po ruské invazi na Ukrajinu a roli v organizaci pomoci ukrajinským válečným uprchlíkům, ale také...
Oct 20 · 59 min 17 sec
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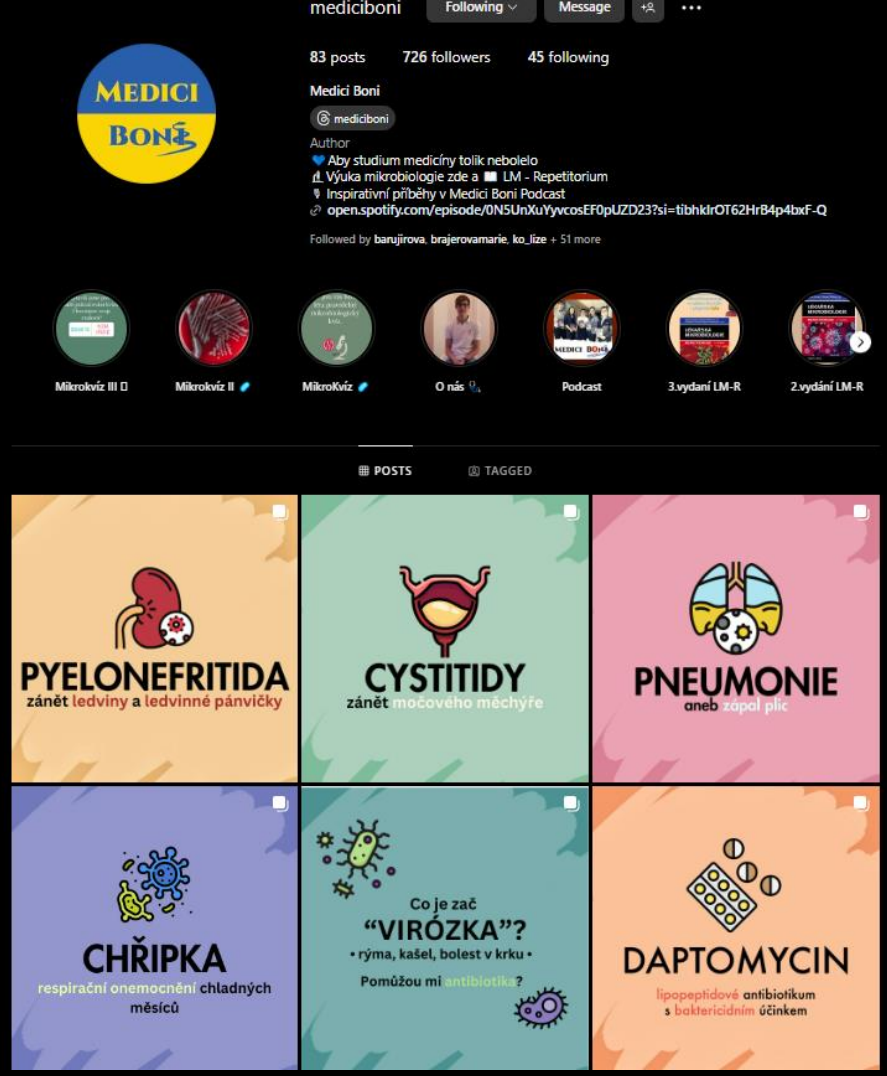
#039 O překážkách na cestě vědou i medicínou | Zuzana Střížová
Tentokrát pozvání do našeho podcastu přijala MUDr. Zuzana Střížová, PhD., oceňovaná vědkyně a lékařka na Ústavu imunologie 2.LF UK a FN Motol.Úspěchy dr. Střížové na poli vědy prakticky nebylo možné nezaznamenat a i my se připojili se zvědavostí, jaký příbě...
Nov 7 - Played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