Diagnostic Solutions laboratory

GI-MAP™ *Advanced Practice Series*

An In-Depth Look at Opportunistic Bacteria

Presented by Thomas Fabian, PhD, CNTP

Opportunistic Bacteria in Disease

- General symptoms: bloating, gas, discomfort
- Post-infection dysbiosis
- Inflammatory Bowel Disease (IBD)
- Autoimmune conditions
- Irritable Bowel Syndrome (IBS)
- Food sensitivities & intolerances
- Small intestinal dysbiosis
- Skin, joint & hormone issues
- Mood & nervous system conditions

OPPORTUNISTIC/OVERGROWTH MICROBES

DYSBIOTIC & OVERGROWTH BACTERIA	Result		Reference
Bacillus spp.	7.10e6	High ↑	< 1.76e6
Enterococcus faecalis	5.10e3		< 1.00e4
Enterococcus faecium	6.89e3		< 1.00e4
Morganella spp.	3.14e7	High ↑	< 1.00e3
Pseudomonas spp.	6.18e5	High ↑	< 1.00e4
Pseudomonas aeruginosa	1.16e4	High ↑	< 5.00e2
Staphylococcus spp.	<dl		< 1.00e4
Staphylococcus aureus	1.16e3	High ↑	< 5.00e2
Streptococcus spp.	7.08e3	High ↑	< 1.00e3
COMMENSAL OVERGROWTH MICROBES			
Desulfovibrio spp.	<dl		< 7.98e8
Methanobacteriaceae (family)	3.32e7		< 3.38e8
INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA			
Citrobacter spp.	<dl		< 5.00e6
Citrobacter freundii	4.25e6	High ↑	< 5.00e5
Klebsiella spp.	3.45e5	High ↑	< 5.00e3
Klebsiella pneumoniae	1.65e6	High ↑	< 5.00e4
M. avium subsp. paratuberculosis	<dl		< 5.00e3
Proteus spp.	1.63e6	High ↑	< 5.00e4
Proteus mirabilis	1.03e6	High ↑	< 1.00e3
COMMENSAL INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA			
Enterobacter spp.	7.06e8	High ↑	< 5.00e7
Escherichia spp.	1.20e10	High ↑	< 3.80e9
Fusobacterium spp.	1.98e8	High ↑	< 1.00e8
Prevotella spp.	4.63e7		< 1.00e8

DYSBIOTIC & OVERGROWTH BACTERIA	Result		Reference
Bacillus spp.	1.68e5		< 1.76e6
Enterococcus faecalis	**7.42e5**	**High ↑**	< 1.00e4
Enterococcus faecium	2.39e3		< 1.00e4
Morganella spp.	<dl		< 1.00e3
Pseudomonas spp.	**4.53e8**	**High ↑**	< 1.00e4
Pseudomonas aeruginosa	**9.19e3**	**High ↑**	< 5.00e2
Staphylococcus spp.	<dl		< 1.00e4
Staphylococcus aureus	<dl		< 5.00e2
Streptococcus spp.	**4.53e4**	**High ↑**	< 1.00e3
COMMENSAL OVERGROWTH MICROBES			
Desulfovibrio spp.	6.54e5		< 7.98e8
Methanobacteriaceae (family)	2.43e6		< 3.38e8

INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA			
Citrobacter spp.	<dl		< 5.00e6
Citrobacter freundii	**3.19e6**	**High ↑**	< 5.00e5
Klebsiella spp.	**4.41e4**	**High ↑**	< 5.00e3
Klebsiella pneumoniae	**7.85e4**	**High ↑**	< 5.00e4
M. avium subsp. paratuberculosis	<dl		< 5.00e3
Proteus spp.	2.66e4		< 5.00e4
Proteus mirabilis	**1.74e5**	**High ↑**	< 1.00e3
COMMENSAL INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA			
Enterobacter spp.	**2.97e8**	**High ↑**	< 5.00e7
Escherichia spp.	6.52e7		< 3.80e9
Fusobacterium spp.	**4.41e8**	**High ↑**	< 1.00e8
Prevotella spp.	3.34e6		< 1.00e8

Interpretive Guide

OPPORTUNISTIC/OVERGROWTH MICROBES

Table 6.

DYSBIOTIC & OVERGROWTH BACTERIA	
***Bacillus* spp.**	Common group of gram-positive bacteria in the *Firmicutes* phylum. Some strains are used as probiotics. High levels may result from reduced digestive function, SIBO, or constipation.
Enterococcus faecalis* *Enterococcus faecium	Gram-positive species in the *Firmicutes* phylum. High levels may result from reduced stomach acid, PPI use, compromised digestive function, SIBO or constipation. High natural resistance to some antibiotics, which may result in overgrowth.
***Morganella* spp.**	Gram-negative group in the *Proteobacteria* phylum. May produce histamine. High levels may indicate increased intestinal inflammatory activity. High levels may cause diarrhea, and may also be associated with SIBO.
Pseudomonas* spp.** ***Pseudomonas aeroginosa	Gram-negative bacteria in the *Proteobacteria* phylum. High levels may indicate increased intestinal inflammatory activity and may cause abdominal cramping and loose stools. Some strains of *P. aeroginosa* may produce toxins that can damage cells.
Staphylococcus* spp.** ***Staphylococcus aureus	Gram-positive bacteria in the *Firmicutes* phylum. High levels may result from reduced digestive capacity, and intestinal inflammatory activity. Some strains may produce toxins and contribute to loose stools or diarrhea.
***Streptococcus* spp.**	Gram-positive bacteria in the *Firmicutes* phylum. *Streptococcus* spp. colonize skin and mucous membranes throughout the body; High levels in the intestine may result from low stomach acid, PPI use, reduced digestive capacity, SIBO or constipation; Elevated levels may also be indicative of intestinal inflammatory activity, and may cause loose stools.

COMMENSAL & OVERGROWTH MICROBES	
***Desulfovibrio* spp.**	A genus of Gram-negative sulfate reducing bacteria. The bacteria produce hydrogen sulfide (H2S), a metabolite which can influence cell signaling and reduce oxidative stress at low concentrations and pose toxicity at higher concentrations.

White Paper

subjects when compared to lean subjects.[149] And when obese subjects lost weight, there was a simultaneous change in the *Firmicutes* to *Bacteroidetes* ratio, favoring that of lean subjects.[35] Some authors have challenged those results, suggesting instead that obese subjects have lower microbial diversity.[148] Overall, it seems clear that there is GI microbial imbalance in people with obesity and this could be a modifiable factor for patients with metabolic disorders.

Diet is one of the most powerful modulators of the GI microbiome. A high fat diet is a driver of microbial changes and can increase the F/B ratio. It is difficult to determine if the characteristic obese microbial pattern is caused by obesity or a diet that promotes obesity. Recent findings suggest that it is the diet, and not obesity itself, that leads to imbalanced GI microbial patterns.[147,148] Patients with a high F/B ratio may benefit from a lower fat diet and probiotics and prebiotics aimed to balance the *Firmicutes* and *Bacteroidetes* phyla. In one study, 30 grams of glutamine taken orally every day for two weeks lowered the F/B ratio.[150]

Opportunistic and Overgrowth Microbes

The GI-MAP was designed to detect pathogenic and opportunistic organisms that may be causing symptoms or illness. Many bacteria measured on the GI-MAP are opportunistic pathogens, meaning that they only cause disease and illness in some individuals, particularly the immune-compromised. Many people come into contact with opportunistic pathogens and experience no symptoms, probably because opportunists are suppressed by the balance of commensal bacteria.[39] Overgrowth and excessive colonization by opportunistic bacteria may occur when the commensal bacteria are impaired by poor diet, antibiotic use, parasitic infection, or a weakened

dsl-academy.teachable.com

Edit in Admin | Preview as student | You can see only published content

Diagnostic Solutions laboratory | Academy | My Products | All Products

DSL Academy

Functional Medicine and Laboratory Evaluations
from Diagnostic Solutions Laboratory

DSL Academy (DSLA) is a self-paced, comprehensive learning platform that will provide you with the skills needed to interpret test results and design individualized protocols for your clients.

DSLA is a growing learning platform taught by our Medical Education Team. As we add educational content, you'll have instant access to every new lecture and module.

This exclusive educational center is offered to practitioners who have active accounts with Diagnostic Solutions Laboratory. Use the button below to request enrollment today!

Request Enrollment

Featured Courses

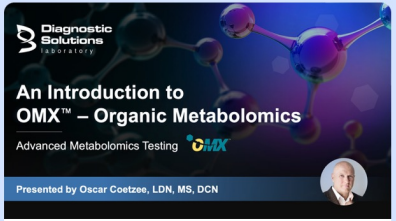
Click any course for more details and begin learning!

GI-MAP Essentials

DSL Academy M... 0% COMPLETE

H. pylori Masterclass Series

Amy Rolfsen, ND 0% COMPLETE



OMX-Organic Metabolomics

Oscar Coetzee, L... 0% COMPLETE

Metabolic Signatures Using OMX Organic Metabolomics

diagnosticsolutionslab.com

Drop-Ship Kits Today – Our Current GI-MAP Turnaround Time: 7–10 Business Days

Diagnostic Solutions laboratory

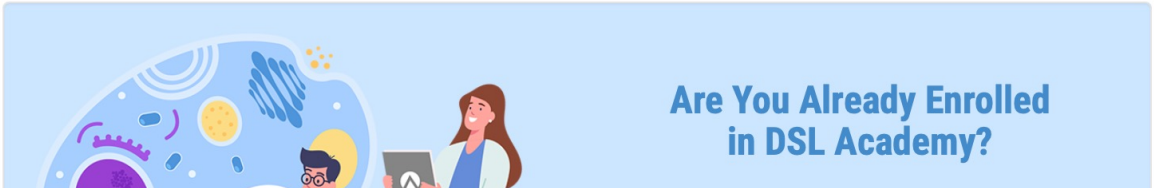
RESEARCH. TECHNOLOGY. RESULTS.

TESTS | CLINICIANS | PATIENTS | ABOUT US | CONTACT | RESULTS PORTAL

DSL Academy

DSL Academy (DSLA) is an exclusive, online learning platform designed to help you better interpret the GI-MAP® and OMX® Organic Metabolomics tests. The DSLA curriculum is taught by the Diagnostic Solutions Laboratory Medical Education Team and is available to all active DSL account holders.*

The educational materials in DSLA will build your knowledge and grow your confidence in interpreting your patient's lab reports. Insights will increase your personalized medicine skillset and help you optimize patient outcomes using testing from Diagnostic Solutions Laboratory.



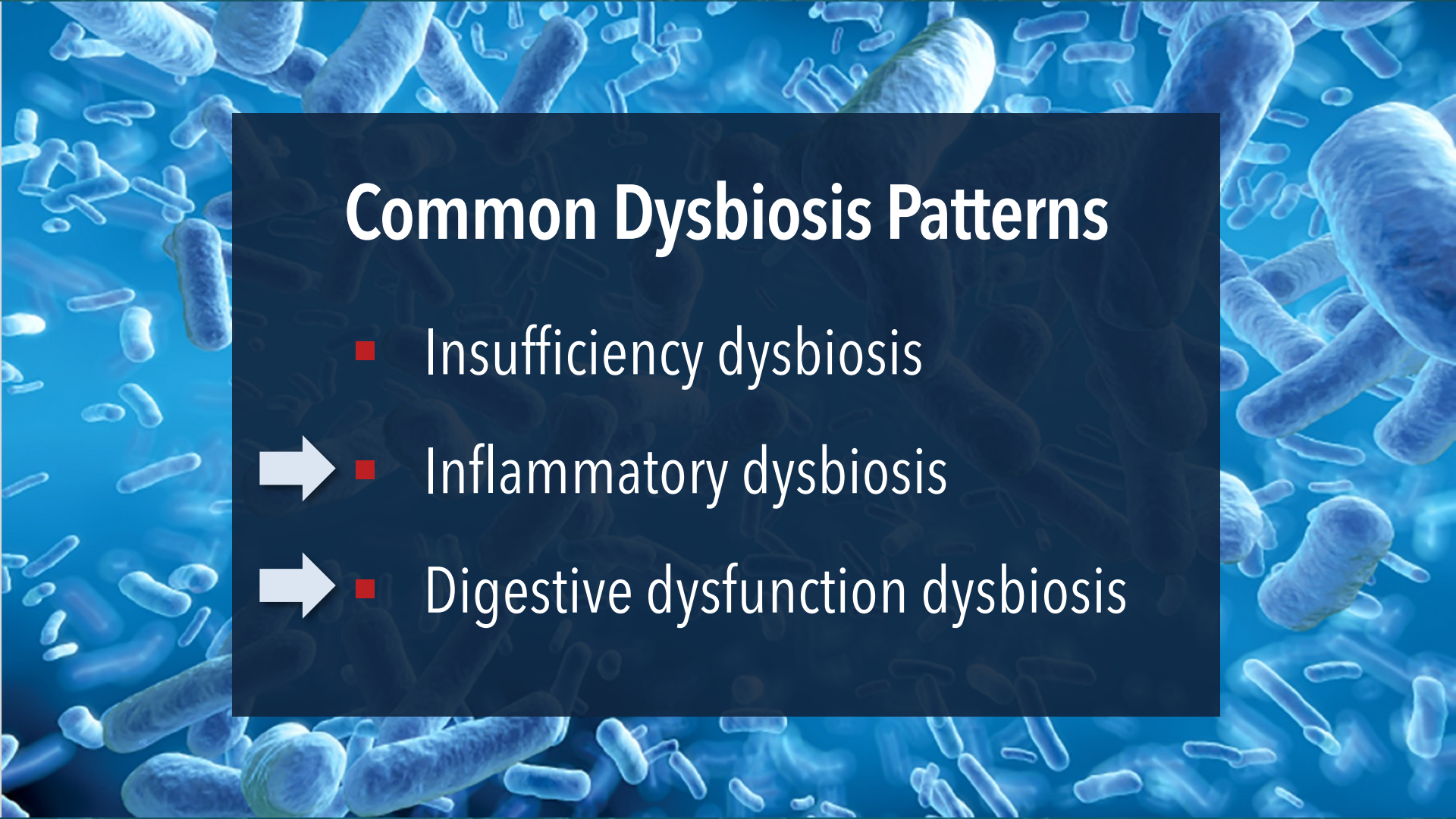
Are You Already Enrolled in DSL Academy?

Are You Already Enrolled in DSL Academy?

ENTER DSL ACADEMY

Please Note: *DSLA is an exclusive educational center offered to practitioners who have active accounts with Diagnostic Solutions Laboratory. See details below.*

Request Enrollment Today!


Common Dysbiosis Patterns
Insufficiency dysbiosis
Inflammatory dysbiosis
Digestive dysfunction dysbiosis

Common Dysbiosis Patterns (Interpretive Guide)

GI-MAP PATTERNS

UNDERSTANDING COMMON DYSBIOSIS PATTERNS WITH GI-MAP

INSUFFICIENCY DYSBIOSIS

Insufficiency dysbiosis is characterized by low levels of beneficial bacteria that provide critical support for healthy intestinal and immune function. Insufficient levels of beneficial bacteria may result in an elevated risk of intestinal infections, increased intestinal barrier permeability, decreased protective factors such as secretory IgA, and increased inflammation. Lack of keystone bacteria is common in autoimmune, allergic, and chronic inflammatory conditions.

Table 9.

Markers Characterizing Insufficiency Dysbiosis	
Commensal/Keystone Bacteria: *(low levels)*	*Bacteroides fragilis* *Bifidobacterium* spp. *Enterococcus* spp. *Escherichia* spp. *Lactobacillus* spp. *Akkermansia muciniphilia* *Faecalbacterium prausnitzii* *Roseburia* spp.
Phyla Microbiota: *(low levels)*	*Bacteroidetes* *Firmicutes*
Associated Intestinal Health Markers:	Secretory IgA *(often low to very low levels)* Zonulin *(sometimes elevated)*

INFLAMMATORY DYSBIOSIS

Inflammatory dysbiosis is characterized by moderate to high levels of certain pathogens, normal microbiota, and opportunistic microbes that promote inflammation and increased intestinal permeability. Many pro-inflammatory microbes are gram-negative bacteria that belong to the Proteobacteria phylum and produce a form of lipopolysaccharide *(LPS)* that is a potent activator of inflammatory responses. This pattern is common in chronic immune and inflammatory conditions.

Table 10.

Markers Characterizing Inflammatory Dysbiosis	
Pathogens *(low to high levels)*	*Campylobacter* *C. difficile* *Pathogenic E. coli* *Salmonella* *Vibrio cholerae* *Yersinia enterocolitica* *Giardia*
Commensal/Keystone Bacteria *(low levels)*	*Escherichia* spp. *Enterobacter* spp.
Opportunistic Bacteria, Yeast, and Protozoa *(moderate to high levels)*	*Morganella* spp. *Pseudomonas* spp. *Pseudomonas aeroginosa* *Desulfovibrio* spp. *Citrobacter* spp. *Citrobacter freundii* *Klebsiella* spp. *Klebsiella pneumoniae* *Proteus* spp. *Proteus mirabilis* *Fusobacterium* spp. *Candida* spp. *Candida albicans* *Parasitic protozoa (specifically Giardia and Blastocystis hominis)*
Associated Intestinal Health Markers:	β-Glucuronidase *(may be elevated)* Occult Blood-FIT *(may be elevated)* Secretory IgA *(often low levels, but sometimes elevated)* Calprotectin *(often elevated, but sometimes very low levels)* Eosinophil Activation Protein (EDN/EPX) *(may be elevated)* Zonulin *(may be elevated in some cases)*

DIGESTIVE DYSFUNCTION DYSBIOSIS

Dysbiosis associated with digestive dysfunction is very common, and is often due to low stomach acid (hypochlorhydria), insufficient bile acids, poor digestion *(pancreatic insufficiency or brush border enzyme deficiency)*, reduced absorption, and altered gastrointestinal motility. Altered digestion and motility can result in imbalances in the microbiome, characterized by overgrowth of certain species. Symptoms associated with digestive dysfunction include but are not limited to: excessive gas and bloating, abdominal discomfort, dyspepsia, heart burn, gastroesophageal reflux (GERD), constipation or diarrhea, food sensitivities and intolerances.

Table 11.

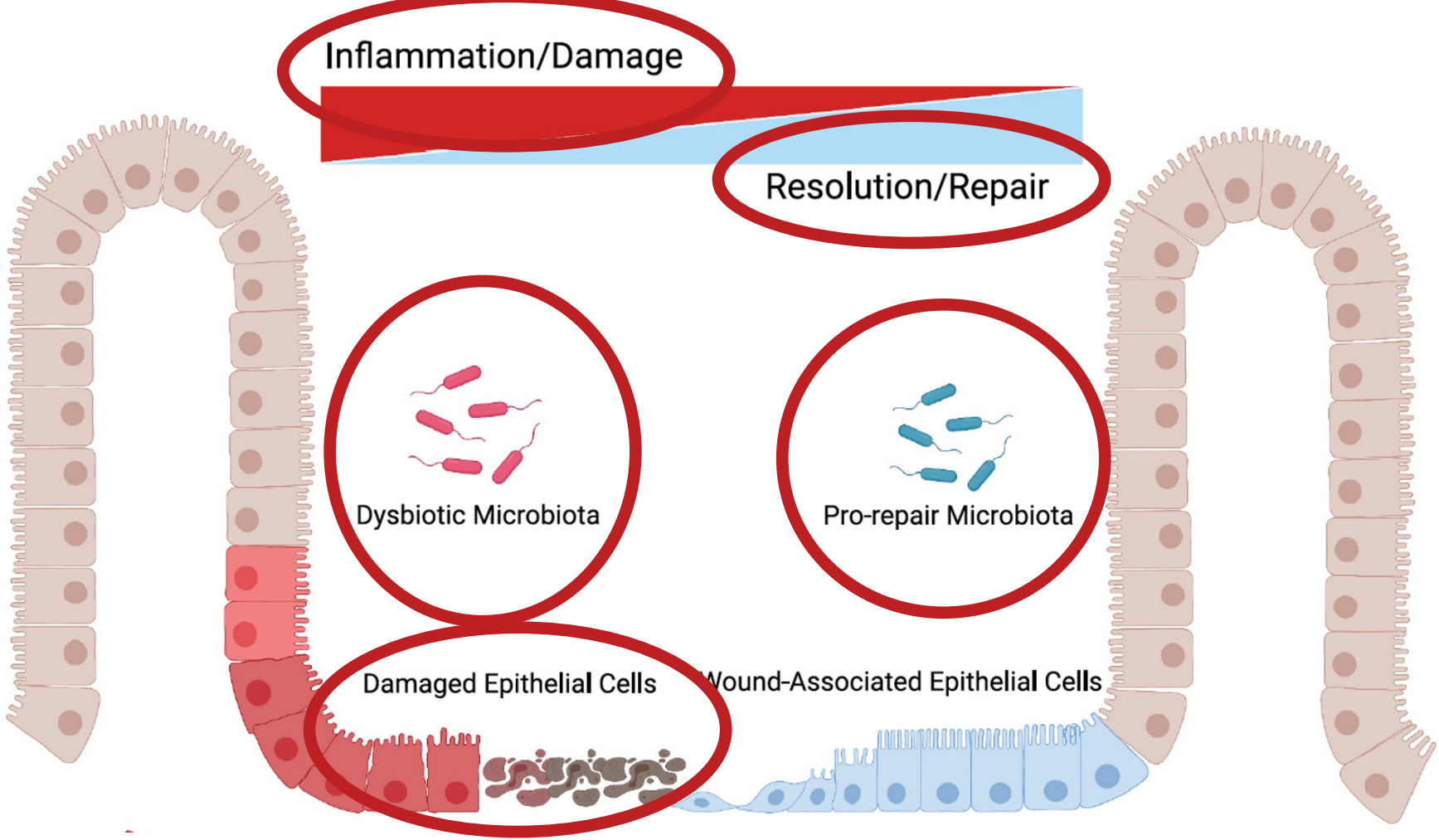
Markers Associated with Digestive Dysfunction	
Pathogens *(low to high levels)*	Most types, especially if multiple pathogens are present
H. pylori *(moderate to high levels)*	*Helicobacter pylori* *(with or without virulence factors)*
Commensal/Keystone Bacteria *(high levels)*	*Enterococcus* *Lactobacillus* *Clostridium*
Phyla Microbiota *(high levels)*	*Bacteroidetes* and/or *Firmicutes*
Opportunistic Bacteria, Yeast, and Protozoa *(moderate to high levels)*	*Bacillus* spp. *Enterococcus faecalis* *Enterococcus faecium* *Morganella* spp. *Staphylococcus* spp. *Staphylococcus aureus* *Streptococcus* spp. *Methanobacteriaceae (family)* *Desulfovibrio* spp. *Klebsiella pneumoniae* *Prevotella* *Candida* spp. *Candida albicans* *Parasitic protozoa*
Intestinal Health Markers:	Elastase-1 *(often low to moderately low levels)* Steatocrit *(sometimes elevated)*

Opportunistic Microbes: Functional Groups

Lipopolysaccharide (LPS) Producing Bacteria
Escherichia spp.
Enterobacter spp.
Morganella spp.
Pseudomonas spp.
Pseudomonas aeruginosa
Citrobacter spp.
Citrobacter freundii
Klebsiella spp.
Klebsiella pneumoniae
Proteus
Proteus mirabilis

Histamine Producing Bacteria
Lactobacillus spp.
Morganella spp.
Pseudomonas
Pseudomonas aeruginosa
Citrobacter freundii
Klebsiella
Klebsiella pneumoniae
Proteus
Proteus mirabilis
Enterobacter spp.
Escherichia spp.
Fusobacterium spp.

Mast Cell-Activating Microbes
H. pylori
Enterococcus faecalis
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus spp.
Candida spp.
Candida albicans
Lipopolysaccharide producers *(see LPS list)*



Am J Physiol Gastrointest Liver Physiol. 2022 Jan 1;322(1):G169-G182.

INTESTINAL HEALTH MARKERS

DIGESTION	Result	Reference
Steatocrit	<dl	< 15 %
Elastase-1	>750	> 200 ug/g
GI MARKERS		
β-Glucuronidase	996	< 2486 U/mL
Occult Blood - FIT	59 H	< 10 ug/g
IMMUNE RESPONSE		
Secretory IgA	>6000 H	510 - 2010 ug/g
Anti-gliadin IgA	178 H	< 175 U/L
Eosinophil Activation Protein (EDN, EPX)	0.21	< 2.34 ug/g
INFLAMMATION		
Calprotectin	722 H	< 173 ug/g
ADD-ON TESTS		
Zonulin	95.9	< 175 ng/g

Comparative Study > BMC Microbiol. 2017 Jul 17;17(1):160. doi: 10.1186/s12866-017-1059-6.

The human jejunum has an endogenous microbiota that differs from those in the oral cavity and colon

Olof H Sundin [1], Antonio Mendoza-Ladd [2], Mingtao Zeng [3], Diana Diaz-Arévalo [3], Elisa Morales [3], B Matthew Fagan [3], Javier Ordoñez [4], Philip Velez [3], Nishaal Antony [2], Richard W McCallum [2]

Affiliations + expand

PMID: 28716079 PMCID: PMC5513040 DOI: 10.1186/s12866-017-1059-6

Free PMC article

Abstract

Background: The upper half of the human small intestine, known as the jejunum, is the primary site for absorption of nutrient-derived carbohydrates, amino acids, small peptides, and vitamins. In contrast to the colon, which contains 10^{11}-10^{12} colony forming units of bacteria per ml (CFU/ml), the normal jejunum generally ranges from 10^3 to 10^5 CFU per ml. Because invasive procedures are required to access the jejunum, much less is known about its bacterial microbiota. Bacteria inhabiting the jejunal lumen have been investigated by classical culture techniques, but not by culture-independent metagenomics.

Results: The lumen of the upper jejunum was sampled during enteroscopy of 20 research subjects.

Table 3 Average composition of the jejunum by genus and its correlation with bacterial load

Rank	Composition Jejunum	Pearson Correlation with Log Bacterial Load	*P*-value of Correlation	Genus	Phylum
1	28.0%	0.036	0.880	Streptococcus	Firmicutes
2	12.5%	−0.296	0.205	Prevotella	Bacteroidetes
3	6.7%	0.158	0.507	Veillonella	Firmicutes
4	6.5%	−0.236	0.316	Escherichia	Proteobacteria
5	5.7%	0.188	0.428	Fusobacterium	Fusobacteria
6	5.2%	0.163	0.492	Haemophilus	Proteobacteria
7	2.8%	0.201	0.396	Actinomyces	Actinobacteria
8	2.3%	−0.554	0.011	Rothia	Actinobacteria
9	2.3%	−0.428	0.060	Leptotrichia	Fusobacteria
10	2.0%	0.101	0.672	Gemella	Firmicutes
11	2.0%	−0.264	0.261	Neisseria	Proteobacteria
12	1.8%	0.336	0.148	Klebsiella	Proteobacteria
13	1.8%	0.345	0.136	Citrobacter	Proteobacteria
14	1.3%	0.196	0.407	Actinobacillus	Proteobacteria
15	0.9%	0.355	0.125	Granulicatella	Firmicutes
16	0.9%	0.381	0.097	Enterobacter	Proteobacteria
17	0.8%	−0.158	0.507	Bacteroides	Bacteroidetes
18	0.8%	−0.483	0.031	Lachnoclostridium	Firmicutes

International Immunology, Vol. 34, No. 9, pp. 485–490
https://doi.org/10.1093/intimm/dxac027
Advance Access publication 18 June 2022

Untangling the oral–gut axis in the pathogenesis of intestinal inflammation

Sho Kitamoto[1,2] and **Nobuhiko Kamada**[1,2]

[1]The World Premier International Research Center (WPI) Immunology Frontier Research Center (IFReC), 1012 IFReC Research Building, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan
[2]Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Michigan, 1150 West Medical Center Drive, Ann Arbor, MI 48109, USA

Correspondence to: S. Kitamoto; E-mail: skitamoto@ifrec.osaka-u.ac.jp or N. Kamada; E-mail: nkamada@umich.edu

Received 6 April 2022, *editorial decision* 14 June 2022; *accepted* 16 June 2022

Abstract

An increasing body of literature reveals that host–microbe networks are well coordinated and impact human health and disease. Recently, it has become evident that an abnormal alteration in bacterial configuration in the oral cavity, namely oral dysbiosis, caused by periodontal inflammation, is associated with various distant inflammatory diseases, including inflammatory bowel disease. However, the extent to which the relationships between oral and distant disorders are merely an association or are causally triggered by oral microorganisms remains debated. In this mini-review, we highlight mechanisms in inter-related organ system diseases, particularly the one between oral and gut inflammation. Further, we discuss clinical perspectives and propose a novel concept of a multi-hit hypothesis in the pathogenesis of gut inflammation, on the basis of our updated knowledge of shared microbiological and immunological pathways between the oral and gut mucosae.

Keywords: inflammatory bowel disease, intermucosal interactions, oral bacteria, periodontitis, systemic organ interactions

REVIEW

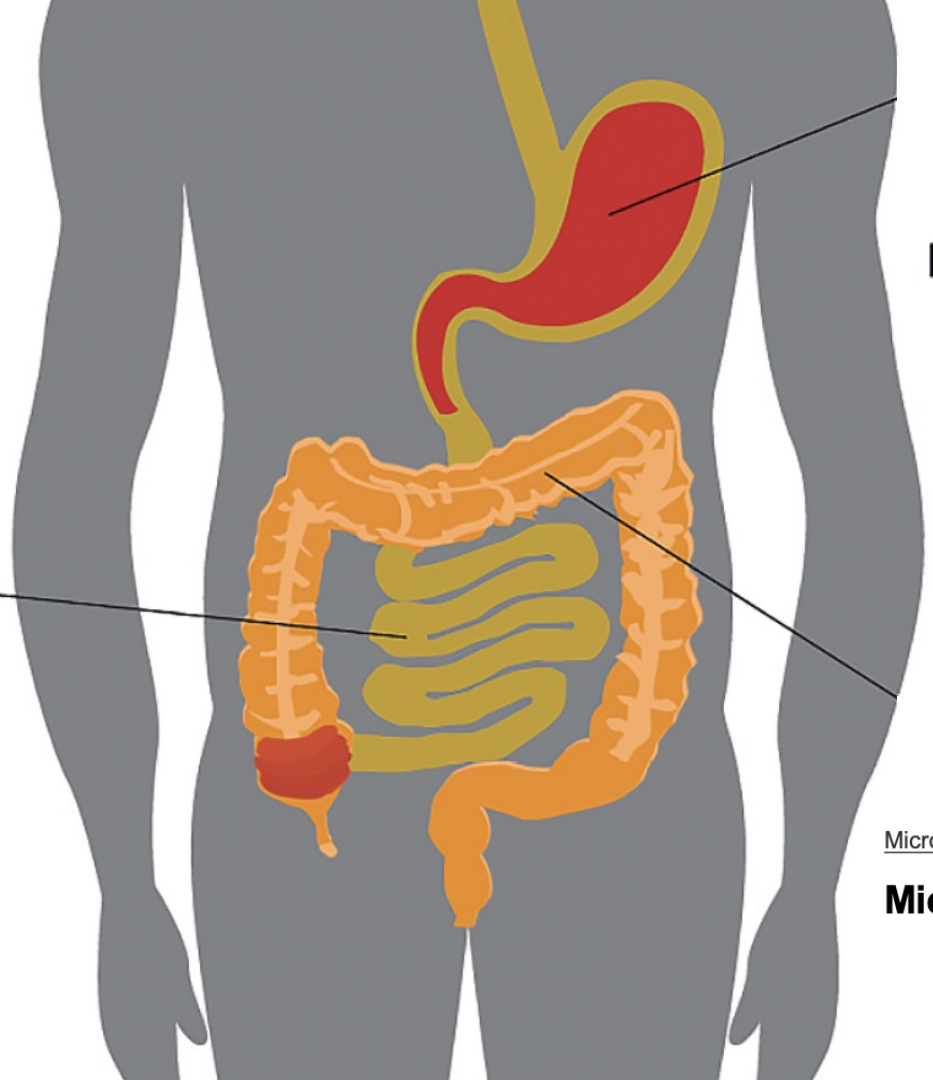
Introduction

The human body is home to 3.8 trillion microorganisms, al-

pathobionts associated with periodontitis, has also been

Abstract

An increasing body of literature reveals that host–microbe networks are well coordinated and impact human health and disease. Recently, it has become evident that an abnormal alteration in bacterial configuration in the oral cavity, namely oral dysbiosis, caused by periodontal inflammation, is associated with various distant inflammatory diseases, including inflammatory bowel disease. However, the extent to which the relationships between oral and distant disorders are merely an association or are causally triggered by oral microorganisms remains debated. In this mini-review, we highlight mechanisms in inter-related organ system diseases, particularly the one between oral and gut inflammation. Further, we discuss clinical perspectives and propose a novel concept of a multi-hit hypothesis in the pathogenesis of gut inflammation, on the basis of our updated knowledge of shared microbiological and immunological pathways between the oral and gut mucosae.



Microbes Environ. 2017 Dec 27;32(4):300-313. doi: 10.1264/jsme2.ME17017. Epub 2017 Nov 10.

Microbial Ecology along the Gastrointestinal Tract.

Klebsiella

a [4Fe-4S] cluster can also enhance the LipA reaction. It remains to be shown how the efficiencies of transfer compare for the two proteins.

The demonstration that NfuA and IscU can reinstall the LipA Fe-S cluster after each turnover explains how Fe-S clusters can serve as a sulfur source in catalytic reactions and dispels controversy surrounding this mechanism (*13*). It is remarkable that an Fe-S cluster can be destroyed and repaired rapidly enough as to not impede catalysis. This strategy is likely applicable to related radical SAM enzymes that contain auxiliary clusters, including biotin synthase, for which a similar mechanism has been proposed (*14*).

A number of questions remain unresolved, however. Further verification that NfuA transfers an Fe-S cluster to LipA, rather than directly providing sulfur for the lipoyl product, is desirable. In addition, it is unclear how NfuA and IscU recognize LipA or other targets that they supply with Fe-S clusters. It may be that in vivo, chaperones, which did not enhance the reaction studied here (*3*), play a role in guiding these processes (*12*). Finally, a major question for this system, and for Fe-S assembly proteins in general, is how the clusters are moved from scaffold to target protein. The molecular details of how the cluster dissociates from one

"It is remarkable that an Fe-S cluster can be destroyed and repaired rapidly enough as to not impede catalysis."

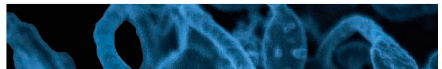
MICROBIOME

Intestinal inflammation induced by oral bacteria

Colonization of intestine by oral bacteria can induce inflammatory disorders

By **Xuetao Cao**

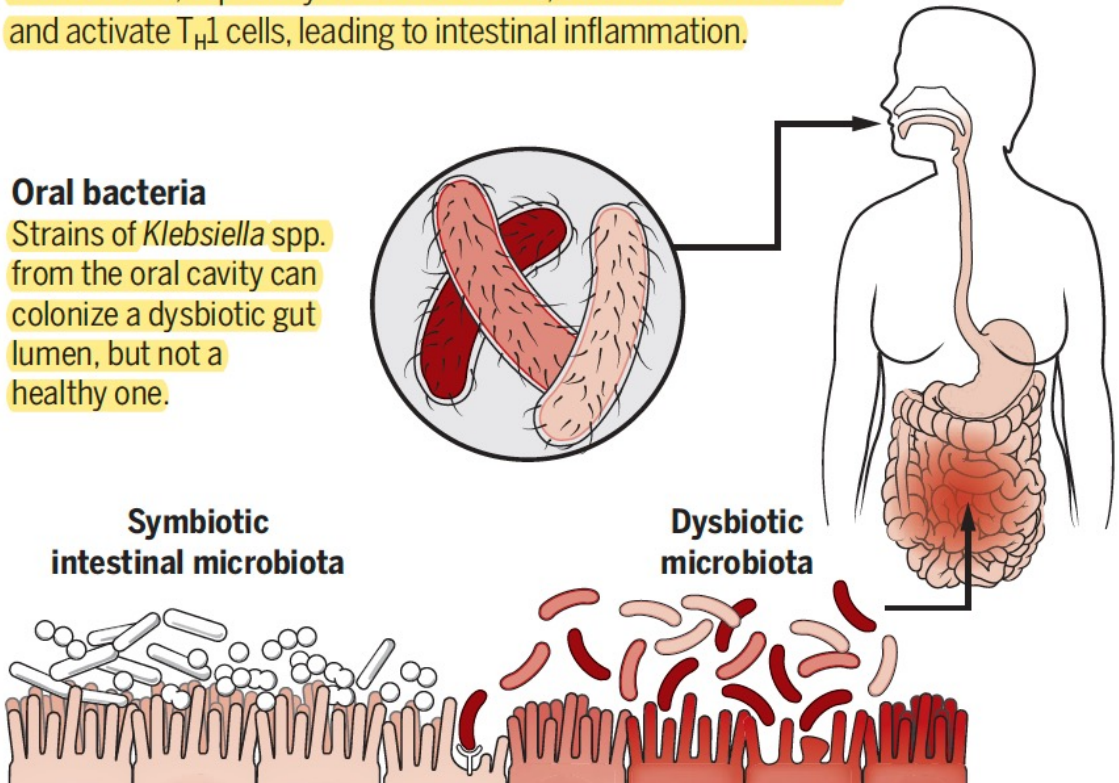
Numerous microorganisms, including bacteria, colonize the intestine where they play important roles in maintaining homeostasis. However, commensal bacteria with pathogenic potential, such as *Helicobacter hepaticus*, can also induce intestinal inflammation. Cross-talk between gut microbiota and the host immune system can prevent or mediate chronic intestinal inflammation, the outcome of which depends on gut microbiota composition, immune response, host genetic factors, and how these factors interact (*1, 2*). Physiologically, the intestine has developed several strategies to resist colonization by non-native bacteria and control the expansion of pathobionts that have the potential to cause pathology. Intestinal colonization by bacteria from the oral cavity has been suggested to be extensively involved in inflammatory diseases (*3, 4*). However, it remains unclear what subset of oral microbiota may ectopically colonize the intestine and whether they induce inflammatory immune responses. On page 359 of this issue, Atarashi *et al.* (*5*) show that strains of *Klebsiella* was identified to be a major inducer of T_H1 cell–mediated inflammatory immune responses. Kp-2H7 was resistant to multiple antibiotics, including ampicillin and tylosin. Antibiotic-naïve, specific pathogen–free (SPF) mice were resistant to intestinal colonization by Kp-2H7, but ampicillin or tylosin treatment allowed Kp-2H7 to persist in the intestine, and this was accompanied by increased colonic T_H1 cells. Although Kp-2H7 could not induce colonic inflammation in wild-type mice, monocolonization of Kp-2H7 caused severe colitis in interleukin-10 (IL-10)–deficient mice, which spontaneously develop intestinal inflammation with a highly polarized T_H1 response. Together, these data demonstrate that intestinal colonization and pathogenic inflammation induced by oral Kp-2H7 occur only under certain circumstances such as antibiotic-induced microbiota perturbation. This indicates that Kp-2H7 acts as a gut pathobiont (but does not induce inflammation in the oral mucosa) in the context of a genetically susceptible host.



Intestinal colonization by oral bacteria induces chronic inflammation

Under certain circumstances, such as antibiotic-induced dysbiosis, ingested oral bacteria, especially *Klebsiella* strains, colonize the intestine and activate T_H1 cells, leading to intestinal inflammation.

Oral bacteria
Strains of *Klebsiella* spp. from the oral cavity can colonize a dysbiotic gut lumen, but not a healthy one.



Biochemical Society Transactions (2023) **51** 887–896
https://doi.org/10.1042/BST20200325

PORTLAND PRESS

Review Article

The role of *Klebsiella* populations in preterm infants

Anne L. McCartney and Lesley Hoyles

Department of Biosciences, School of Science & Technology, Nottingham Trent University, Nottingham, U.K.

Correspondence: Lesley Hoyles (lesley.hoyles@ntu.ac.uk)

OPEN ACCESS

The preterm infant microbiota is dominated by *Enterobacteriaceae* (*Escherichia*, *Klebsiella* or *Enterobacter* spp.), *Enterococcus* and *Staphylococcus* spp. Recent work has demonstrated the development of this microbiota is predictable and driven by simple microbe–microbe interactions. Because of their systemic immaturity, including an underdeveloped immune system, preterm infants are susceptible to a range of infections. Numerous retrospective studies have examined the association of the preterm gut microbiota with diseases such as necrotizing enterocolitis (NEC), early-onset sepsis and late-onset sepsis. To date, no single bacterium has been associated with infection in these infants, but a *Klebsiella*/*Enterococcus*-dominated faecal microbiota is associated with an increased risk of developing NEC. Staphylococci aid and enterococci inhibit establishment/maintenance of gastrointestinal *Klebsiella* populations in preterm infants, though the mechanisms underlying these interactions are poorly understood. *Klebsiella* spp. recovered from healthy and sick preterm infants display similar antimicrobial resistance and virulence profiles, giving no clues as to why some infants develop potentially life-threatening diseases while others do not. The identification of cytotoxin-producing *Klebsiella oxytoca sensu lato* in the gut microbiota of some preterm infants has led to the suggestion that these

The preterm infant microbiota is dominated by *Enterobacteriaceae* (*Escherichia*, *Klebsiella* or *Enterobacter* spp.), *Enterococcus* and *Staphylococcus* spp. Recent work has demonstrated the development of this microbiota is predictable and driven by simple microbe–microbe interactions. Because of their systemic immaturity, including an underdeveloped immune system, preterm infants are susceptible to a range of infections. Numerous retrospective studies have examined the association of the preterm gut microbiota with diseases such as necrotizing enterocolitis (NEC), early-onset sepsis and late-onset sepsis. To date, no single bacterium has been associated with infection in these infants, but a *Klebsiella*/*Enterococcus*-dominated faecal microbiota is associated with an increased risk of developing NEC. Staphylococci aid and enterococci inhibit establishment/maintenance of gastrointestinal *Klebsiella* populations in preterm infants, though the mechanisms underlying these interactions are poorly understood. *Klebsiella* spp. recovered from healthy and sick preterm infants display similar antimicrobial resistance and virulence profiles, giving no clues as to why some infants develop potentially life-threatening diseases while others do not. The identification of cytotoxin-producing *Klebsiella oxytoca sensu lato* in the gut microbiota of some preterm infants has led to the suggestion that these bacteria may contribute to NEC in a subset of neonates. This mini review highlights current knowledge on *Klebsiella* spp. contributing to the preterm gut microbiota and provides insights into areas of research that warrant further attention.

AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

RESEARCH ARTICLE

Modelling the Gastrointestinal Carriage of *Klebsiella pneumoniae* Infections

Ricardo Calderon-Gonzalez,[a] Alix Lee,[a] Guillermo Lopez-Campos,[a] Steven J. Hancock,[a] Joana Sa-Pessoa,[a] Amy Dumigan,[a] Ronan McMullan,[a] Eric L. Campbell,[a] Jose A. Bengoechea[a]

[a]Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom

ABSTRACT *Klebsiella pneumoniae* is a leading cause of nosocomial and community acquired infections, making *K. pneumoniae* the pathogen that is associated with the second largest number of deaths attributed to any antibiotic resistant infection. *K. pneumoniae* colonizes the nasopharynx and the gastrointestinal tract in an asymptomatic manner without dissemination to other tissues. Importantly, gastrointestinal colonization is a requisite for infection. Our understanding of *K. pneumoniae* colonization is still based on interrogating mouse models in which animals are pretreated with antibiotics to disturb the colonization resistance imposed by the gut microbiome. In these models, infections disseminate to other tissues. Here, we report a murine model to allow for the study of the gastrointestinal colonization of *K. pneumoniae* without tissue dissemination. Hypervirulent and antibiotic resistant strains stably colonize the gastrointestinal tract of in an inbred mouse population without

ABSTRACT *Klebsiella pneumoniae* is a leading cause of nosocomial and community acquired infections, making *K. pneumoniae* the pathogen that is associated with the second largest number of deaths attributed to any antibiotic resistant infection. *K. pneumoniae* colonizes the nasopharynx and the gastrointestinal tract in an asymptomatic manner without dissemination to other tissues. Importantly, gastrointestinal colonization is a requisite for infection. Our understanding of *K. pneumoniae* colonization is still based on interrogating mouse models in which animals are pretreated with antibiotics to disturb the colonization resistance imposed by the gut microbiome. In these models, infections disseminate to other tissues. Here, we report a murine model to allow for the study of the gastrointestinal colonization of *K. pneumoniae* without tissue dissemination. Hypervirulent and antibiotic resistant strains stably colonize the gastrointestinal tract of in an inbred mouse population without antibiotic treatment. The small intestine is the primary site of colonization and is followed by a transition to the colon over time, without dissemination to other tissues. Our model recapitulates the disease dynamics of the metastatic *K. pneumoniae* strains that are able to disseminate from the gastrointestinal tract to other sterile sites. Colonization is associated with mild to moderate histopathology, no significant

Significant knowledge gaps exist regarding the host factors that influence gastrointestinal colonization, although recent data indicate that age and alcohol consumption are associated with increased colonization (9). In addition, antibiotic treatment seems to predispose individuals to colonization (9) and, in the clinical setting, may result in the dissemination of *K. pneumoniae* from the gastrointestinal tract to other tissues, thereby resulting in sepsis and other life-threatening complications (3, 4, 10). These observations suggest that the commensal gut microbiota provide a barrier to *K. pneumoniae* colonization. Indeed, a number of studies in mice demonstrate that antibiotic pretreatment facilitates *K. pneumoniae* colonization (11).

Comment > Nat Rev Gastroenterol Hepatol. 2022 Oct;19(10):623.
doi: 10.1038/s41575-022-00681-z.

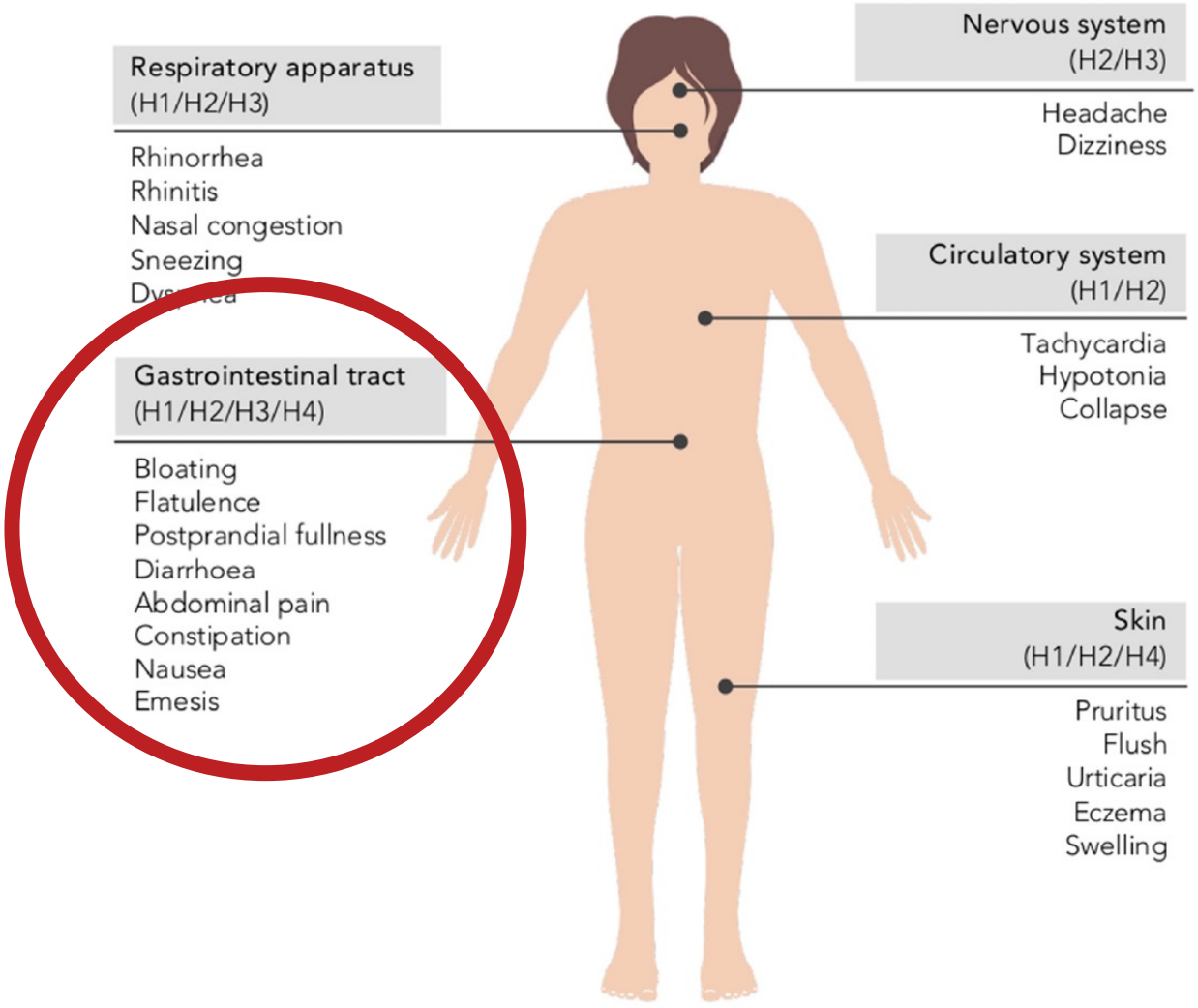
Bacterial histamine and abdominal pain in IBS

"***Bacterium-produced histamine induces abdominal pain sensitivity*** via histamine H4 receptor signalling, leading to the ***accumulation and activation of mast cells in the colon***. The study pinpoints ***Klebsiella aerogenes as a major producer of histamine*** and a potential therapeutic target in the management of pain in irritable bowel syndrome (IBS)."

Review > Biomolecules. 2020 Aug 14;10(8):1181. doi: 10.3390/biom10081181.

Histamine Intolerance: The Current State of the Art

"Specifically, the Enterobacteriaceae species Hafnai aluei, ***Morganella morganii*** and ***Klebsiella pneumoniae*** have been identified as some of the most ***prolific histamine-forming bacteria*** … "


Respiratory apparatus
(H1/H2/H3)
Rhinorrhea
Rhinitis
Nasal congestion
Sneezing
Gastrointestinal tract
(H1/H2/H3/H4)
Bloating
Flatulence
Postprandial fullness
Diarrhoea
Abdominal pain
Constipation
Nausea
Emesis
Nervous system
(H2/H3)
Headache
Dizziness
Circulatory system
(H1/H2)
Tachycardia
Hypotonia
Collapse
Skin
(H1/H2/H4)
Pruritus
Flush
Urticaria
Eczema
Swelling

bioRxiv. 2023 Jun 11;2023.05.25.542283. doi: 10.1101/2023.05.25.542283. Preprint

Colonization and Dissemination of *Klebsiella pneumoniae* is Dependent on Dietary Carbohydrates

Aaron L Hecht, Lisa C Harling, Elliot S Friedman, Ceylan Tanes, Junhee Lee, Jenni Firrman, Vincent Tu, LinShu Liu, Kyle Bittinger, Mark Goulian, Gary D Wu

PMID: 37292978 PMCID: PMC10245944 DOI: 10.1101/2023.05.25.542283
Free PMC article

Abstract

Dysbiosis of the gut microbiota is increasingly appreciated as both a consequence and precipitant of human disease. The outgrowth of the bacterial family *Enterobacteriaceae* is a common feature of dysbiosis, including the human pathogen *Klebsiella pneumoniae* . Dietary interventions have proven efficacious in the resolution of dysbiosis, though the specific dietary components involved remain poorly defined. Based on a previous human diet study, we hypothesized that dietary nutrients serve as a key resource for the growth of bacteria found in dysbiosis. Through human sample testing, and *ex-vivo* , and *in vivo* modeling, we find that nitrogen is not a limiting resource for the growth of *Enterobacteriaceae* in the gut, contrary to previous studies. Instead, we identify dietary simple carbohydrates as critical in colonization of *K. pneumoniae* . We additionally find that dietary fiber is necessary for colonization resistance against *K. pneumoniae* , mediated by recovery of the commensal microbiota, and protecting the host against dissemination from the gut microbiota during colitis. Targeted dietary therapies based on these findings may offer a therapeutic strategy in susceptible patients with dysbiosis.

nature microbiology

ARTICLES

https://doi.org/10.1038/s41564-019-0640-1

Commensal Bacteroidetes protect against *Klebsiella pneumoniae* colonization and transmission through IL-36 signalling

Richard P. Sequeira[1], Julie A. K. McDonald[2], Julian R. Marchesi[2,3] and Thomas B. Clarke[1*]

The microbiota primes immune defences but the identity of specific commensal microorganisms that protect against infection is unclear. Conversely, how pathogens compete with the microbiota to establish their host niche is also poorly understood. In the present study, we investigate the antagonism between the microbiota and *Klebsiella pneumoniae* during colonization and transmission. We discover that maturation of the microbiota drives the development of distinct immune defence programmes in the upper airways and intestine to limit *K. pneumoniae* colonization within these niches. Immune protection in the intestine depends on the development of Bacteroidetes, interleukin (IL)-36 signalling and macrophages. This effect of Bacteroidetes requires the polysaccharide utilization locus of their conserved commensal colonization factor. Conversely, in the upper airways, Proteobacteria prime immunity through IL-17A, but *K. pneumoniae* overcomes these defences through encapsulation to effectively colonize this site. Ultimately, we find that host-to-host spread of *K. pneumoniae* occurs principally from its intestinal reservoir, and that commensal-colonization-factor-producing Bacteroidetes are sufficient to prevent transmission between hosts through IL-36. Thus, our study provides mechanistic insight into when, where and how commensal Bacteroidetes protect against *K. pneumoniae* colonization and contagion, providing insight into how these protective microorganisms could be harnessed to confer population-level protection against *K. pneumoniae* infection.

The microbiota enhances immune defences to protect against pathogenic microorganisms[1–4]. Identification of members of the microbiota that protect against pathogens could therefore provide an alternative way of treating infections resistant to current antimicrobial therapies[5]. Of these antimicrobial-resistant is poorly understood, hampering the use of microbiota-based approaches to protect against *K. pneumoniae*.

Once colonization has been established, these hosts serve as reservoirs for the transmission of *K. pneumoniae* within a population. The

nutrients

MDPI

Article

Biotin Deficiency Induces Intestinal Dysbiosis Associated with an Inflammatory Bowel Disease-like Phenotype

Julianne C. Yang [1], Jonathan P. Jacobs [1,2], Michael Hwang [3], Subrata Sabui [3], Fengting Liang [1], Hamid M. Said [3,4,5] and Jonathan Skupsky [4,5,*]

[1] The Vatche and Tamar Manoukian Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA
[2] Division of Gastroenterology, Department of Medicine, VA Greater Los Angeles Healthcare System, Los Angeles, CA 90073, USA
[3] Department of Physiology and Biophysics, University of California, Irvine, CA 92697, USA
[4] Department of Medicine, University of California, Irvine, CA 92697, USA
[5] Division of Gastroenterology, Department of Medicine, Tibor Rubin VA Medical Center, Long Beach, CA 90822, USA
* Correspondence: jonathan.skupsky@va.gov

Abstract: Biotin is an essential vitamin and critical cofactor in several metabolic pathways, and its deficiency has been linked to several disorders including inflammatory bowel disease (IBD). We previously reported that biotin deficiency (BD) in mice, whether modeled through intestine-specific deletion of biotin transporter (SMVT-icKO) or through a biotin-deficient diet, resulted in intestinal inflammation consistent with an IBD-like phenotype. To assess whether the gut microbiome is associated with these BD-induced changes, we collected stool and intestinal samples from both of these mouse models and utilized them for 16S rRNA gene sequencing. We find that both diet-mediated and deletion-mediated BD result in the expansion of opportunistic microbes including *Klebsiella*, *Enterobacter*, and *Helicobacter*, at the expense of mucus-resident microbes including *Akkermansia*. Ad-

Pseudomonas

J Med Microbiol. 2011 Feb;60(Pt 2):236-245. doi: 10.1099/jmm.0.022848-0. Epub 2010 Oct 14.

Molecular analysis of faecal and duodenal samples reveals significantly higher prevalence and numbers of Pseudomonas aeruginosa in irritable bowel syndrome

"This study shows that P. aeruginosa is detected more frequently and at higher levels in IBS patients than in healthy subjects, suggesting its potential role in the pathophysiology of IBS."

› Gastroenterology. 2016 Oct;151(4):670-83. doi: 10.1053/j.gastro.2016.06.041. Epub 2016 Jun 30.

Duodenal Bacteria From Patients With Celiac Disease and Healthy Subjects Distinctly Affect Gluten Breakdown and Immunogenicity

Alberto Caminero [1], Heather J Galipeau [1], Justin L McCarville [1], Chad W Johnston [2], Steve P Bernier [1], Amy K Russell [3], Jennifer Jury [1], Alexandra R Herran [4], Javier Casqueiro [4], Jason A Tye-Din [5], Michael G Surette [6], Nathan A Magarvey [2], Detlef Schuppan [7], Elena F Verdu [8]

Affiliations + expand

PMID: 27373514 DOI: 10.1053/j.gastro.2016.06.041

Abstract

Background & aims: Partially degraded gluten peptides from cereals trigger celiac disease (CD), an autoimmune enteropathy occurring in genetically susceptible persons. Susceptibility genes are necessary but not sufficient to induce CD, and additional environmental factors related to unfavorable alterations in the microbiota have been proposed. We investigated gluten metabolism by opportunistic pathogens and commensal duodenal bacteria and characterized the capacity of

Nat Commun. 2019 Mar 13;10(1):1198. doi: 10.1038/s41467-019-09037-9.

Duodenal bacterial proteolytic activity determines sensitivity to dietary antigen through protease-activated receptor-2.

"Here, we found a correlation between Pseudomonas relative abundance and increased proteolytic activity against gluten in the small intestine of patients with CeD."

"These results demonstrate that proteases expressed by ***opportunistic pathogens*** impact host immune responses that are ***relevant to the development of food sensitivities***, independently of the trigger antigen."

Comment Immunity. 2022 May 10;55(5):824-826. doi: 10.1016/j.immuni.2022.04.011.

Virulence triggered allergies: Pseudomonas gets the Las laugh

Justin L McCarville [1], Janelle S Ayres [2]

Affiliations

PMID: 35545032 DOI: 10.1016/j.immuni.2022.04.011

Abstract

The mechanisms of how infectious diseases contribute to allergy remain unanswered. In this issue of Immunity, Agaronyan et al. (2022) show that Pseudomonas aeruginosa drives immune deviation through induction of type 2 immune responses, resulting in niche remodeling that incites allergic responses to innocuous antigens.

Inflammation Research (2019) 68:397–413
https://doi.org/10.1007/s00011-019-01227-x

Inflammation Research

ORIGINAL RESEARCH PAPER

Pseudomonas aeruginosa biofilm is a potent inducer of phagocyte hyperinflammation

Marta Ciszek-Lenda[1] · Magdalena Strus[2] · Maria Walczewska[1] · Grzegorz Majka[1] · Agnieszka Machul-Żwirbla[2] · Diana Mikołajczyk[2] · Sabina Górska[3] · Andrzej Gamian[3] · Benjamin Chain[4] · Janusz Marcinkiewicz[1]

Received: 7 March 2019 / Accepted: 10 March 2019 / Published online: 18 March 2019

Abstract

Objective *Pseudomonas aeruginosa* effectively facilitate resistance to phagocyte killing by biofilm formation. However, the cross talk between biofilm components and phagocytes is still unclear. We hypothesize that a biofilm provides a concentrated extracellular source of LPS, DNA and exopolysaccharides (EPS), which polarize neighbouring phagocytes into an adverse hyperinflammatory state of activation.

Methods We measured the release of a panel of mediators produced in vitro by murine neutrophils and macrophages exposed to various biofilm components of *P. aeruginosa* cultures.

Results We found that conditioned media from a high biofilm-producing strain of *P. aeruginosa*, PAR5, accumulated high concentrations of extracellular bacterial LPS, DNA and EPS by 72 h. These conditioned media induced phagocytes to release a hyperinflammatory pattern of mediators, with enhanced levels of TNF-α, IL-6, IL12p40, PGE_2 and NO. Moreover, the phagocytes also upregulated COX-2 and iNOS with no influence on the expression of arginase-1.

Conclusions Phagocytes exposed to biofilm microenvironment, called by us biofilm-associated neutrophils/macrophages (BANs/BAMs), display secretory properties similar to that of N1/M1-type phagocytes. These results suggest that in vivo high concentrations of LPS and DNA, trapped in biofilm by EPS, might convert infiltrating phagocytes into cells responsible for tissue injury without direct contact with bacteria and phagocytosis.

Keywords Biofilm · Hyperinflammation · Neutrophils · *P. aeruginosa* · LPS · DNA

ISME J. 2020 Aug 19. doi: 10.1038/s41396-020-00741-9. Online ahead of print.

Trophic cooperation promotes bacterial survival of Staphylococcus aureus and Pseudomonas aeruginosa

Laura Camus [1], Paul Briaud [1], Sylvère Bastien [1], Sylvie Elsen [2], Anne Doléans-Jordheim [3 4], François Vandenesch [1 3 5], Karen Moreau [6]

Affiliations

PMID: 32814867 DOI: 10.1038/s41396-020-00741-9

Abstract

In the context of infection, Pseudomonas aeruginosa and Staphylococcus aureus are frequently co-isolated, particularly in cystic fibrosis (CF) patients. Within lungs, the two pathogens exhibit a range of competitive and coexisting interactions. In the present study, we explored the impact of S. aureus on the physiology of P. aeruginosa in the context of coexistence. Transcriptomic analyses showed that S. aureus significantly and specifically affects the expression of numerous genes involved in P. aeruginosa carbon and amino acid metabolism. In particular, 65% of the strains presented considerable overexpression of the genes involved in the acetoin catabolic (aco) pathway. We demonstrated that acetoin is (i) produced by clinical S. aureus strains, (ii) detected in sputa from CF patients and (iii) involved in P. aeruginosa's aco system induction. Furthermore, acetoin is catabolized by P. aeruginosa, a metabolic process that improves the survival of both pathogens by providing a new carbon source for P. aeruginosa and avoiding the toxic accumulation

Biotechnology Advances 49 (2021) 107734

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Pseudomonas aeruginosa biofilms and their partners in crime

Maria del Mar Cendra [a,**], Eduard Torrents [a,b,*]

[a] Bacterial Infections and Antimicrobial therapies Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 15-21, 08028 Barcelona, Spain

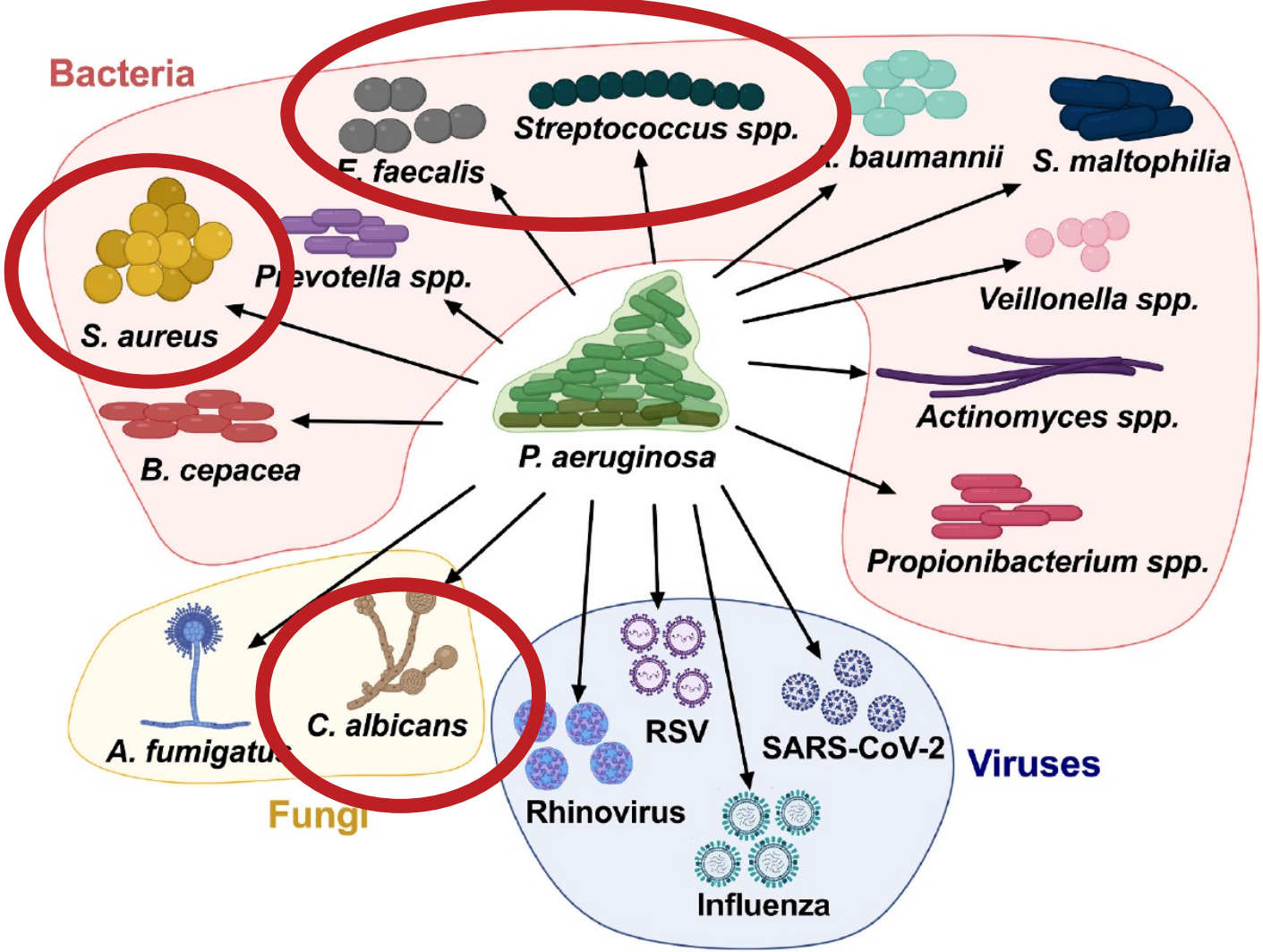
[b] Microbiology Section, Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, 643 Diagonal Ave., 08028 Barcelona, Spain

ARTICLE INFO

Keywords:
Pseudomonas aeruginosa
Biofilms
Polymicrobial
Chronic infections
P. aeruginosa models
Antimicrobials

ABSTRACT

Pseudomonas aeruginosa biofilms and the capacity of the bacterium to coexist and interact with a broad range of microorganisms have a substantial clinical impact. This review focuses on the main traits of *P. aeruginosa* biofilms, such as the structural composition and regulatory networks involved, placing particular emphasis on the clinical challenges they represent in terms of antimicrobial susceptibility and biofilm infection clearance. Furthermore, the ability of *P. aeruginosa* to grow together with other microorganisms is a significant pathogenic attribute with clinical relevance; hence, the main microbial interactions of *Pseudomonas* are especially highlighted and detailed throughout this review. This article also explores the infections caused by single and polymicrobial biofilms of *P. aeruginosa* and the current models used to recreate them under laboratory conditions. Finally, the antimicrobial and antibiofilm strategies developed against *P. aeruginosa* mono and multispecies biofilms are detailed at the end of this review.


Bacteria
E. faecalis
Streptococcus spp.
A. baumannii
S. maltophilia
S. aureus
Prevotella spp.
Veillonella spp.
Actinomyces spp.
B. cepacea
P. aeruginosa
Propionibacterium spp.
A. fumigatus
C. albicans
Fungi
RSV
SARS-CoV-2
Viruses
Rhinovirus
Influenza

Genet Mol Res. 2015 Dec 16;14(4):17044-58. doi: 10.4238/2015.December.16.5.

Lactic acid bacteria protect human intestinal epithelial cells from Staphylococcus aureus and Pseudomonas aeruginosa infections

S Affhan [1], W Dachang [1], Y Xin [1], D Shang [2]

Affiliations

PMID: 26681052 DOI: 10.4238/2015.December.16.5

Free article

Abstract

Staphylococcus aureus and Pseudomonas aeruginosa are opportunistic pathogens that cause nosocomial and food-borne infections. They promote intestinal diseases. Gastrointestinal colonization by S. aureus and P. aeruginosa has rarely been researched. These organisms spread to extra gastrointestinal niches, resulting in increasingly progressive infections. Lactic acid bacteria are Gram-positive bacteria that produce lactic acid as the major end-product of carbohydrate fermentation. These bacteria inhibit pathogen colonization and modulate the host immune response. This study aimed to investigate the effects of Lactobacillus acidophilus and Lactobacillus rhamnosus on enteric infections caused by the paradigmatic human pathogens S. aureus

Staphylococcus

Randomized Controlled Trial > J Allergy Clin Immunol. 2019 Aug;144(2):494-503.
doi: 10.1016/j.jaci.2019.04.025. Epub 2019 May 31.

Association of Staphylococcus aureus colonization with food allergy occurs independently of eczema severity

Olympia Tsilochristou [1], George du Toit [2], Peter H Sayre [3], Graham Roberts [4], Kaitie Lawson [5], Michelle L Sever [5], Henry T Bahnson [6], Suzana Radulovic [2], Monica Basting [2], Marshall Plaut [7], Gideon Lack [8], Immune Tolerance Network Learning Early About Peanut Allergy Study Team

Collaborators, Affiliations + expand

PMID: 31160034 DOI: 10.1016/j.jaci.2019.04.025

Abstract

Background: Staphylococcus aureus has been implicated in the pathophysiology of eczema, allergic rhinitis, asthma, and food allergy. S aureus is a marker of more severe eczema, which is a risk factor for food sensitization/allergy. Therefore it might be that the association between S aureus and food allergy in eczematous patients is related to eczema severity.

Objective: We sought to investigate the association of S aureus colonization with specific IgE

toxins

MDPI

Review

Allergy—A New Role for T Cell Superantigens of *Staphylococcus aureus*?

Goran Abdurrahman [1], Frieder Schmiedeke [1], Claus Bachert [2,3], Barbara M. Bröker [1] and Silva Holtfreter [1,*]

1 Department of Immunology, University Medicine Greifswald, 17475 Greifswald, Germany; goran.abdurrahman@uni-greifswald.de (G.A.); frieder.schmiedeke@uni-greifswald.de (F.S.); broeker@uni-greifswald.de (B.M.B.)

2 Upper Airways Research Laboratory, Department of Otorhinolaryngology, Ghent University, 9000 Ghent, Belgium; Claus.Bachert@UGent.be

3 Current address: Department of Medicine Solna, Immunology and Allergy Research Unit, Karolinska Institute, 171 77 Stockholm, Sweden

* Correspondence: silva.holtfreter@med.uni-greifswald.de; Tel.: +49-3834-86-5518

Received: 15 February 2020; Accepted: 10 March 2020; Published: 12 March 2020

Abstract: *Staphylococcus aureus* superantigens (SAgs) are among the most potent T cell mitogens known. They stimulate large fractions of T cells by cross-linking their T cell receptor with major histocompatibility complex class-II molecules on antigen presenting cells, resulting in T cell proliferation and massive cytokine release. To date, 26 different SAgs have been described in the

> Nature. 2013 Nov 21;503(7476):397-401. doi: 10.1038/nature12655. Epub 2013 Oct 30.

Staphylococcus δ-toxin induces allergic skin disease by activating mast cells

"These studies identify ***delta-toxin as a potent inducer of mast cell degranulation*** and suggest a mechanistic link between S. aureus colonization and allergic skin disease."

Review > Front Immunol. 2021 Jun 15;12:685865. doi: 10.3389/fimmu.2021.685865. eCollection 2021.

Responses of Mast Cells to Pathogens: Beneficial and Detrimental Roles

Microbes known to stimulate mast cell responses:

Staphylococcus aureus
Streptococcus spp.
Pseudomonas aeruginosa
Enterococcus faecalis
Candida
H. pylori
Histamine & LPS-producing microbes

› Gut Pathog. 2011 Apr 26;3(1):6. doi: 10.1186/1757-4749-3-6.

Real-time PCR analysis of enteric pathogens from fecal samples of irritable bowel syndrome subjects

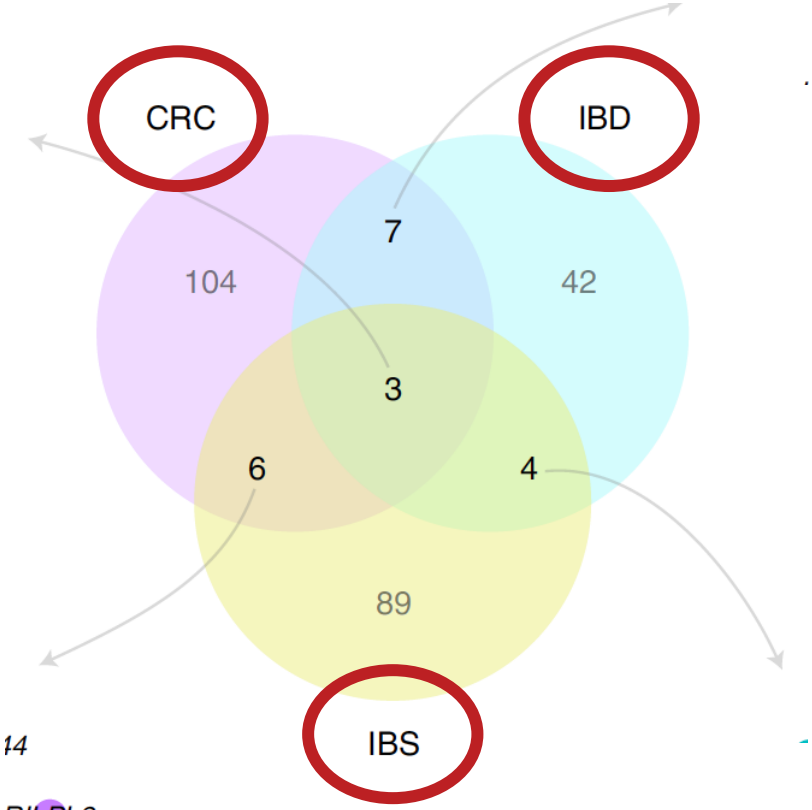
Background: Growing amount of scientific evidence suggests that microbes are involved in the pathophysiology of irritable bowel syndrome (IBS). The predominant fecal microbiota composition of IBS subjects has been widely studied with DNA-based techniques but less research has been focused on the intestinal pathogens in this disorder. Here, we optimized a highly sensitive panel of 12 quantitative real-time PCR (qPCR) assays to shed light on the putative presence of intestinal pathogens in IBS sufferers. The panel was used to screen fecal samples from 96 IBS subjects and 23 healthy controls.

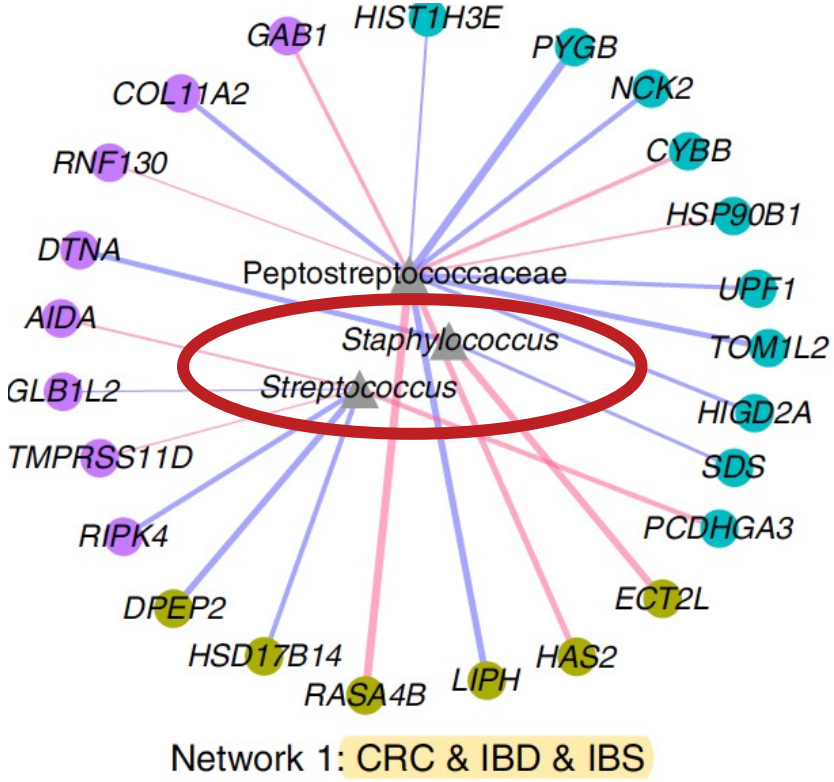
Results: Fifteen IBS samples (17%) tested positive for *Staphylococcus aureus* with a thermonuclease (*nuc*) gene-targeting qPCR assay, whereas none of the healthy controls were positive for *S. aureus* ($p < 0.05$). The *S. aureus* -positive IBS samples were confirmed by sequencing of the PCR amplicons. *Clostridium perfringens* was detected from IBS and control groups with a similar frequency (13% and 17%, respectively) with α-toxin (*plc*) gene -targeting qPCR assay while none of the samples tested positive for the *Cl. perfringens* enterotoxin-encoding gene (*cpe*).

Conclusions: The qPCR panel consisting of 12 assays for an extensive set of pathogenic microorganisms provides an efficient alternative to the conventional detection of gastrointestinal pathogens and could accelerate the initiation of targeted antibiotic therapy reducing the risk of post-infectious IBS (PI-IBS). *S. aureus* has not been previously reported to be associated with the onset of IBS. Although we discovered significant differences in the prevalence of *S. aureus* between the study groups, its importance in giving rise to IBS symptoms requires further studies.

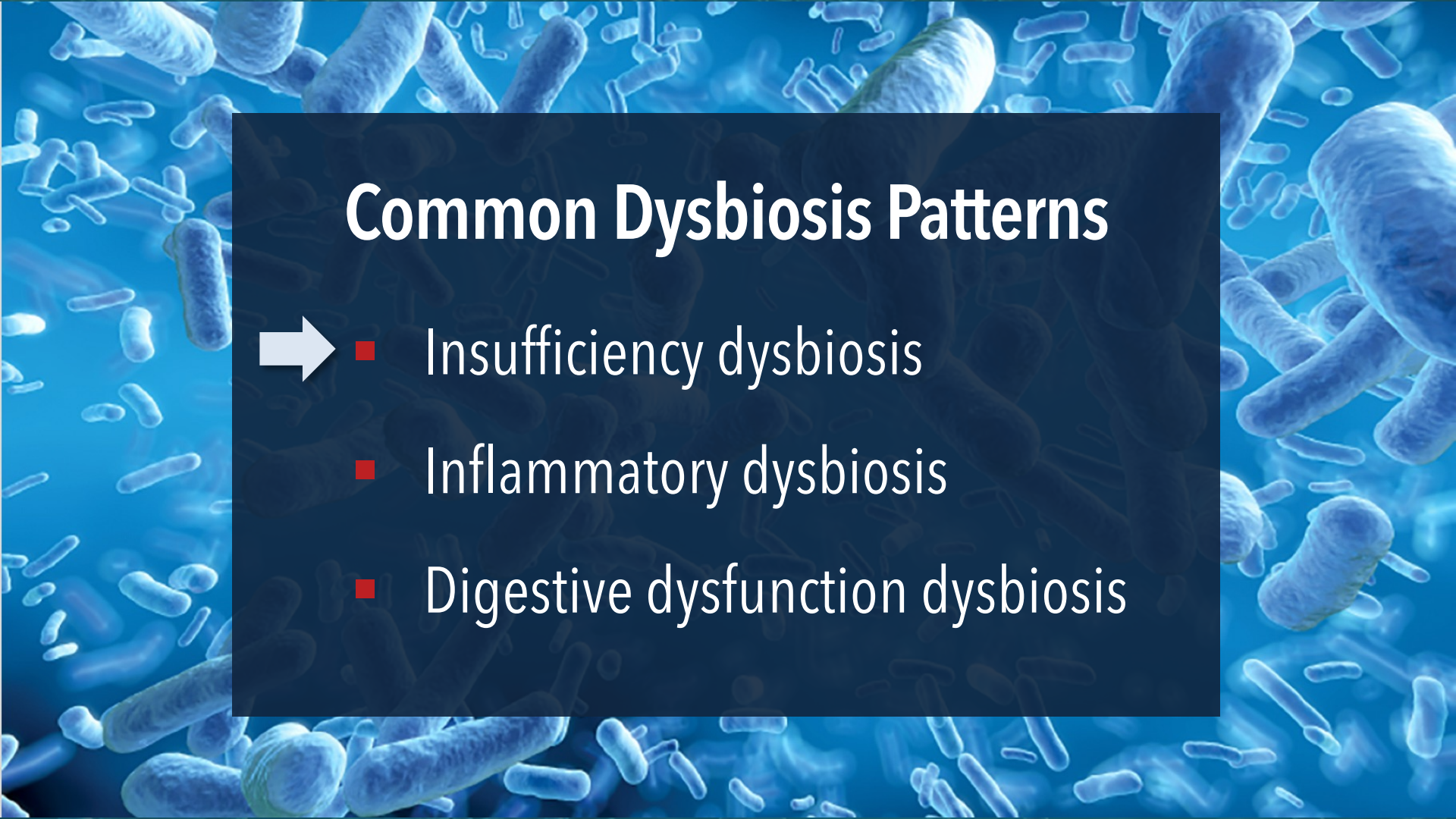
Nat Microbiol. 2022 Jun;7(6):780-795. doi: 10.1038/s41564-022-01121-z. Epub 2022 May 16.

Identification of shared and disease-specific host gene-microbiome associations across human diseases using multi-omic integration

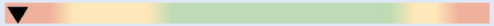
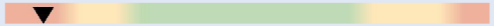
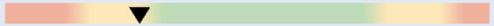
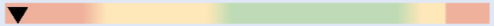
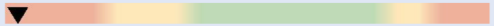


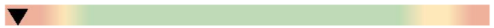
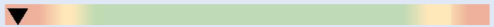


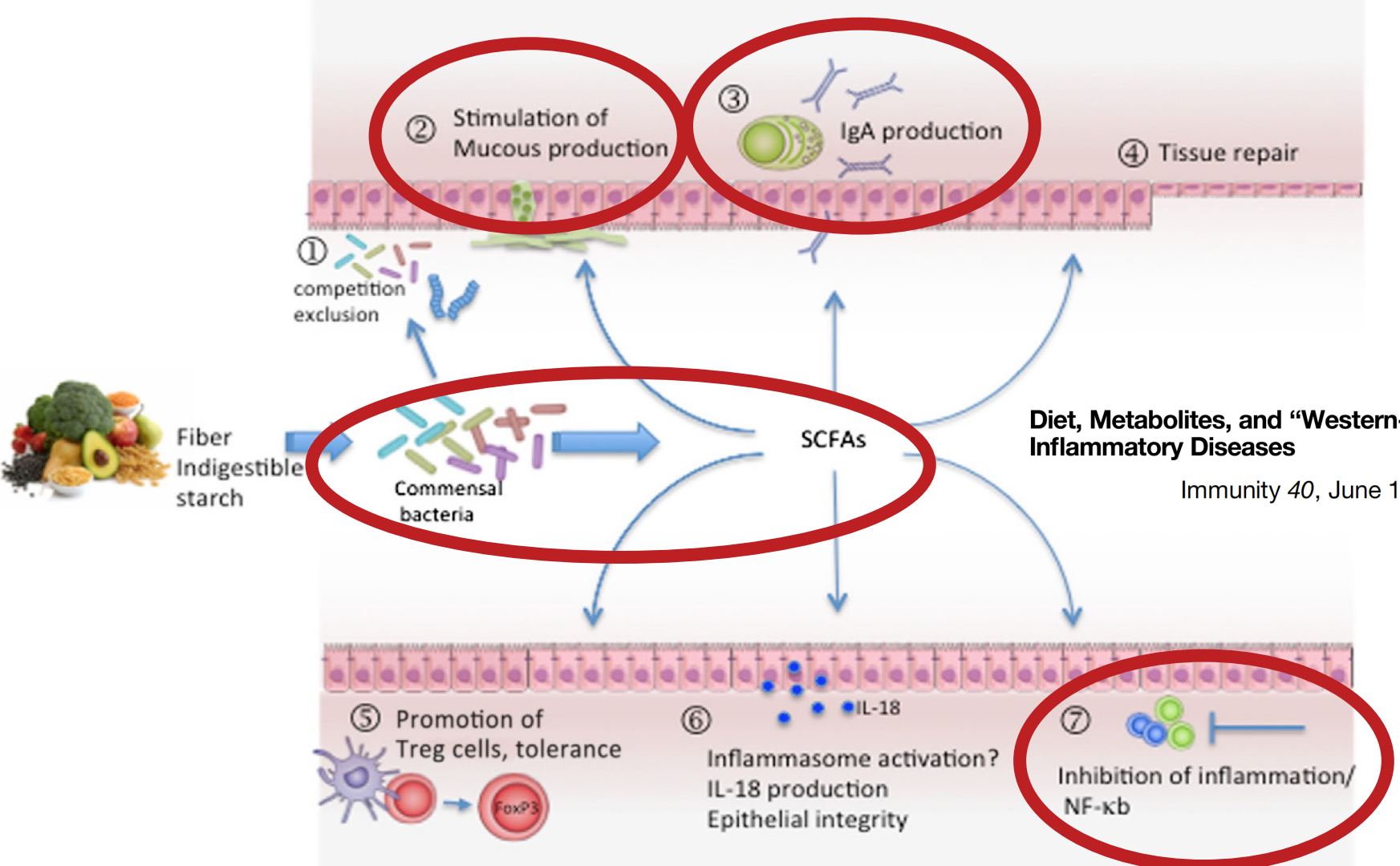
Network 1: CRC & IBD & IBS


Common Dysbiosis Patterns
Insufficiency dysbiosis
Inflammatory dysbiosis
Digestive dysfunction dysbiosis

COMMENSAL/KEYSTONE BACTERIA

COMMENSAL BACTERIA	Result		Reference
Bacteroides fragilis	5.84e8 L		1.6e9 - 2.5e11
Bifidobacterium spp.	1.95e9		> 6.7e7
Enterococcus spp.	1.36e5 L		1.9e5 - 2.0e8
Escherichia spp.	1.32e6 L		3.7e6 - 3.8e9
Lactobacillus spp.	2.85e6		8.6e5 - 6.2e8
Enterobacter spp.	5.54e6		1.0e6 - 5.0e7
Akkermansia muciniphila	<dl L		1.0e1 - 8.2e6
Faecalibacterium prausnitzii	<dl L		1.0e3 - 5.0e8
Roseburia spp.	4.56e6 L		5.0e7 - 2.0e10

BACTERIAL PHYLA			
Bacteroidetes	2.17e10 L		8.6e11 - 3.3e12
Firmicutes	1.38e9 L		5.7e10 - 3.0e11
Firmicutes:Bacteroidetes Ratio	0.06		< 1.0



Diet, Metabolites, and "Western-Lifestyle" Inflammatory Diseases

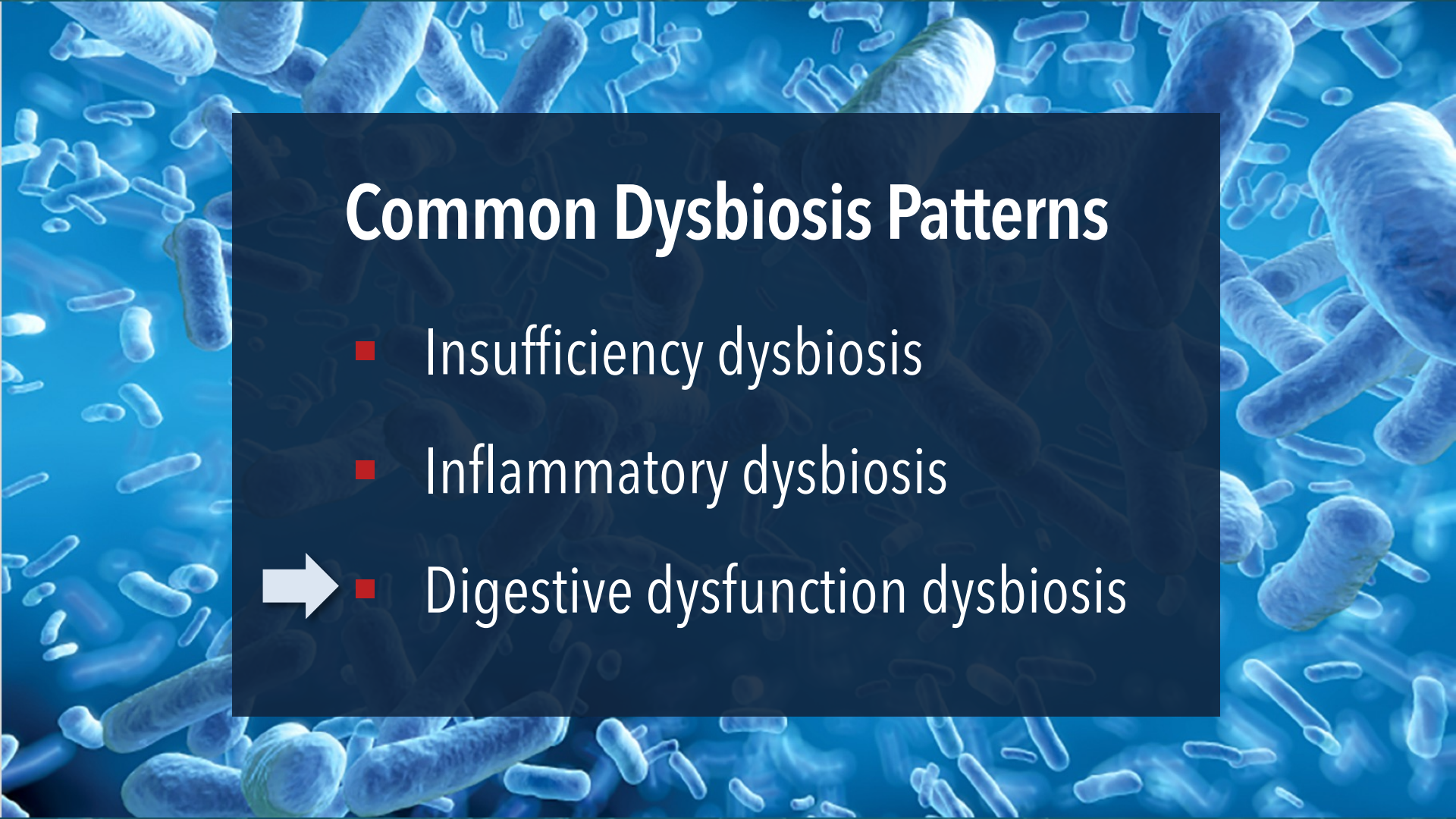
Immunity *40*, June 19, 2014

INTESTINAL HEALTH MARKERS

DIGESTION	Result	Reference
Steatocrit	<dl	< 15 %
Elastase-1	163 L	> 200 ug/g
GI MARKERS		
β-Glucuronidase	1204	< 2486 U/mL
Occult Blood - FIT	<dl	< 10 ug/g
IMMUNE RESPONSE		
Secretory IgA	403 L	510 - 2010 ug/g
Anti-gliadin IgA	71	< 175 U/L
Eosinophil Activation Protein (EDN, EPX)	0.07	< 2.34 ug/g
INFLAMMATION		
Calprotectin	<dl	< 173 ug/g
ADD-ON TESTS		
Zonulin	119.2	< 175 ng/g

Supporting Commensals (4P's)

- Prebiotics
- Probiotics
- Polyphenols
- Postbiotics
  (butyrate, vitamins, indoles, urolithin A, bile acid metabolites, etc.)


Common Dysbiosis Patterns
Insufficiency dysbiosis
Inflammatory dysbiosis
Digestive dysfunction dysbiosis

INTESTINAL HEALTH MARKERS

DIGESTION	Result	Reference
Steatocrit	<dl	< 15 %
Elastase-1	163 L	> 200 ug/g
GI MARKERS		
β-Glucuronidase	1204	< 2486 U/mL
Occult Blood - FIT	<dl	< 10 ug/g
IMMUNE RESPONSE		
Secretory IgA	403 L	510 - 2010 ug/g
Anti-gliadin IgA	71	< 175 U/L
Eosinophil Activation Protein (EDN, EPX)	0.07	< 2.34 ug/g
INFLAMMATION		
Calprotectin	<dl	< 173 ug/g
ADD-ON TESTS		
Zonulin	119.2	< 175 ng/g

> Aliment Pharmacol Ther. 2020 Mar;51(5):505-526. doi: 10.1111/apt.15604. Epub 2020 Jan 28.

Systematic review: the effects of proton pump inhibitors on the microbiome of the digestive tract–evidence from next-generation sequencing studies

- In a review of 19 eligible studies, higher levels of the following were found in stool of PPI users:
  - ***Streptococcus***
  - ***Enterococcus***
  - ***Staphylococcus***
  - ***Bacillus***
  - Lactobacillus
  - Enterobacteriaceae (E. coli, Klebsiella, etc.)

> Gastroenterology. 2019 Mar;156(4):1010-1015. doi: 10.1053/j.gastro.2018.10.047. Epub 2018 Nov 2.

Impaired Exocrine Pancreatic Function Associates With Changes in Intestinal Microbiota Composition and Diversity

"Differences in pancreatic elastase levels associated with significantly ($P < .0001$) greater changes in microbiota diversity than with participant age, body mass index, sex, smoking, alcohol consumption, or dietary factors."

The Gut Microbiome in Patients With Chronic Pancreatitis Is Characterized by Significant Dysbiosis and Overgrowth by Opportunistic Pathogens

Fabian Frost, MD[1], Frank U. Weiss, PhD[1], Matthias Sendler, PhD[1], Tim Kacprowski, PhD[2,3], Malte Rühlemann, PhD[4], Corinna Bang, PhD[4], Andre Franke, PhD[4], Uwe Völker, PhD[2], Henry Völzke, MD[5], Georg Lamprecht, MD[6], Julia Mayerle, MD[7], Ali A. Aghdassi, MD[1], Georg Homuth, PhD[2] and Markus M. Lerch, MD[1]

INTRODUCTION: **Exocrine pancreatic function is a critical host factor in determining the intestinal microbiota composition. Diseases affecting the exocrine pancreas could therefore influence the gut microbiome. We investigated the changes in gut microbiota of patients with chronic pancreatitis (CP).**

METHODS: **Patients with clinical and imaging evidence of CP (n = 51) were prospectively recruited and compared with twice the number of nonpancreatic disease controls matched for distribution in age, sex, body mass index, smoking, diabetes mellitus, and exocrine pancreatic function (stool elastase). From stool samples of these 153 subjects, DNA was extracted, and intestinal microbiota composition was determined by bacterial 16S ribosomal RNA gene sequencing.**

RESULTS: **Patients with CP exhibited severely reduced microbial diversity (Shannon diversity index and Simpson diversity number, $P < 0.001$) with an increased abundance of facultative pathogenic organisms ($P < 0.001$) such as *Enterococcus* ($q < 0.001$), *Streptococcus* ($q < 0.001$), and *Escherichia.Shigella* ($q = 0.002$). The CP-associated changes were independent of exocrine pancreatic insufficiency. Short-chain fatty acid producers, considered protective for epithelia such as *Faecalibacterium* ($q < 0.001$), showed reduced abundance in patients with CP. Of 4 additional patients with CP previously treated with antibiotics (ceftriaxone and metronidazole), 3 patients were characterized by distinct *Enterococcus***

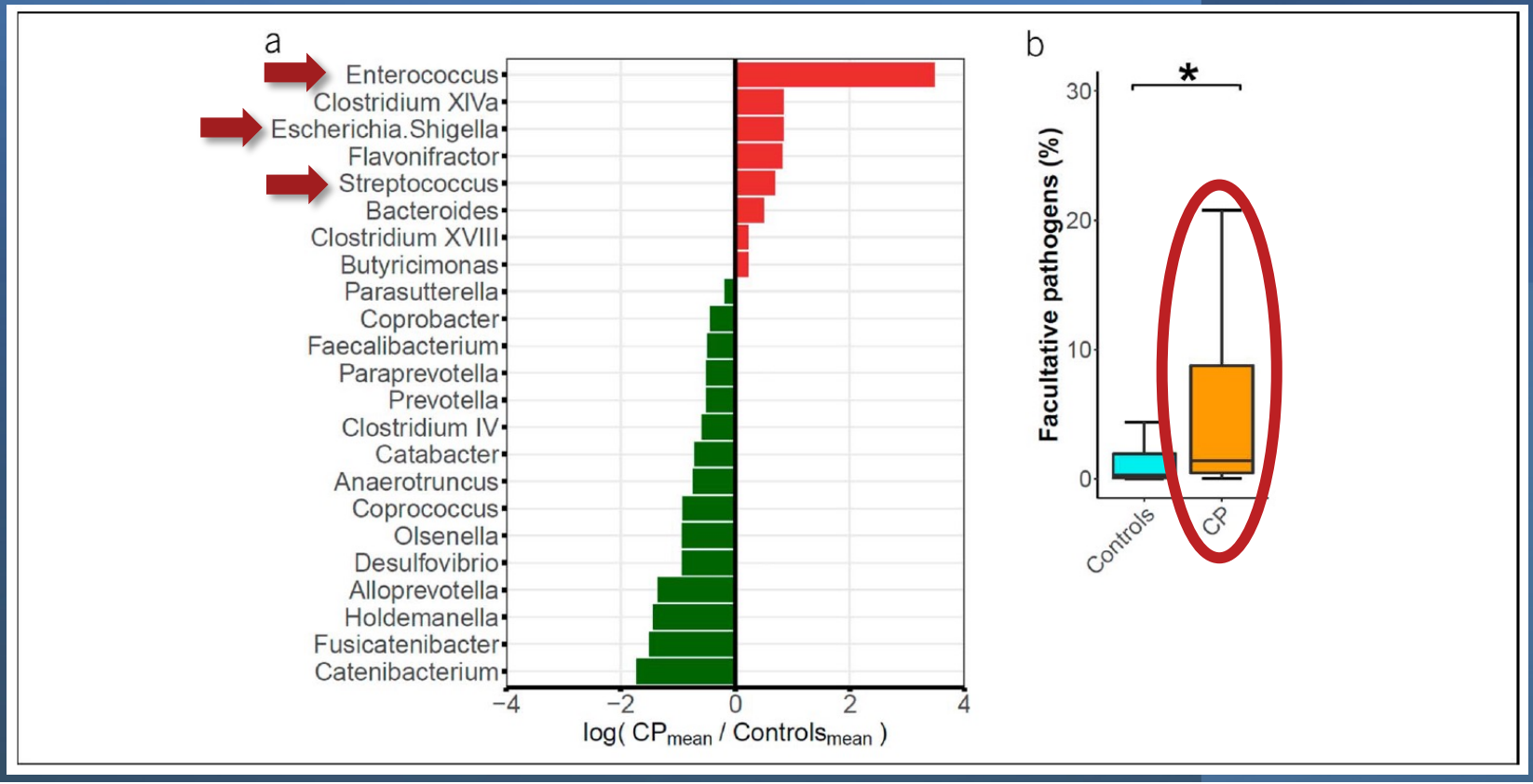


Figure 3. Intestinal microbiota alterations in CP cases. (**a**) Shown are all genera with significant differential abundance between CP cases and controls according to the Mann-Whitney test ($q < 0.05$). Abundance changes are depicted as log-fold change of mean abundance ratio (CP/controls). (**b**) Boxplot shows the distribution of important facultative pathogenic bacteria (summarized *Citrobacter*, *Enterobacter*, *Enterococcus*, *Enterobacteriaceae*, *Escherichia. Shigella*, *Klebsiella*, *Pseudomonas*, *Proteus*, *Staphylococcus*, and *Streptococcus* counts) in CP cases compared with controls. * Indicates a significant difference ($P < 0.05$). CP, chronic pancreatitis.

Case Example: Crohn's Disease

- 70-year old F, Dx several decades ago, chronic diarrhea, worse recently
- Herbal antimicrobials, butyrate, probiotics, polyphenols, emphasizing more plant foods

PATHOGENS

The testing includes pathogens (bacterial, parasitic and viral) commonly known to cause gastroenteritis. Note that not all individuals with positive findings will present with symptoms. Many factors, including the health of the individual (such as immune health, digestive function, and microbiome balance), the transient nature of most pathogens, and the presence and expression of virulence factors, all contribute to pathogen virulence and individual symptoms.

BACTERIAL PATHOGENS	Result	Reference
Campylobacter	<dl	< 1.00e3
C. difficile Toxin A	<dl	< 1.00e3
C. difficile Toxin B	<dl	< 1.00e3
Enterohemorrhagic E. coli	<dl	< 1.00e3
E. coli O157	<dl	< 1.00e3
Enteroinvasive *E. coli/Shigella*	<dl	< 1.00e3
Enterotoxigenic *E. coli* LT/ST	<dl	< 1.00e3
Shiga-like Toxin *E. coli* stx1	<dl	< 1.00e3
Shiga-like Toxin *E. coli* stx2	<dl	< 1.00e3
Salmonella	<dl	< 1.00e4
Vibrio cholerae	<dl	< 1.00e5
Yersinia enterocolitica	<dl	< 1.00e5
PARASITIC PATHOGENS		
Cryptosporidium	<dl	< 1.00e6
Entamoeba histolytica	<dl	< 1.00e4
Giardia	<dl	< 5.00e3
VIRAL PATHOGENS		
Adenovirus 40/41	<dl	< 1.00e10
Norovirus GI/II	<dl	< 1.00e7

HELICOBACTER PYLORI

H. PYLORI & VIRULENCE FACTORS	Result	Reference
Helicobacter pylori	<dl	< 1.00e3
Virulence Factor, babA	N/A	Negative
Virulence Factor, cagA	N/A	Negative
Virulence Factor, dupA	N/A	Negative
Virulence Factor, iceA	N/A	Negative
Virulence Factor, oipA	N/A	Negative
Virulence Factor, vacA	N/A	Negative
Virulence Factor, virB	N/A	Negative
Virulence Factor, virD	N/A	Negative

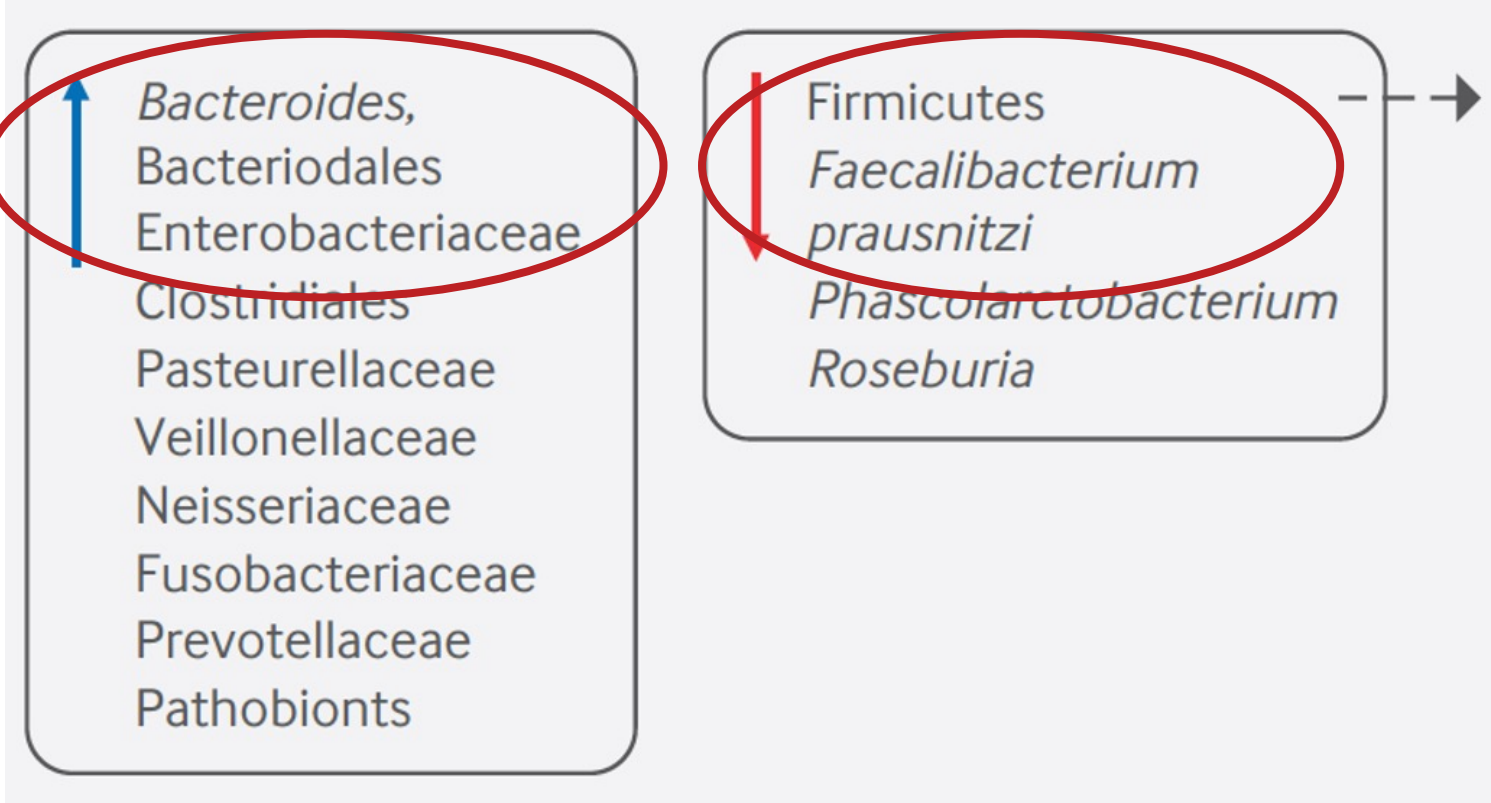
COMMENSAL/KEYSTONE BACTERIA

COMMENSAL BACTERIA	Result	Reference
Bacteroides fragilis	1.70e11	1.6e9 - 2.5e11
Bifidobacterium spp.	1.10e11	> 6.7e7
Enterococcus spp.	1.61e9 H	1.9e5 - 2.0e8
Escherichia spp.	7.01e9 H	3.7e6 - 3.8e9
Lactobacillus spp.	2.06e7	8.6e5 - 6.2e8
Enterobacter spp.	8.83e5 L	1.0e6 - 5.0e7
Akkermansia muciniphila	1.16e4	1.0e1 - 8.2e6
Faecalibacterium prausnitzii	<dl L	1.0e3 - 5.0e8
Roseburia spp.	1.41e9	5.0e7 - 2.0e10

BACTERIAL PHYLA		
Bacteroidetes	3.10e12	8.6e11 - 3.3e12
Firmicutes	6.27e10	5.7e10 - 3.0e11
Firmicutes:Bacteroidetes Ratio	0.02	< 1.0

BMJ. 2018 Jan 8;360:j5145. doi: 10.1136/bmj.j5145.

The role of the gut microbiome in systemic inflammatory disease.



OPPORTUNISTIC/OVERGROWTH MICROBES

DYSBIOTIC & OVERGROWTH BACTERIA	Result		Reference
Bacillus spp.	**6.95e7**	**High ↑**	< 1.76e6
Enterococcus faecalis	4.12e2		< 1.00e4
Enterococcus faecium	**1.36e7**	**High ↑**	< 1.00e4
Morganella spp.	**8.68e8**	**High ↑**	< 1.00e3
Pseudomonas spp.	<dl		< 1.00e4
Pseudomonas aeruginosa	<dl		< 5.00e2
Staphylococcus spp.	2.30e3		< 1.00e4
Staphylococcus aureus	**9.40e2**	**High ↑**	< 5.00e2
Streptococcus spp.	**2.19e3**	**High ↑**	< 1.00e3
COMMENSAL OVERGROWTH MICROBES			
Desulfovibrio spp.	7.91e5		< 7.98e8
Methanobacteriaceae (family)	2.10e7		< 3.38e8

> Front Cell Infect Microbiol. 2022 Oct 4;12:1015890. doi: 10.3389/fcimb.2022.1015890. eCollection 2022.

Overrepresentation of Enterobacteriaceae and *Escherichia coli* is the major gut microbiome signature in Crohn's disease and ulcerative colitis; a comprehensive metagenomic analysis of IBDMDB datasets

Results: Compared to the gut microbiome of HCs, six Enterobacteriaceae species were significantly elevated in both CD and UC patients, including *Escherichia coli*, *Klebsiella variicola*, *Klebsiella quasipneumoniae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter freundii*, and *Citrobacter youngae*, while *Klebsiella oxytoca*, *Morganella morganii*, and *Citrobacter amalonaticus* were uniquely differentially abundant and enriched in the CD cohort. Four species were uniquely differentially abundant and enriched in the UC cohort, including *Citrobacter portucalensis*, *Citrobacter pasteurii*, *Citrobacter werkmanii*, and *Proteus hauseri*. Our analysis also showed a dramatically increased abundance of *E. coli* in their intestinal bacterial community. Biosynthetic pathways of aerobactin siderophore, LPS, enterobacterial common antigen, nitrogen metabolism, and sulfur relay systems encoded by *E. coli* were significantly elevated in the CD samples compared to the HCs. Menaquinol biosynthetic pathways were associated with UC that belonged to *K. pneumoniae* strains.

INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA			
Citrobacter spp.	<dl		< 5.00e6
Citrobacter freundii	<dl		< 5.00e5
Klebsiella spp.	<dl		< 5.00e3
Klebsiella pneumoniae	5.45e3		< 5.00e4
M. avium subsp. paratuberculosis	<dl		< 5.00e3
Proteus spp.	<dl		< 5.00e4
Proteus mirabilis	<dl		< 1.00e3
COMMENSAL INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA			
Enterobacter spp.	8.83e5		< 5.00e7
Escherichia spp.	**7.01e9**	**High ↑**	< 3.80e9
Fusobacterium spp.	**7.16e8**	**High ↑**	< 1.00e8
Prevotella spp.	**1.03e8**	**High ↑**	< 1.00e8

FUNGI/YEAST

FUNGI/YEAST	Result	Reference
Candida spp.	<dl	< 5.00e3
Candida albicans	<dl	< 5.00e2
Geotrichum spp.	<dl	< 3.00e2
Microsporidium spp.	<dl	< 5.00e3
Rhodotorula spp.	<dl	< 1.00e3

VIRUSES

VIRUSES	Result	Reference
Cytomegalovirus	<dl	< 1.00e5
Epstein-Barr Virus	<dl	< 1.00e7

PARASITES

PROTOZOA	Result	Reference
Blastocystis hominis	<dl	< 2.00e3
Chilomastix mesnili	<dl	< 1.00e5
Cyclospora spp.	<dl	< 5.00e4
Dientamoeba fragilis	<dl	< 1.00e5
Endolimax nana	<dl	< 1.00e4
Entamoeba coli	<dl	< 5.00e6
Pentatrichomonas hominis	<dl	< 1.00e2

WORMS		
Ancylostoma duodenale	Not Detected	Not Detected
Ascaris lumbricoides	Not Detected	Not Detected
Necator americanus	Not Detected	Not Detected
Trichuris trichiura	Not Detected	Not Detected
Taenia spp.	Not Detected	Not Detected

INTESTINAL HEALTH MARKERS

DIGESTION	Result	Reference
Steatocrit	<dl	< 15 %
Elastase-1	472	> 200 ug/g
GI MARKERS		
β-Glucuronidase	292	< 2486 U/mL
Occult Blood - FIT	28 H	< 10 ug/g
IMMUNE RESPONSE		
Secretory IgA	1286	510 - 2010 ug/g
Anti-gliadin IgA	198 H	< 175 U/L
Eosinophil Activation Protein (EDN, EPX)	1.52	< 2.34 ug/g
INFLAMMATION		
Calprotectin	7	< 173 ug/g