MIKROBIOM ČOVJEKA
(u zdravlju i bolesti)

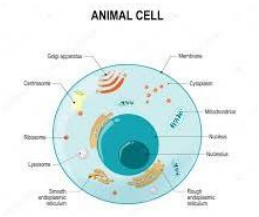
1. PREDAVANJE

14.03.2024.

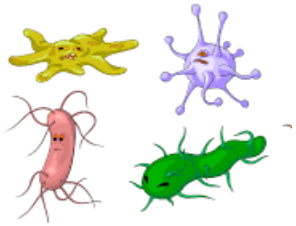
We are not alone in our bodies!

TIJELO SVAKOG POJEDINCA UDOMLJAVA OKO $39x10^{12}$ BAKTERIJA (+virusi, gljive i drugi mikrobi). Zbirni naziv - **MIBROBIOTA**

Omjer „vlastitih” i bakterijskih stanica?



1:1,3



1-3%
mase tijela

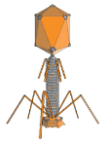
Omjer „vlastitih” i bakterijskih gena?

1:100

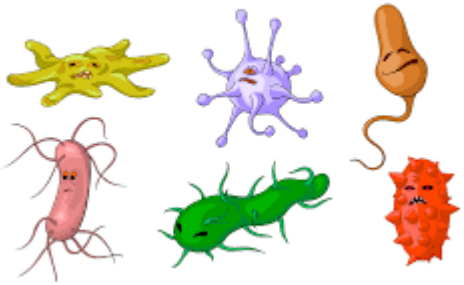
(preko 1000 bakterijskih vrsta s oko 2000 gena svaka)

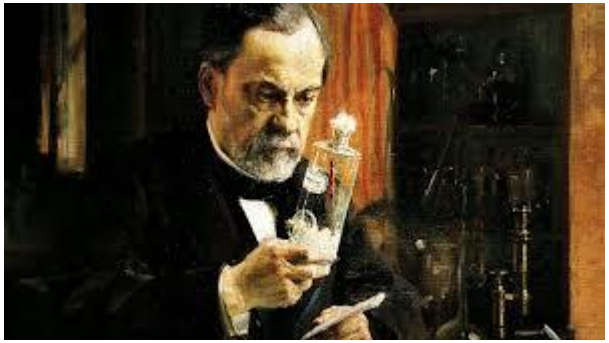
Omjer bakterija i virusa (važna uloga u regulaciji mikrobiote)

1:10



Ima li života bez mikroba?



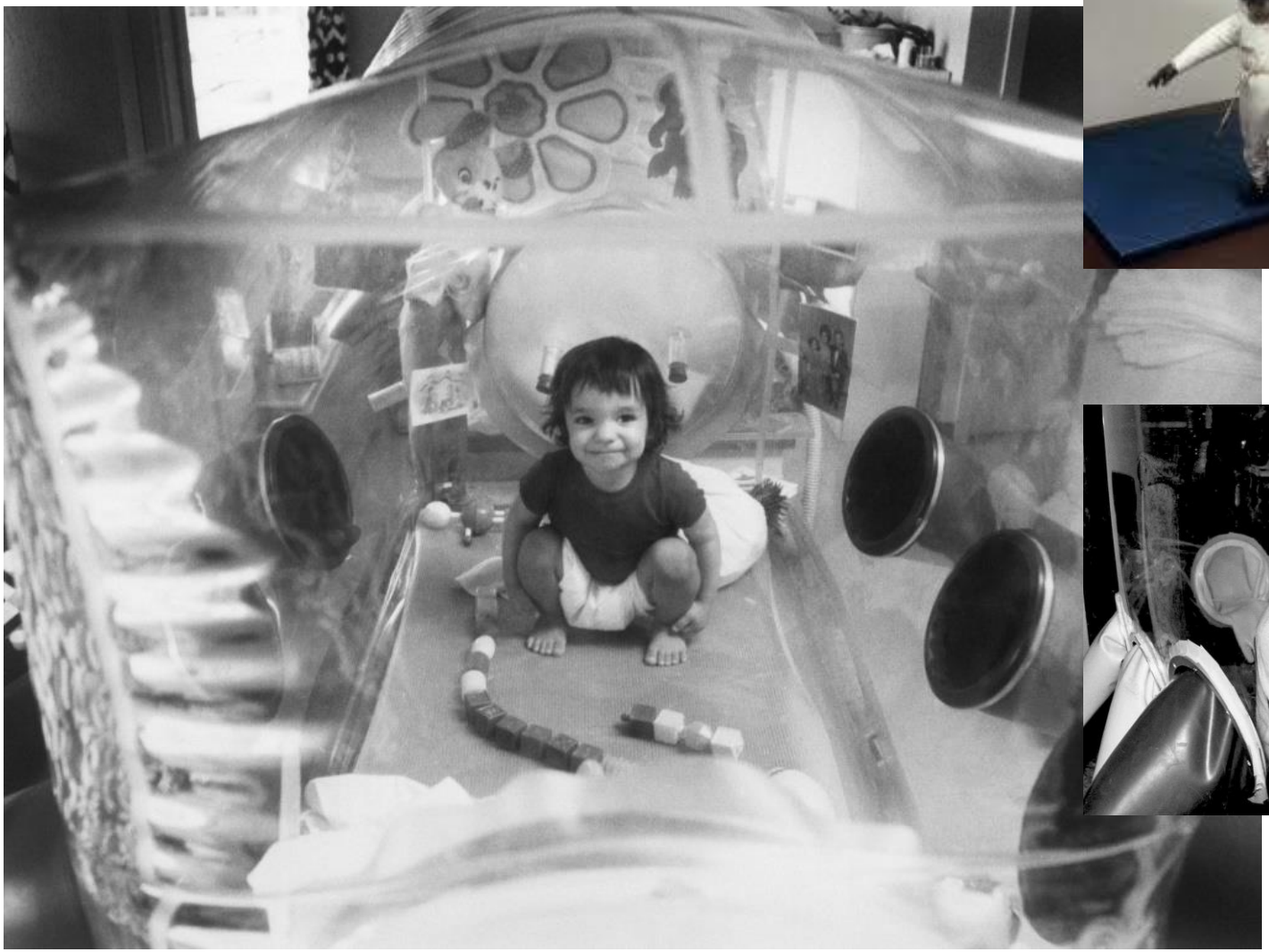


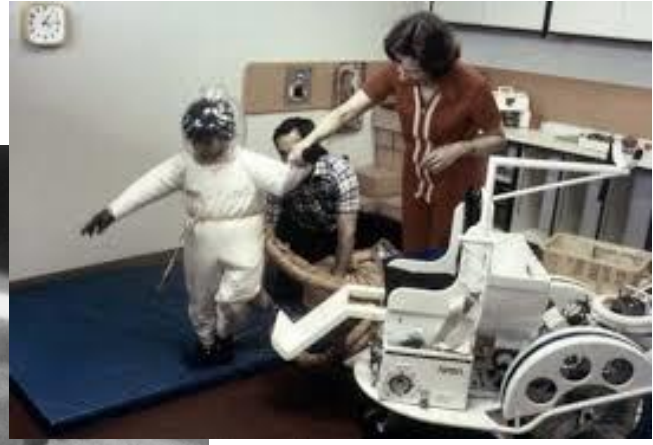
???

1885. L. Pasteur
život bez mikroba nije moguć

1928.-1958. J. A. Reyniers
„germ free" životinje

Život bez mikroba JE MOGUĆ!





David Vetter „bubble boy” 1971.-1984.

Tko je bio u pravu?

MIKROBIOTA

DOBROBITI:

- „treniranje” imunosnog sustava, inhibicija patogena
- razgradnja hrane
- proizvodnja vitamina, masnih kiselina, amino kiselina...

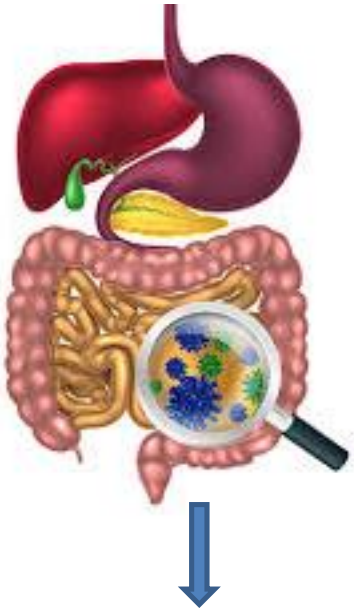
PROBAVILO ČOVJEKA

- više od tisuću različitih bakterijskih vrsta

(dodatno: arheje, gljive, protozoa i virusi)

- najveći broj i raznolikost bakterija u kolonu

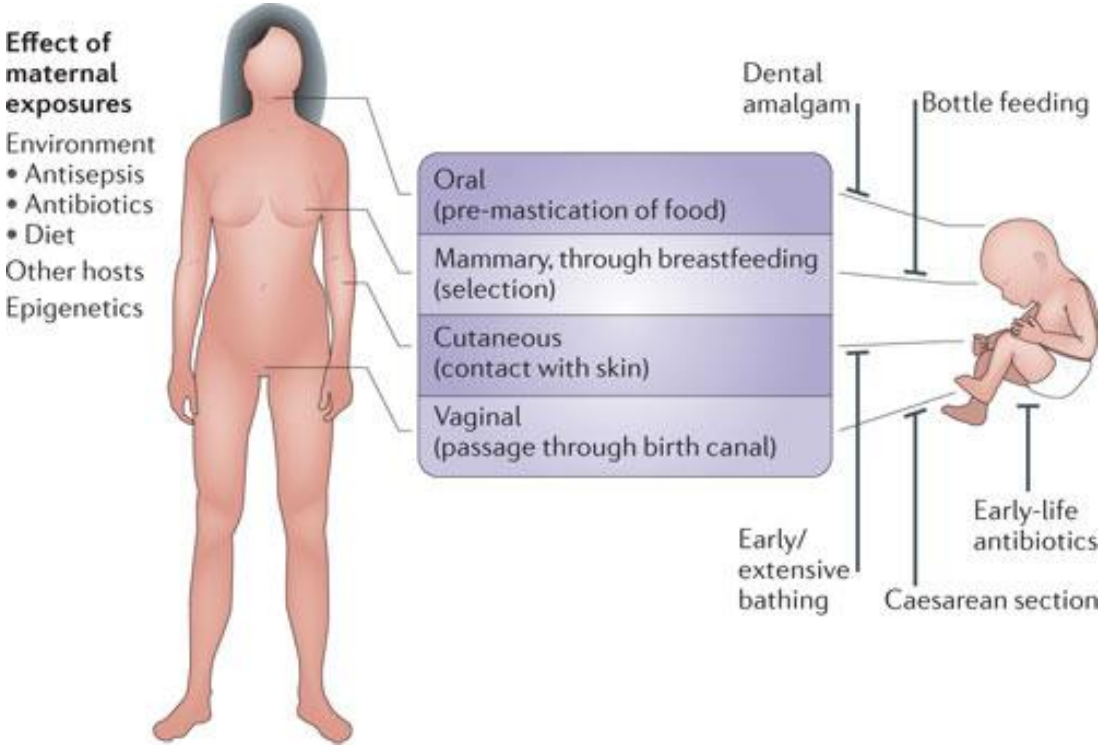
(anaerobni uvjeti, manje nutrijenata, prehrana sekretima...)



100 milijardi mikroba/ 1g fecesa

INTRAUTERINI RAZVOJ – sterilni uvjeti ???

TIJEKOM PORODA I NAKON ROĐENJA – kolonizacija bakterijama, arhejama, gljivama i virusima putem kontakta s majkom i okolišem



Nekoliko godina po rođenju normalna mikrobna flora, tzv. MIKROBIOTA naseljava: POVRŠINU KOŽE, NOSNU I USNU ŠUPLJINU, PROBAVNI I URO-GENITALNI TRAKT

uloga u zdravlju i bolesti?

faktori koji utječu na njen sastav?

PROJEKT LJUDSKOG MIKROBIOMA (ciljni mikrobi - bakterije)

Terminologija:
MIKROBIOM – zbir mikrobnih genoma unutar mikrobne zajednice

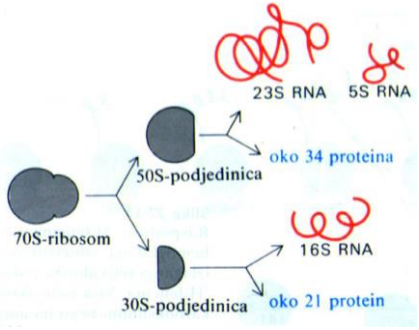
2003. završen projekt ljudskog genoma
(3 milijarde nt, ≈ 23 000 protein-kodirajućih gena)

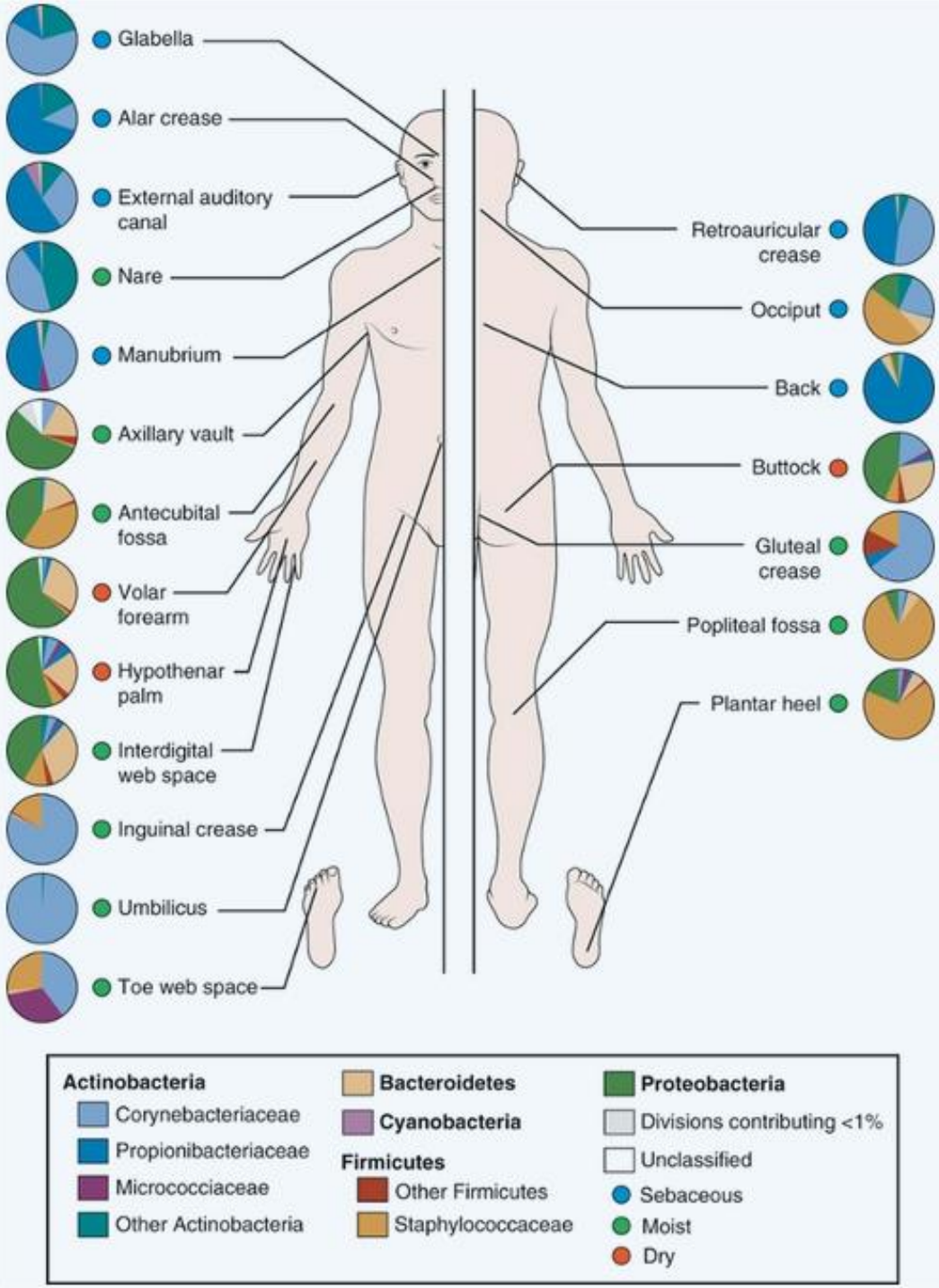
C. Venter
F. Collins

RAZVOJ TEHNOLOGIJA I INFORMATIČKIH ALATA ZA MASOVNU ANALIZU DNA i mRNA

2007. započinje višegodišnji multinacionalni **PROJEKT LJUDSKOG MIKROBIOMA**

- uzorkovani zdravi odrasli dobrovoljci (nos, usta, koža, crijevo i vagina)
- ukupno 300 x više protein-kodirajućih gena
- sekvencirane ciljne regije - geni za 16S rRNA (identifikacija)
- sekvenciranje čitavih genoma pojedinih uzoraka (genska raznolikost)



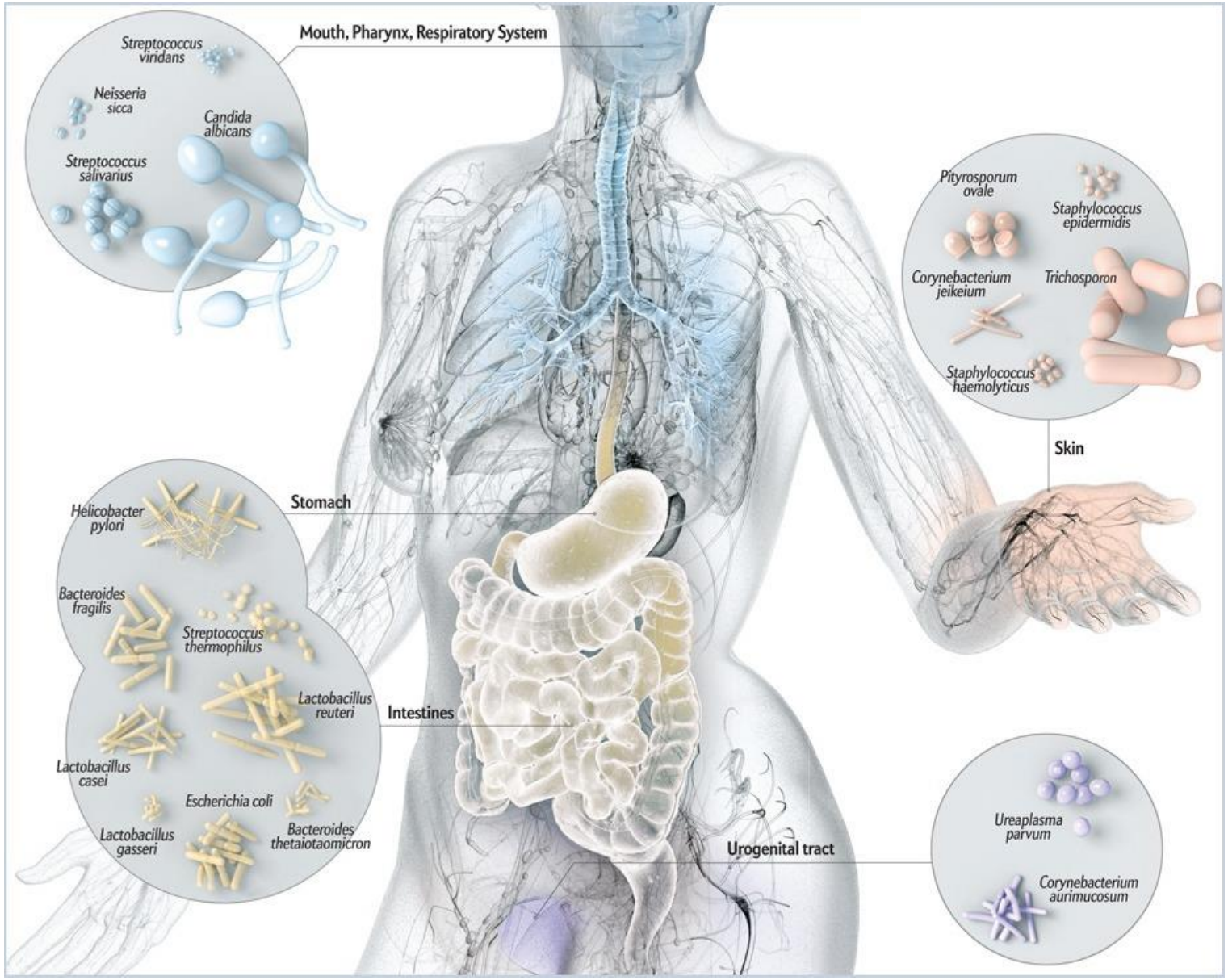


OSNOVNE SPOZNAJE:

- bitne razlike između mikrobnih populacija na različitim dijelovima tijela
- razlike i između pojedinaca
- najveća genska i taksonomska raznovrsnost među bakterijama crijeva
- najmanja genska i taksonomska raznovrsnost među bakterijama vaginalnih uzoraka
- sastav ovisan i o mikrookolišu (pr. različiti dijelovi crijeva imaju karakterističan mikrobiom)

DISTRIBUCIJA BAKTERIJA NA KOŽI

Grice E, Segre J: The skin microbiome, Nat Rev Microbiol 9:244–253, 2011.


Mouth, Pharynx, Respiratory System
Streptococcus viridans
Neisseria sicca
Candida albicans
Streptococcus salivarius
Pityrosporum ovale
Staphylococcus epidermidis
Corynebacterium jeikeium
Trichosporon
Staphylococcus haemolyticus
Skin
Stomach
Helicobacter pylori
Bacteroides fragilis
Streptococcus thermophilus
Lactobacillus reuteri
Intestines
Lactobacillus casei
Escherichia coli
Lactobacillus gasseri
Bacteroides thetaiotaomicron
Urogenital tract
Ureaplasma parvum
Corynebacterium aurimucosum

TEMELJNI MIKROBIOM čine mikrobne vrste prisutne u najmanje 95% pojedinaca (manji broj vrsta, velika brojčana zastupljenost pojedine vrste)

- najveći broj „zajedničkih" vrsta prisutan je redom u ustima, nosu, crijevu i na koži
- najmanji broj „zajedničkih" vrsta prisutan je u vaginalnim uzorcima

SEKUNDARNI MIKROBIOM čine mikrobne vrste karakteristične za pojedinca (veći broj vrsta, mala brojčana zastupljenost pojedine vrste)

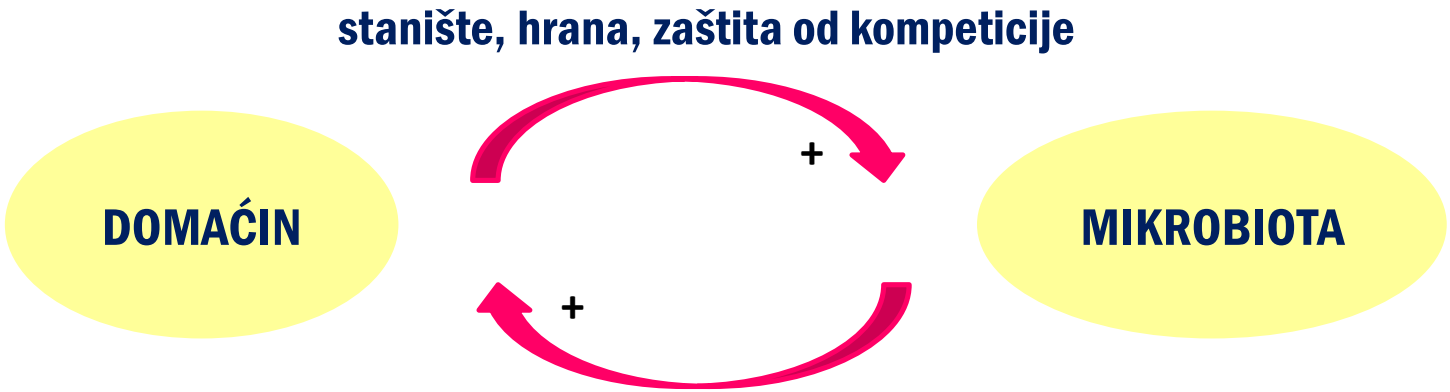
ZAKLJUČAK:
Mikrobi temeljnog mikrobioma esencijalni su za funkcioniranje organizma (probava, stimulacija imunosnog odgovora...), dok je uloga sekundarnog mikrobioma također važna, ali nije nužno vezana uz određenu mikrobnu vrstu (različite mikrobne vrste mogu obavljati istu funkciju*).

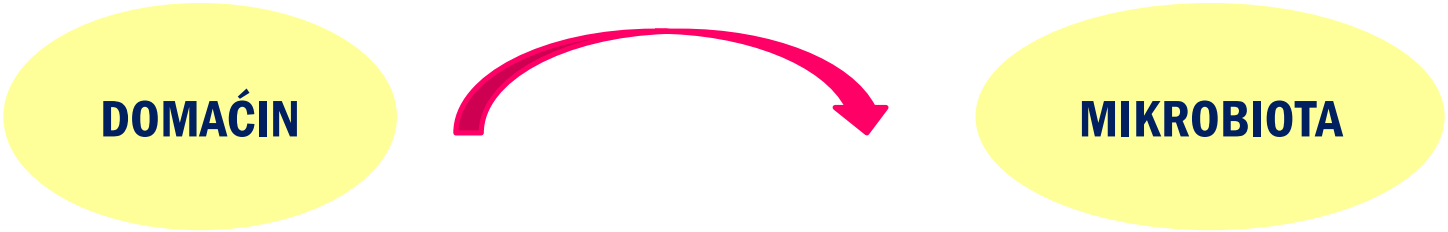
IAKO JE TAKSONOMSKA RAZNOLIKOST VELIKA, FUNKCIONALNE KARAKTERISTIKE SU VISOKO KONZERVIRANE!!!

Razlike između mikrobioma zdravih pojedinaca su prihvatljive sve dok je njihova funkcija očuvana!

- **FUNKCIONALNA REDUNDANCIJA** - višak istovrsnih komponenata u nekom složenom sustavu bez kojih bi sustav mogao raditi, ali se funkcije umnožavaju zbog sigurnosti (ista funkcija osigurana je različitim članovima mikrobiote).

EVOLUCIJA MIKROBIOMA





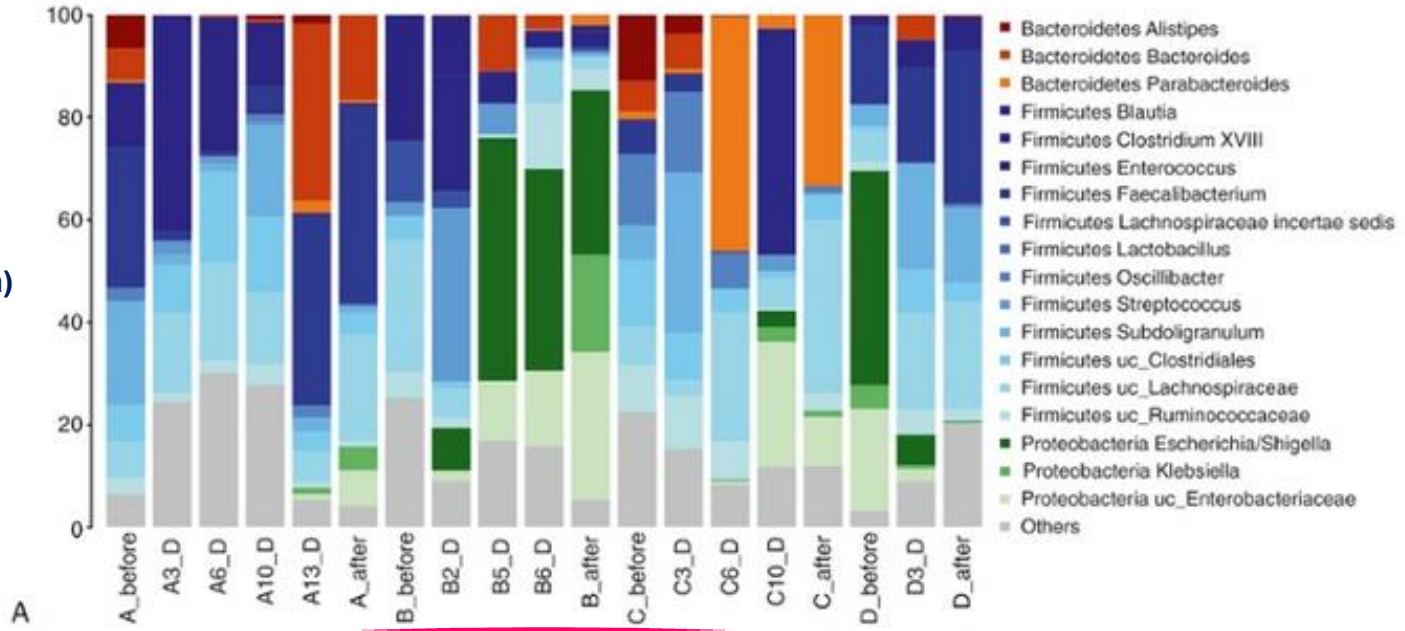
BROJNOST I SASTAV MIKROBIOMA U RAZLIČITIM DIJELOVIMA TIJELA OVISI O:
količini dostupnog kisika, pH, koncentraciji soli i dostupnosti nutrijenata

UTJECAJ DODATNIH FAKTORA:
higijenske navike (sapuni, antiperspiranti, vodice za ispiranje usta...),
prehrana (vlakna, šećeri, masnoće)
stupanj kloriranosti pitke vode
izloženost toksinima iz okoliša
lijekovi* - nužna kontrolirana primjena antibiotika ne samo zbog razvoja rezistencije, nego i zbog ugrožavanja funkcije mikrobiote > BOLEST

*Utjecaj antibiotika na mikrobnu floru crijeva

(slovima su označeni pacijenti tretirani različitim antibioticima)

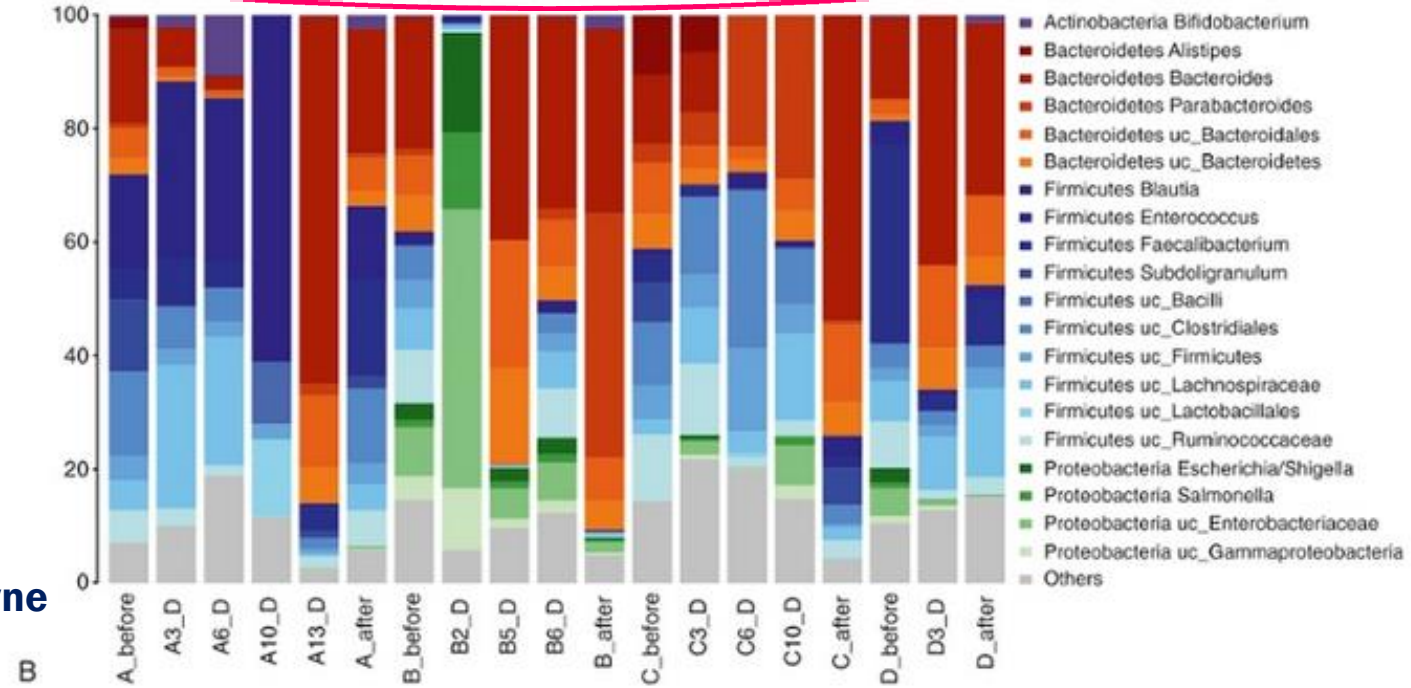
ukupne bakterije



A

cell replication inhibitor | protein synthesis inhibitor | cell envelope synthesis inhibitor

metabolički aktivne bakterije



B

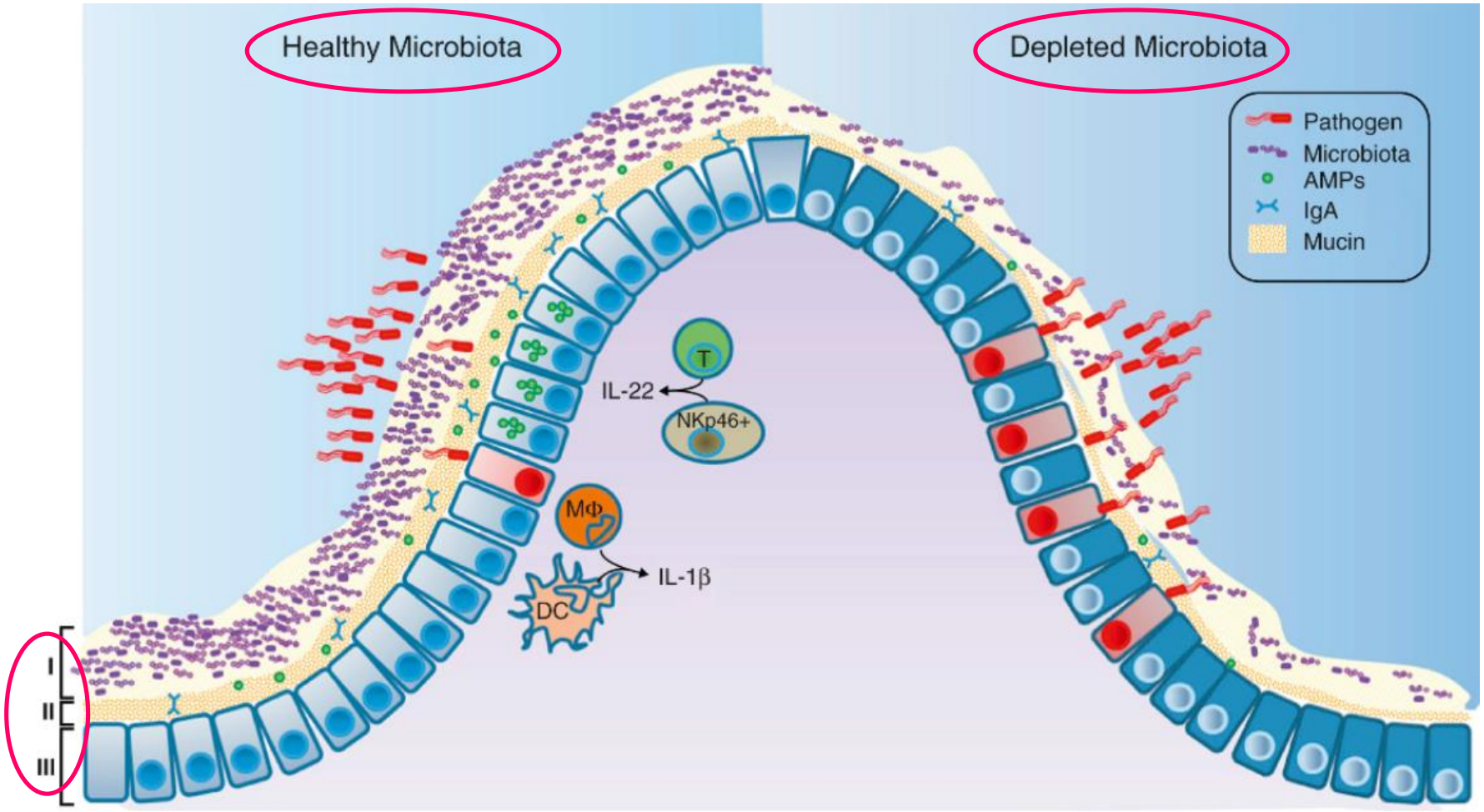
- crijevo čovjeka kolonizira preko tisuću bakterijskih vrsta (uloga u metabolizmu)

- razgradnjom složenih ugljikohidrata (npr. celuloza, pektini…) nastaju masne kiseline (acetati, propionati i butirati) koje služe kao izvor energije*, a imaju brojne pozitivne efekte (npr. inhibiraju rast nekih patogenih bakterija)

- sposobnost *de novo* sinteze vitamina B12, K2, folne kiseline…

- „borba" bakterija različitih vrsta za ekološku nišu lučenjem toksina (npr. kolicin *E.coli*) koji inhibiraju rast konkurentskih vrsta, a istovremeno štite domaćina od patogena (*Salmonella, Shigella*, …)

*Pojedine skupine bakterija (koljeno *Firmicutes)* su posebno efikasne u razgradnji složenih ugljikohidrata što rezultira većom pohranom produkata metabolizma te može dovesti do pretilosti (potencijalna terapija kod pothranjenosti)



Uloga crijevne mikrobiote u prevenciji crijevnih infekcija:

I – populacijska zasićenost (velika potrošnja nutrijenata) sprječava pristup patogena domaćinskom tkivu

II – mikrobiota pomaže nespecifičnu imunost stimulacijom proizvodnje mucina, antimikrobnih peptida (AMP) te IgA

III - mikrobiota stimulira proizvodnju interleukina IL-22 (povećava rezistenciju epitela) i IL-1β (regrutira stanice upalnog odgovora)

ULOGA MIKROBIOTE U BOLESTI – još uvijek nedovoljno spoznaja

(1843-1910)

Kochovi postulati

koncept jedan patogen – jedna bolest

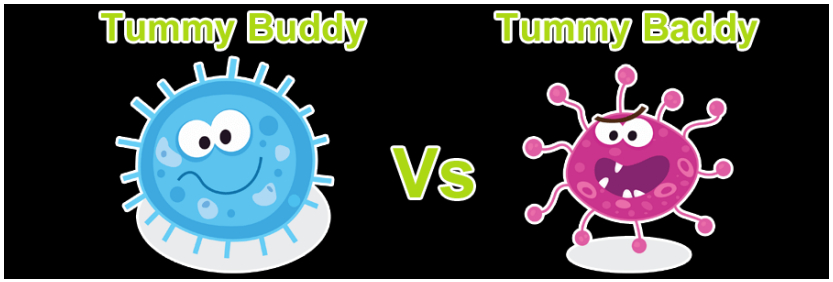
1. u bolesnom organizmu mora biti prisutan mikrob-uzročnik
2. mikroorganizam se iz bolesnika mora izolirati u čistoj kulturi
3. pokusna životinja zaražena čistom kulturom mikroorganizma mora pokazati iste znakove oboljenja
4. iz pokusno zaraženog organizma treba ponovno izdvojiti uzročnika u čistoj kulturi

Istraživanja mikrobiote rezultirala NOVIM KONCEPTOM za brojne bolesti:

Bolest može biti uzrokovana poremećajem u ZAJEDNICI mikroba, što može dovesti do imunosnih i metaboličkih poremećaja (pr. pretilost, dijabetes tipa 2, celijakija, upalne bolesti crijeva, ulcerozni kolitis, Chronova bolest...).

POTREBA REDEFINICIJE ?

DISBIOZA (poremećaj normalne mikroflore)



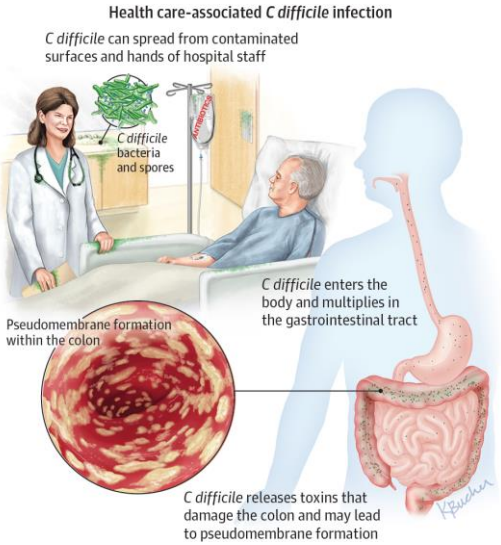
eliminacija poželjnih mikroba

kolonizacija nepoželjnih mikroba

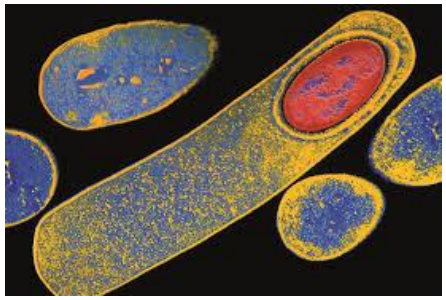
BOLEST

PRIMJERI BOLESTI UZROKOVANIH DISBIOZAMA:

- **upalna bolest crijeva uzrokovana antibioticima**
  je posljedica redukcije normalne crijevne flore zbog čega se povećava populacija bakterije *Clostridioides difficile* (endogena ili češće egzogena infekcija) koja izlučuje upalne enterotoksine što dovodi do upale sluznice crijeva uz obilnu sekreciju tekućine (proljevi).

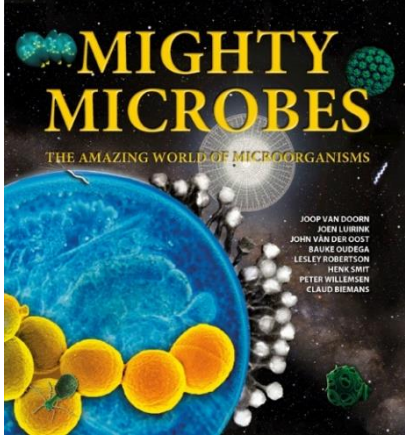


SAD – 223 900 infekcija godišnje
12 800 smrtnih ishoda!
CDC report 2019.



Gram +, sporogeni, anaerobni bacil

TERAPIJSKI PRISTUP: ciljana antibiotska terapija ili kod lošeg odgovora (ponovljene infekcije) transplantacija crijevne mikrobiote fekalnim uzorkom zdravog donora ili sintetskim pripravcima mikrobnih fekalnih kultura, efikasnost >90%!!!



bes. These communi-
nd genes. In the intes-
n close to our hearts.
nteract with the gut,
r maintaining health.
used in translational
trains, or mixtures of

become a simple but
tinal microbiota of the
robiota from a healthy
cribed over 1000 years
revived and. especially

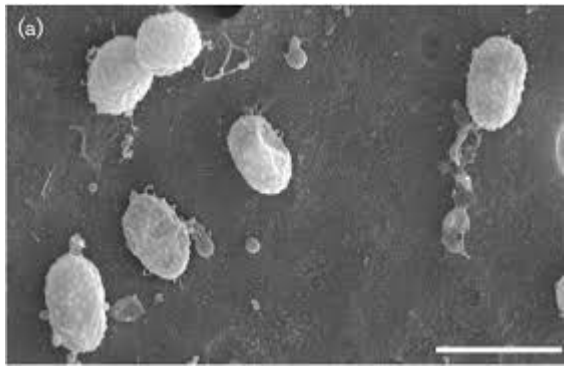
MC) to cure an 80-year

The authors of this chapter simulating Faecal Microbiota Transplantation via the gastro-duodenal route.

- **kronične upalne bolesti crijeva (ulcerozni kolitis, Chronova bolest) i kolorektalni tumori**

Ulcerozni kolitis i Chronova bolest karakterizirani su pojavom čireva na sluznici crijeva što uzrokuje bolove, proljeve, krvarenja i može dovesti do brojnih komplikacija uključujući razvoj karcinoma.

Utvrđena je veza navedenih bolesti s porastom populacije bakterija koje proizvode mucin-razgrađujuće sulfataze što rezultira razgradnjom mukoznog sloja stijenke crijeva i upalnim procesom te bakterija koje potiču lučenje kemokina i nastanak upalne reakcije.



Akkermansia muciniphila

- **nekrotizirajući enterokolitis**

Bolest se javlja kod nedonoščadi i drugi je po redu najčešći uzrok smrti nedonoščadi, a karakterizira ga nekroza crijevne sluznice nejasne etiologije.

Istraživanja pokazuju da razvoju bolesti prethodi izražena disbioza!

- **sklonost dijabetesu tipa 2 i pretilosti**

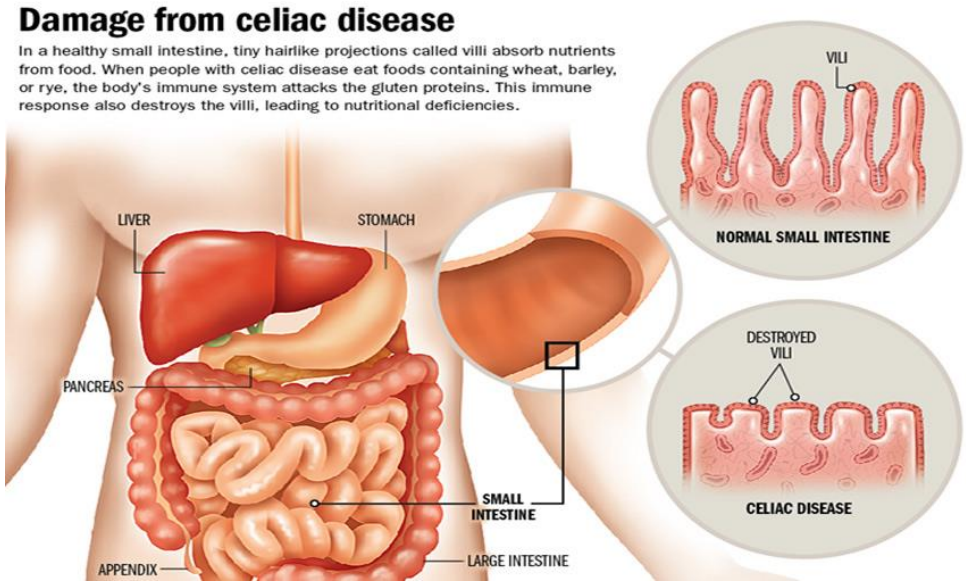
  osobe čija je crijevna mikrobiota efikasnija u razgradnji složenih ugljikohidrata mogu imati veću sklonost razvoju dijabetesa tipa 2, dok pojačana sinteza masnih kiselina dovodi do povećanog skladištenja nutrijenata i eventualno pretilosti

- **celijakija**

  osobe čija je crijevna mikrobiota uspješna u razgradnji glutena ne obolijevaju nužno od celijakije unatoč genetskoj predispoziciji

- **atopijski dermatitis, vaginitis**

  posljedica promjene sastava mikrobioma



2014. druga faza projekta ljudskog mikrobioma
„The Integrative Human Microbiome Project"

cilj: razumjeti kakav je utjecaj mikrobiote na zdravlje i razvoj bolesti

https://hmpdacc.org/ihmp/overview/

Project 1: Pregnancy & Preterm Birth

Project 2: Onset of Inflammatory Bowel Disease (IBD)

Project 3: Onset of Type 2 Diabetes

DISBIOZE – TERAPIJSKI PRISTUPI I PREVENCIJA?

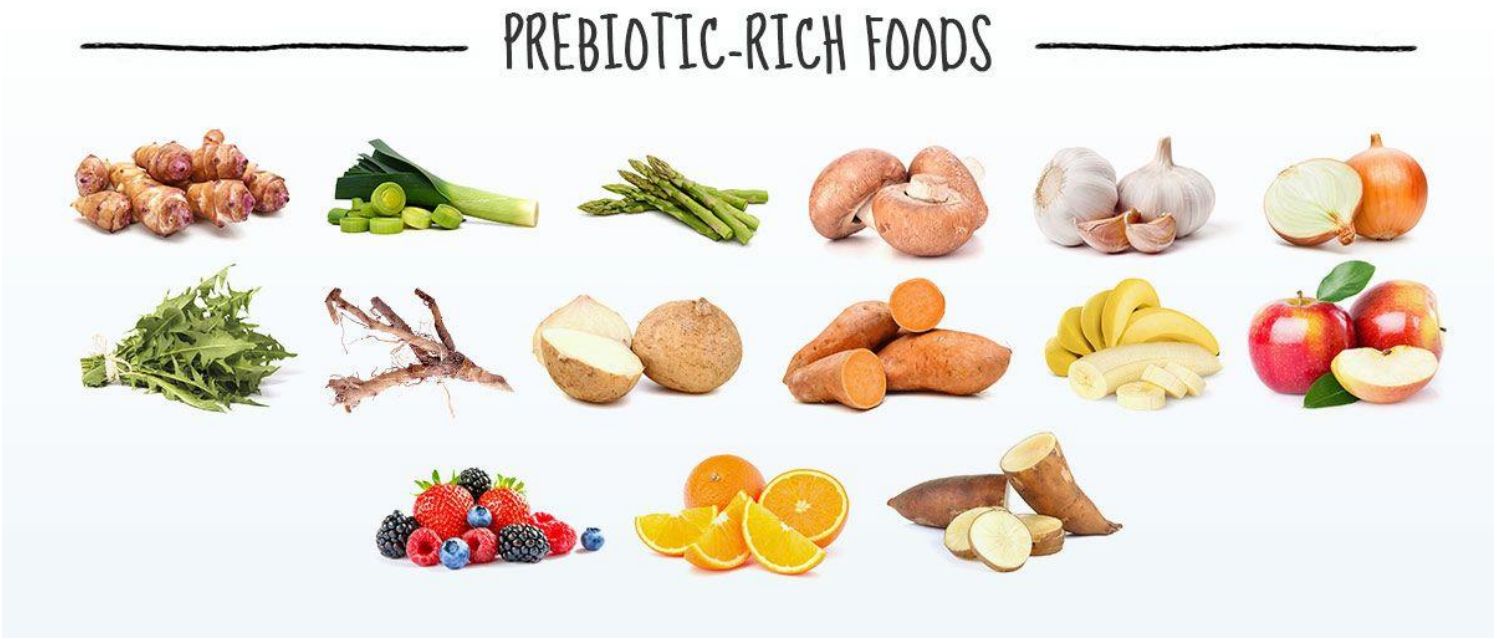
PROBIOTICI su miješane mikrobne kulture bakterija i kvasaca (najčešće *Bifidobacterium*, *Lactobacillus* i *Saccharomyces*) koje mogu (barem privremeno) kolonizirati crijevo ili drugi dio tijela i tamo se umnožavati.

Terapijska učinkovitost im je nedovoljno istražena, a potrošači ističu pozitivan utjecaj na probavu hrane, podnošenje laktoze i imunosni odgovor.

Predložene indikacije:
-zaštita od upalnih bolesti crijeva tijekom uzimanja antibiotika
-zaštita od infekcije bakterijom *H. pylori* (potencijalni karcinogen)
-prevencija atopijskog dermatitisa i autoimunih bolesti u najranijoj dobi

Učinkovitost ovisi o:
sastavu i vijabilnosti mikrobne kulture, dozi, načinu konzumacije...

Budućnost u „pametnim" probioticima???



„Gut Fertilizers” – izvor vlakana, hrana za probiotike (potiće replikaciju jednog ili više članova mikrobiote)

PERSPEKTIVA

Dostupnost, brzina i niska cijena DNA sekvenciranja mogla bi u budućnosti dovesti do rutinske izrade osobnih mikrobioma kao dijagnostičkih testova za brojne bolesti!

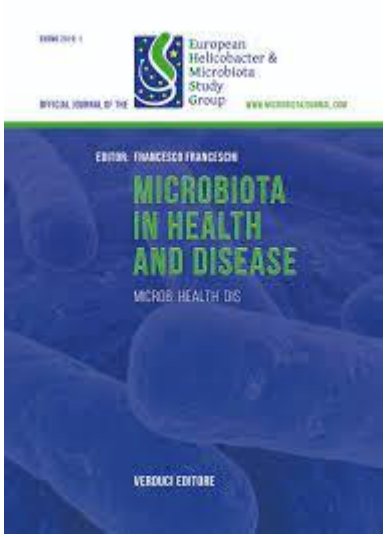
Brojna pitanja:

- **Možemo li uistinu predvidjeti bolest proučavajući promjene mikrobioma?**
- **Koje su promjene mikrobioma relevantne za razvoj bolesti (taksonomske ili funkcionalne)?**
- **Može li ponovna uspostava ravnoteže mikrobioma prevenirati bolest i kojim postupkom?**
- **Koja je uloga domaćinskog genoma, okolišnih faktora i higijenskih navika u održanju zdrave mikrobiote?**
- **.....**


ELSEVIER
HUMAN
MICROBIOME
JOURNAL


GUTMICROBIOME


Microbiome
BMC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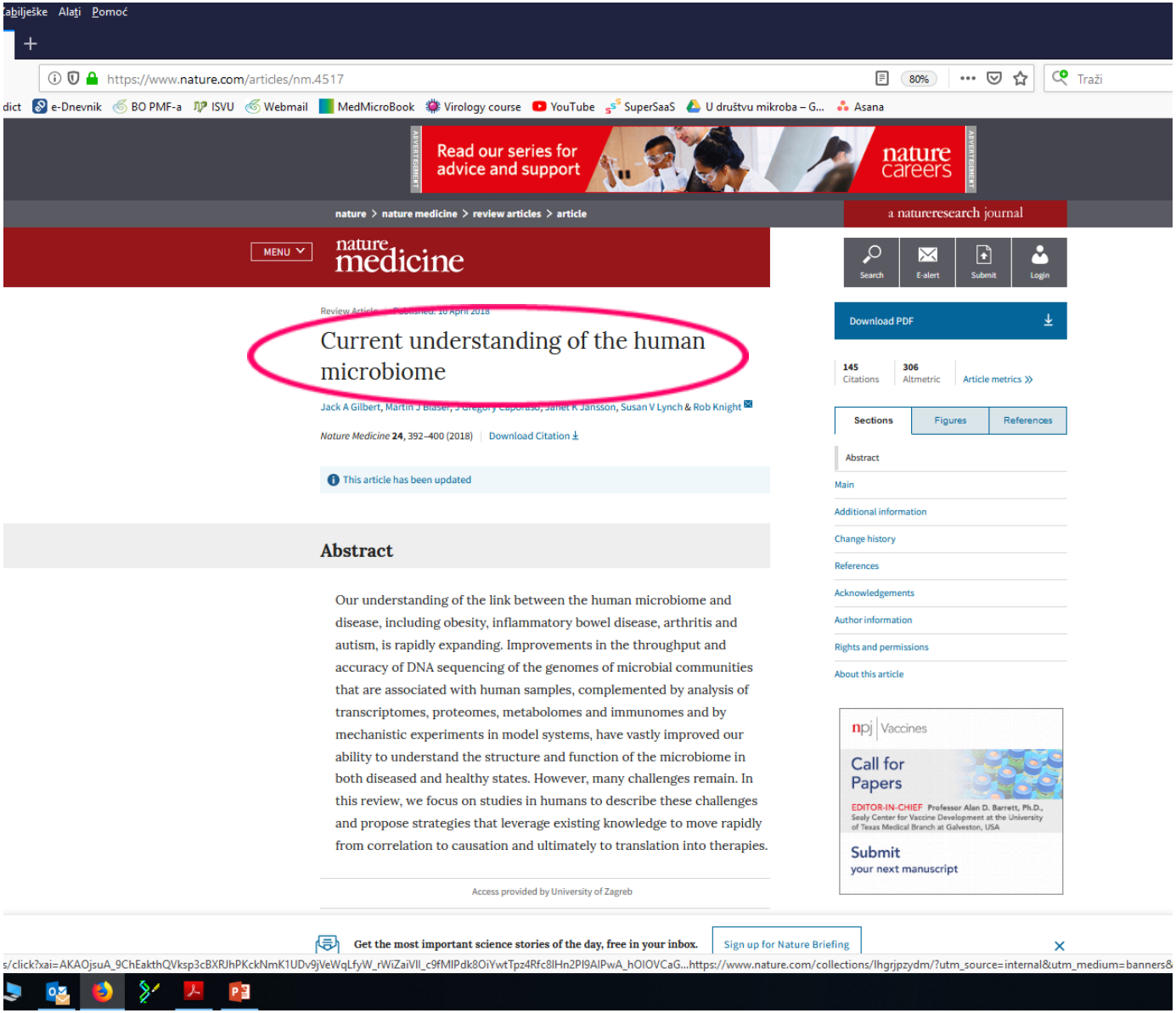

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Microbiota: a key orchestrator of cancer therapy

Soumen Roy and Giorgio Trinchieri

Abstract | The microbiota is composed of commensal bacteria and other microorganisms that live on the epithelial barriers of the host. The commensal microbiota is important for the health and survival of the organism. Microbiota influences physiological functions from the maintenance of barrier homeostasis locally to the regulation of metabolism, haematopoiesis, inflammation, immunity and other functions systemically. The microbiota is also involved in the initiation, progression and dissemination of cancer both at epithelial barriers and in sterile tissues. Recently, it has become evident that microbiota, and particularly the gut microbiota, modulates the response to cancer therapy and susceptibility to toxic side effects. In this Review, we discuss the evidence for the ability of the microbiota to modulate chemotherapy, radiotherapy and immunotherapy with a focus on the microbial species involved, their mechanism of action and the possibility of targeting the microbiota to improve anticancer efficacy while preventing toxicity.

Germ-free animals
Animals raised in strict sterile conditions that have no microorganisms living in or on them.

Commensalism
A symbiotic relationship between two species in which one species benefits without causing harm to the other.

Pathobionts
Resident commensal microorganisms that under certain conditions may acquire pathogenic potential.

Mutualism
A symbiotic relationship between two species that is beneficial for both species.

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The human microbiota is the ensemble of bacteria and other microorganisms (archaea, fungi, protozoa, as well as human, fungal, bacterial and protozoan viruses) that inhabit the epithelial barrier surfaces of our body[1]. The microbiota affects physiological functions, particularly metabolism, neurological and cognitive functions, haematopoiesis, inflammation and immunity[2,3]. Germ-free animals are functionally immature in many physiological systems, including innate immunity, and they are highly susceptible to infection by pathogens[4]. However, the germ-free condition in itself is not lethal. Germ-free rodents maintained in a sheltered sterile environment and fed with a controlled diet containing factors, such as vitamins that are normally supplied by the intestinal microbiota, can survive significantly longer than conventionally raised animals[4–6]. The local microbiota affects the functions and regulates the immunity of the epithelial barrier on which it resides[7–10]. In addition, the microbiota also exerts systemic effects[10,11] (FIG. 1) through mechanisms that are less well understood than those mediating the local effects.

The gut microbiota comprises approximately 3×10^{13} bacterial cells that mostly exhibit commensalism with the host[12]. However, when the intestinal ecology is altered, commensal bacteria that are referred to as pathobionts (for example, *Clostridium difficile* or vancomycin-resistant *Enterococcus*) may expand and acquire pathogenic characteristics[13]. The gut microbiota also exhibits mutualism with the host that modulates immunity, promotes bone marrow haematopoiesis and also regulates maturation and function of tissue-resident haematopoietic cells of yolk sac origin such as the microglia in the central nervous system[14–16]. The gut microbiota interacting with epithelial and stromal intestinal cells regulates barrier functions, mucosal immune homeostasis[7–9], host–microbiota symbiosis, prevention of infestation by pathogens, control of overgrowth by pathobionts, metabolism of indigestible dietary fibre, synthesis of vitamins and regulation of metabolism, including the prevention of obesity[7–9,17–19]. An intact and functional gut epithelium maintains a healthy body, and gut epithelial homeostasis is maintained by continuous crosstalk between the gut microbiota, immune cells and the mucosal barrier[20].

The composition of the microbiota is shaped by host genetics, colonization at the time of birth, type of birth delivery, an individual's lifestyle, incidence of diseases and exposure to antibiotics[21–23]. Microbiota composition evolves during the first few years of human life before maturation into an adult-like microbiota[22]. After that, the composition of the microbiota in the gut and other epithelial barriers remains relatively constant throughout adult life, although it could still be affected by diet, changes in lifestyle, disease and disease treatment[24,25]. The microbiota present at the epithelial barrier and particularly in the gut influences local and systemic metabolic functions, inflammation and adaptive immunity, which modulate cancer initiation, progression and response to anticancer treatment. Host genetics and environmental factors, including nutrition, modify the composition of the microbiota both in humans and in the mouse[26–29]. In the mouse, the pro-carcinogenic

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The Microbiota of Breast Tissue and Its Association with Breast Cancer

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ABSTRACT

In the United States, 1 in 8 women will be diagnosed with breast cancer in her lifetime. Along with genetics, the environment contributes to disease development, but what these exact environmental factors are remains unknown. We have previously shown that breast tissue is not sterile but contains a diverse population of bacteria. We thus believe that the host's local microbiome could be modulating the risk of breast cancer development. Using 16S rRNA amplicon sequencing, we show that bacterial profiles differ between normal adjacent tissue from women with breast cancer and tissue from healthy controls. Women with breast cancer had higher relative abundances of *Bacillus*, *Enterobacteriaceae* and *Staphylococcus*. *Escherichia coli* (a member of the *Enterobacteriaceae* family) and *Staphylococcus epidermidis*, isolated from breast cancer patients, were shown to induce DNA double-stranded breaks in HeLa cells using the histone-2AX (H2AX) phosphorylation (γ-H2AX) assay. We also found that microbial profiles are similar between normal adjacent tissue and tissue sampled directly from the tumor. This study raises important questions as to what role the breast microbiome plays in disease development or progression and how we can manipulate this for possible therapeutics or prevention.

IMPORTANCE

This study shows that different bacterial profiles in breast tissue exist between healthy women and those with breast cancer. Higher relative abundances of bacteria that had the ability to cause DNA damage *in vitro* were detected in breast cancer patients, as was a decrease in some lactic acid bacteria, known for their beneficial health effects, including anticarcinogenic properties. This study raises important questions as to the role of the mammary microbiome in modulating the risk of breast cancer development.

Bacteria inhabit numerous body sites, and this collective microbiota plays an integral role in human development. Changes in the composition of one's microbiota at various body sites may promote disease progression, as individuals with periodontitis (1, 2), inflammatory bowel disease (3), psoriasis (4), asthma (5), diabetes (6), bacterial vaginosis (7), and colorectal cancer (8) have different bacterial communities than healthy individuals. While it is still unclear whether these microbial differences are a consequence or a cause of the disease, there is evidence in favor of the latter, as healthy animals transplanted with feces from those with obesity, colitis, or colorectal cancer then go on to develop disease (9–11).

In the United States, 1 in 8 women will be diagnosed with breast cancer in her lifetime. While the etiology of breast cancer is still unknown, it is believed to be due to a combination of both genetic and environmental factors. Support for environmental factors comes from migration studies showing an increased incidence of breast cancer among migrants and their descendants after they move from a region of low breast cancer risk to a region of high risk (12, 13). Bacterial communities within the host could be one such environmental factor which has not been considered to date.

We have previously shown that a breast tissue microbiome exists in a cohort of Canadian and Irish women (14). To determine whether this local microbiome could have a role in modulating the risk of breast cancer development, we examined the breast microbiota of 70 women who had either breast cancer (normal adjacent tissue collected) or benign tumors (normal adjacent tissue collected) or were disease free. Bacteria isolated from cancer patients were characterized and examined for their abilities to induce DNA damage.

MATERIALS AND METHODS

Microbiome analysis. (i) Tissue collection and processing. Fresh breast tissue was collected from 71 women (ages 19 to 90 years) undergoing breast surgery at St. Joseph's Hospital in London, Ontario, Canada. Ethical approval was obtained from the Western Research Ethics Board and Lawson Health Research Institute, London, Ontario, Canada. Subjects provided written consent for sample collection and subsequent analyses. Fifty-eight women underwent lumpectomies or mastectomies for either benign ($n = 13$) or cancerous ($n = 45$) tumors, and 23 were free of disease and underwent either breast reductions or enhancements. For those

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The human microbiome and COVID-19: A systematic review

Shinya Yamamoto, Makoto Saito, Azumi Tamura, Diki Prawisuda, Taketoshi Mizutani, Hiroshi Yotsuyanagi

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Abstract

Background

Human microbiotas are communities of microorganisms living in symbiosis with humans. They play an important role in the host immune response to respiratory viral infection. However, evidence on the human microbiome and coronavirus disease (COVID-19) relationship is insufficient. The aim of this systematic literature review was to evaluate existing evidence on the association between the microbiome and COVID-19 in humans and summarize these data in the pandemic era.

Methods

We conducted a systematic literature review on the association between the microbiome and COVID-19 in humans by searching PubMed, Embase, and the Cochrane Library, CINAHL, and Web of Science databases for articles in English published up to October 31, 2020. The results were analyzed qualitatively. This study is registered with PROSPERO (CRD42020195982).

ISTRAŽIVANJA MIKROBIOTE

-nužan oprez pri dizajniranju eksperimenata i zaključivanju

-što je „normalna” mikrobiota?

-brojne se bolesti (gotovo sve) dovode u vezu sa sastavom mikrobiote

-ukazuje li ovo na njenu važnost u nastanku bolesti ili je promjena mikrobiote posljedica same bolesti ???

-što je s multifaktorijalnim bolestima?

...

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