Investigating and managing infectious diseases in the microbiome era.

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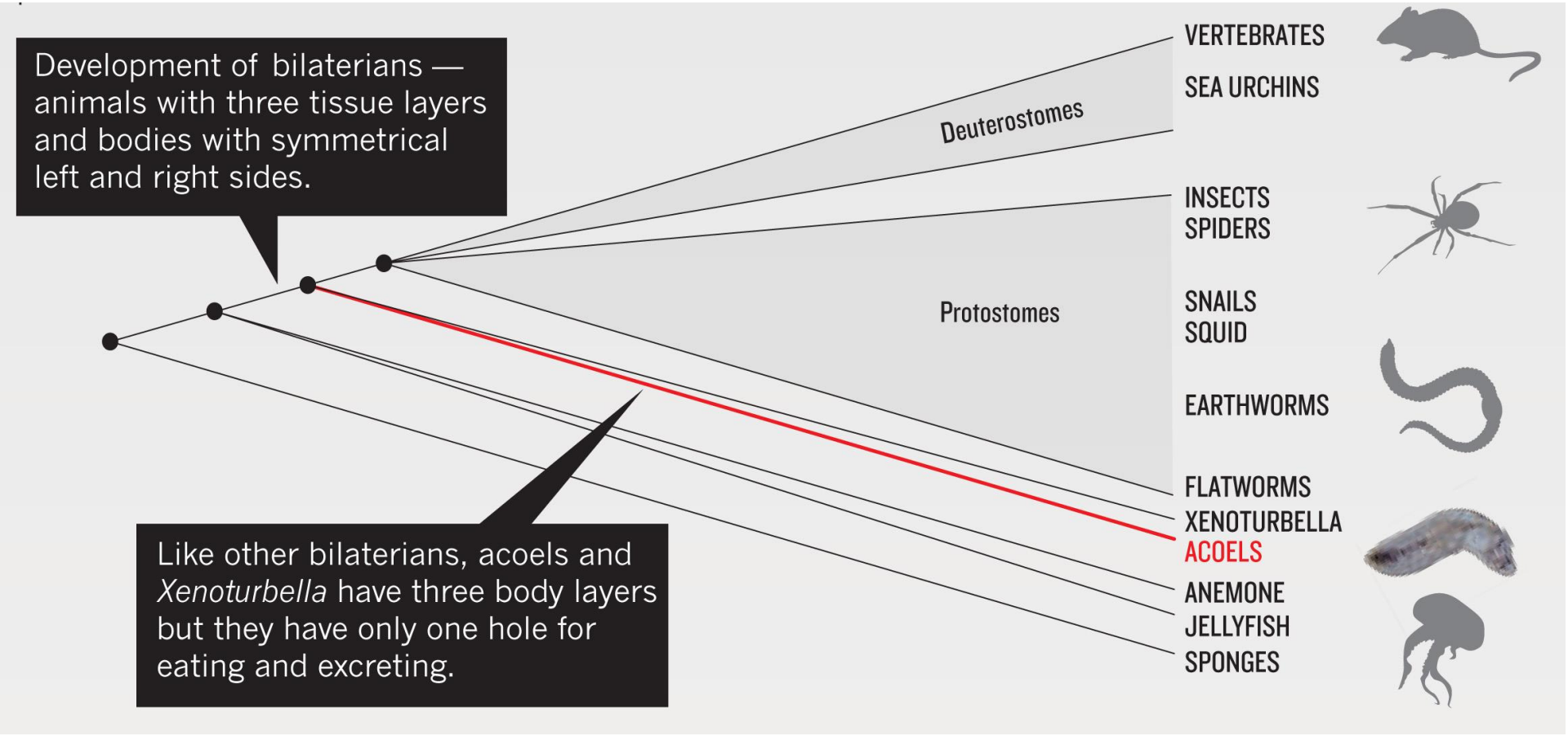
Microbes and the gut: 560 million years of co-evolution



Nature (2011) 470:161

Diversity of the Human Intestinal Microbial Flora

Paul B. Eckburg,[1*] Elisabeth M. Bik,[2] Charles N. Bernstein,[3] Elizabeth Purdom,[4] Les Dethlefsen,[2] Michael Sargent,[3] Steven R. Gill,[5] Karen E. Nelson,[5] David A. Relman[1,2,6*]

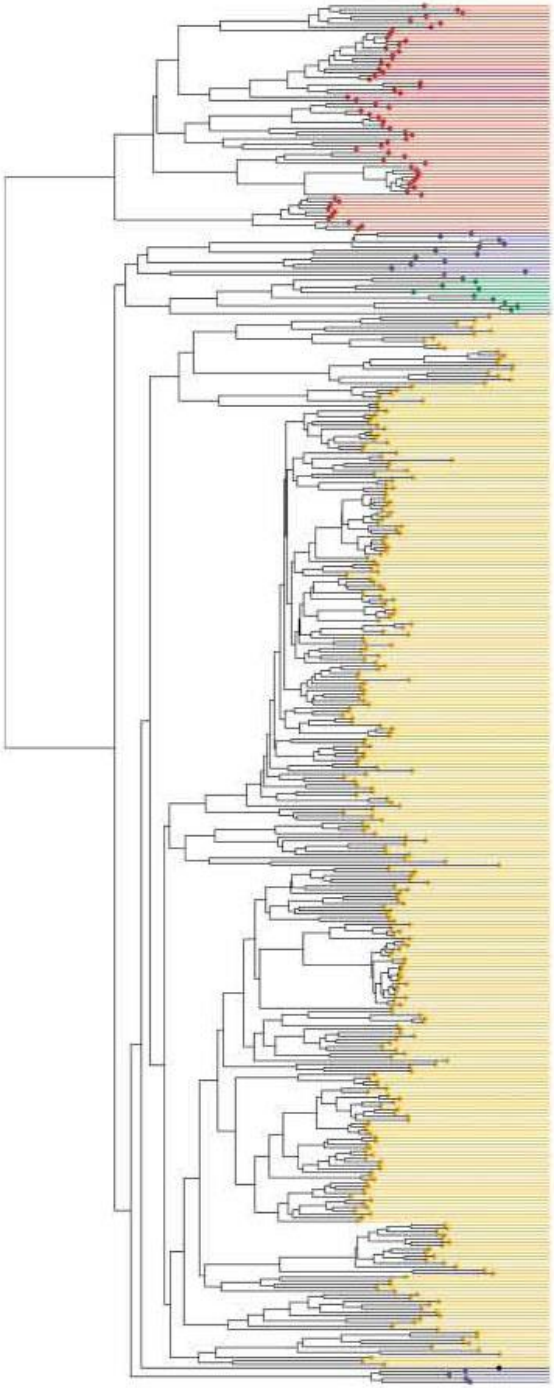
The human endogenous intestinal microflora is an essential "organ" in providing nourishment, regulating epithelial development, and instructing innate immunity; yet, surprisingly, basic features remain poorly described. We examined 13,355 prokaryotic ribosomal RNA gene sequences from multiple colonic mucosal sites and feces of healthy subjects to improve our understanding of gut microbial diversity. A majority of the bacterial sequences corresponded to uncultivated species and novel microorganisms. We discovered significant intersubject variability and differences between stool and mucosa community composition. Characterization of this immensely diverse ecosystem is the first step in elucidating its role in health and disease.

SCIENCE VOL 308 10 JUNE 2005

Most organisms identified by 16S sequencing had not been cultured.

Most organisms had not been associated with human disease.

Organisms inhabiting different individuals vary markedly.



ARTICLE

doi:10.1038/nature11234

Structure, function and diversity of the healthy human microbiome

The Human Microbiome Project Consortium*

Studies of the human microbiome have revealed that even healthy individuals differ remarkably in the microbes that occupy habitats such as the gut, skin and vagina. Much of this diversity remains unexplained, although diet, environment, host genetics and early microbial exposure have all been implicated. Accordingly, to characterize the ecology of human-associated microbial communities, the Human Microbiome Project has analysed the largest cohort and set of distinct, clinically relevant body habitats so far. We found the diversity and abundance of each habitat's signature microbes to vary widely even among healthy subjects, with strong niche specialization both within and among individuals. The project encountered an estimated 81–99% of the genera, enzyme families and community configurations occupied by the healthy Western microbiome. Metagenomic carriage of metabolic pathways was stable among individuals despite variation in community structure, and ethnic/racial background proved to be one of the strongest associations of both pathways and microbes with clinical metadata. These results thus delineate the range of structural and functional configurations normal in the microbial communities of a healthy population, enabling future characterization of the epidemiology, ecology and translational applications of the human microbiome.

Nature (2012) 486:207

Diverse microbial populations in different healthy humans.

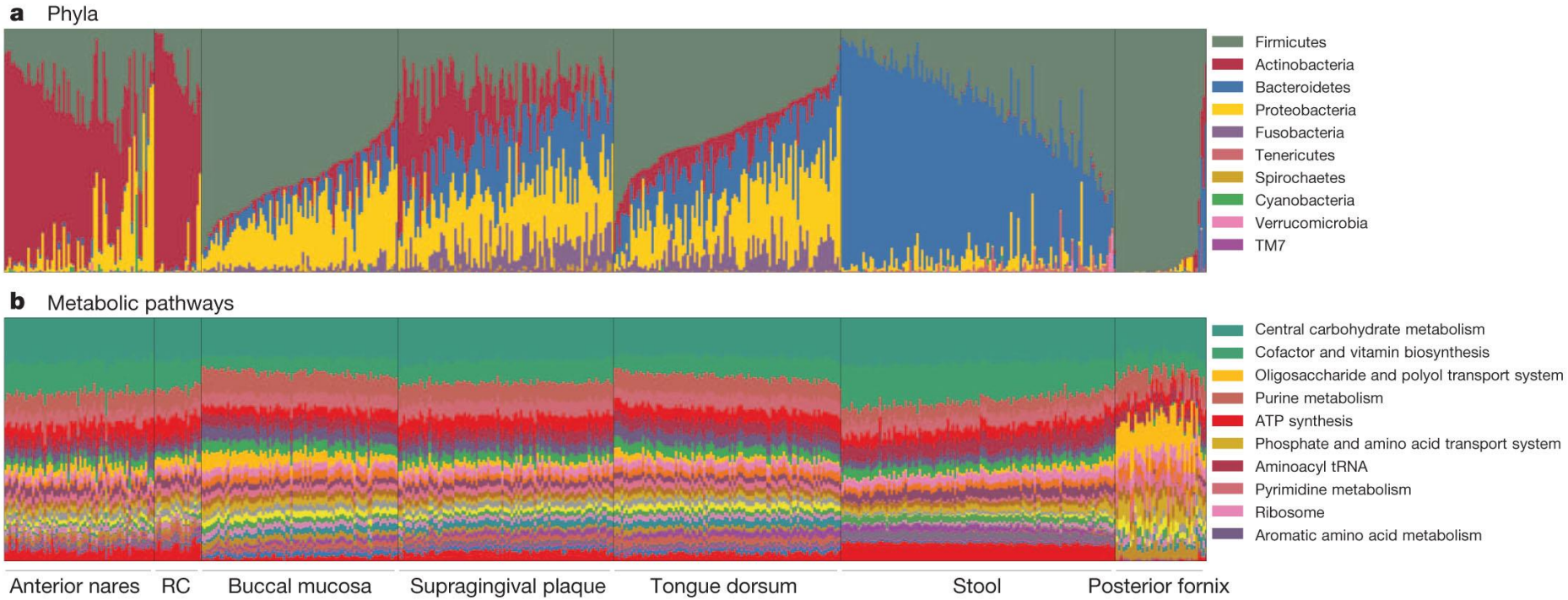


Figure 2 | Carriage of microbial taxa varies while metabolic pathways remain stable within a healthy population. **a**, **b**, Vertical bars represent microbiome samples by body habitat in the seven locations with both shotgun and 16S data; bars indicate relative abundances colored by microbial phyla from binned OTUs (**a**) and metabolic modules (**b**). Legend indicates most abundant phyla/pathways by average within one or more body habitats; RC, retroauricular crease. A plurality of most communities' memberships consists of a single dominant phylum (and often genus; see Supplementary Fig. 2), but this is universal neither to all body habitats nor to all individuals. Conversely, most metabolic pathways are evenly distributed and prevalent across both individuals and body habitats.

Nature (2012) 486:207

IN VIVO AND IN VITRO ANTAGONISM OF INTESTINAL BACTERIA AGAINST *SHIGELLA FLEXNERI*

I. CORRELATION BETWEEN VARIOUS TESTS*

DAVID J. HENTGES AND ROLF FRETER

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Antagonism against pathogenic bacteria exerted by microorganisms of the normal human body flora has been the subject of numerous studies ever since the time of Metchnikoff's original ideas on the benefits attributable to the presence of lactobacilli in the intestine. This degree of interest is only natural since, for many different reasons, the ability to control the bacterial flora of patients would be of great value to the physician.

Hentges, D. J. and Freter, R. 1962, J Infect Dis 110:30–37.

RESISTANCE OF THE MOUSE'S INTESTINAL TRACT TO EXPERIMENTAL SALMONELLA INFECTION

I. Factors Which Interfere with the Initiation of Infection by Oral Inoculation*

By MARJORIE BOHNHOFF, C. PHILLIP MILLER, M.D., and WILLIAM R. MARTIN,‡ Ph.D.

(*From the Departments of Medicine and Microbiology, University of Chicago, Chicago*)

(Received for publication, July 2, 1964)

Described below are observations which seem to account, in large part at least, for the normal resistance of the mouse's intestinal tract to infection with *Salmonella enteritidis* introduced by mouth, the natural route of infection. Thus inoculated, about 10^6 microorganisms are required to infect 50 per cent of young adult CF-1 mice (1). Their resistance, however, can be sharply reduced by the oral administration of a single, large dose of streptomycin, for during the following 24 hours, <10 microorganisms of the same strain suffice to initiate infection (2, 3). Of the changes in the mouse's enteric microflora resulting from streptomycin treatment, the most consequential was thought to be the elimination of certain obligate anaerobes belonging to the genus *Bacteroides* (4, 5).

Journal of Experimental Medicine (1964) 120:805-16.

Urgent Threats

- Carbapenem-resistant *Acinetobacter*
- *Candida auris (C. auris)*
- *Clostridioides difficile (C. difficile)*
- Carbapenem-resistant Enterobacteriaceae (CRE)
- Drug-resistant *Neisseria gonorrhoeae (N. gonorrhoeae)*

Serious Threats

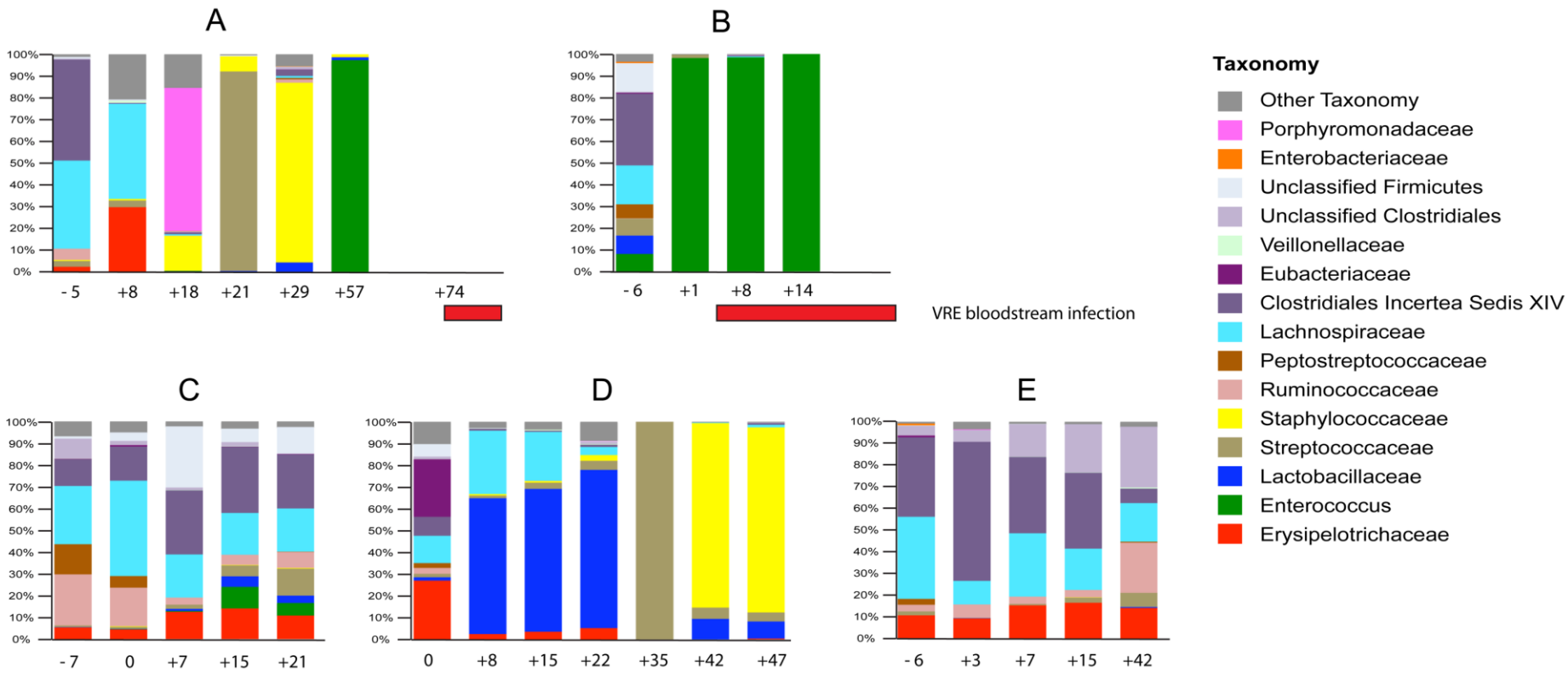
- Drug-resistant *Campylobacter*
- Drug-resistant *Candida*
- Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae
- Vancomycin-resistant *Enterococci* (VRE)
- Multidrug-resistant *Pseudomonas aeruginosa (P. aeruginosa)*
- Drug-resistant nontyphoidal *Salmonella*
- Drug-resistant *Salmonella* serotype Typhi
- Drug-resistant *Shigella*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Drug-resistant *Streptococcus pneumoniae (S. pneumoniae)*
- Drug-resistant Tuberculosis (TB)

Concerning Threats

- Erythromycin-resistant group A *Streptococcus*
- Clindamycin-resistant group B *Streptococcus*

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

VRE Domination of the GI tract occurs in some patients following allogeneic hematopoietic stem cell transplantation and is associated with VRE bacteremia.



Ubeda et al. (2010) Journal of Clinical Investigation 120:4332.

Antibiotic Risk Factors for Bacterial Domination

Clinical Predictor	*Enterococcus* Domination		*Streptococcus* Domination		Proteobacteria Domination	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Vancomycin	2.10 (067 10.14)	0.228	0.95 (0.33 – 3.75)	0.931	5.08 (0.52 – 693.3)	0.196
Metronidazole	3.40 (1.66 – 6.75)	0.001	1.94 (0.81-4.31)	0.130	1.73 (0.41 – 6.04)	0.425
Fluoroquinolones	1.09 (0.49-2.25)	0.824	1.19 (0.52 – 2.61)	0.673	0.09 (0 - 0.75)	0.020
Beta-lactam	1.19 (0.47 - 3.45)	0.724	3.56 (0.83 - 33.20)	0.094	0.64 (0.15 - 3.27)	0.574

Taur et al. (2012) Clinical Infectious Diseases

Domination as Risk Factors for Subsequent Bacteremia

Domination by	VRE bacteremia		Gram negative bacteremia	
	Haz Ratio (95% CI)	P-value	Haz Ratio (95% CI)	P-value
Enterococcus	9.47 (2.46 – 46.0)	0.001	1.53 (0.28 – 5.97)	0.583
Streptococcus	0.22 (0.00 – 1.77)	0.188	0.92 (0.10 – 4.17)	0.925
Proteobacteria	0.76 (0.01 – 6.20)	0.842	6.20 (1.15 – 23.37)	0.036

Taur et al. (2012) Clinical Infectious Diseases

Domination as Risk Factors for Subsequent Bacteremia

Clinical Infectious Diseases

MAJOR ARTICLE

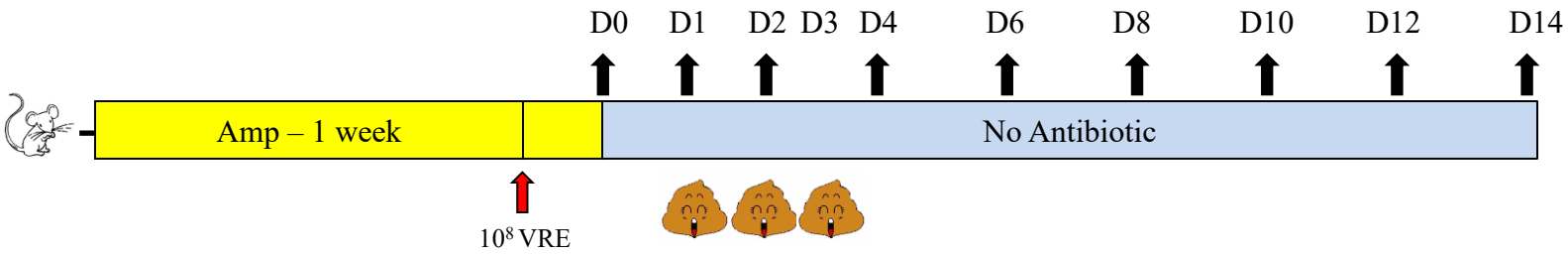
Compositional Flux Within the Intestinal Microbiota and Risk for Bloodstream Infection With Gram-negative Bacteria

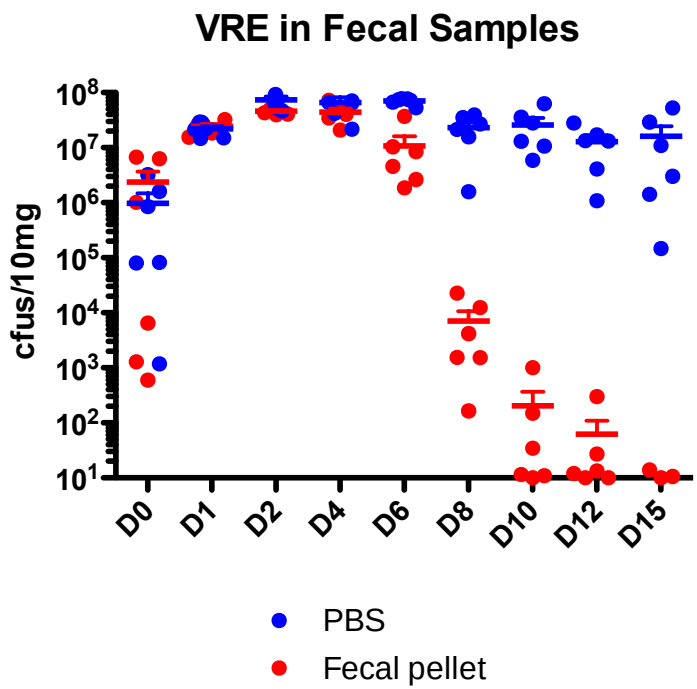
Igor Stoma,[1,2] Eric R. Littmann,[1] Jonathan U. Peled,[3,4] Sergio Giralt,[3,4] Marcel R. M. van den Brink,[3,4] Eric G. Pamer,[1,4,5] and Ying Taur[1,4,5]

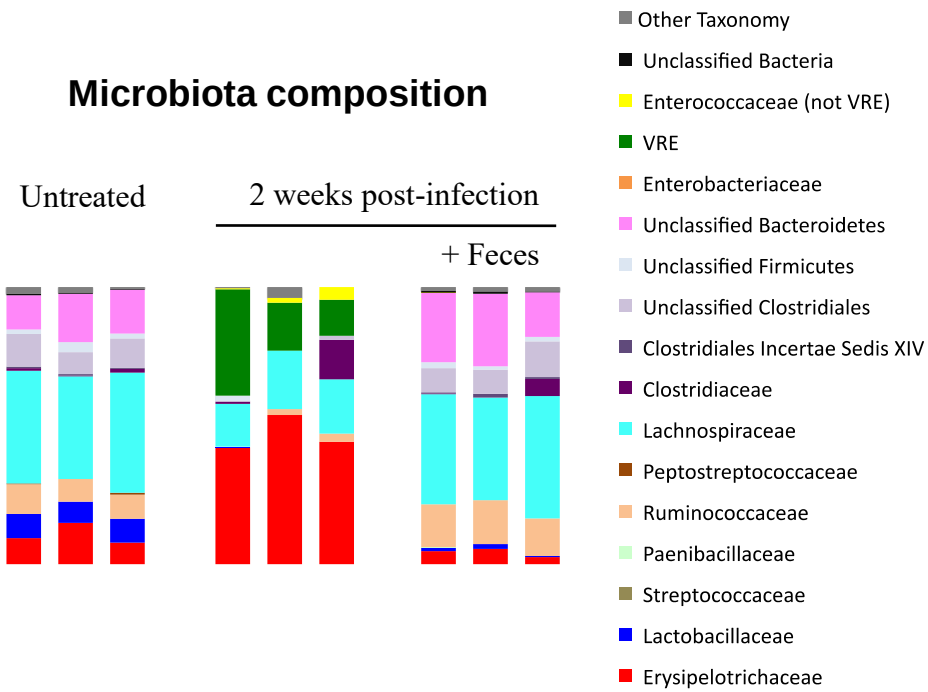
[1]Center for Microbes, Inflammation and Cancer, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, New York, USA; [2]Department of Infectious Diseases, Belarusian State Medical University, Minsk, Belarus; [3]Adult Bone Marrow Transplantation Service, Memorial Hospital, Memorial Sloan Kettering Cancer Center, New York, New York, USA; [4]Weill Cornell Medical College, New York, New York, USA; and [5]Infectious Diseases Service, Memorial Hospital, Memorial Sloan Kettering Cancer Center, New York, New York, USA

	Gram-negative BSIs, OR (95% CI)					
Intestinal Domination[a]	*Escherichia coli* BSI (n = 32)	*Klebsiella* BSI (n = 10)	*Enterobacter* BSI (n = 3)	*Stenotrophomonas* BSI (n = 2)	*Pseudomonas* BSI (n = 3)	*Citrobacter* BSI (n = 1)
Escherichia coli (n = 50)	21.7 (10.1–47.3) $P < .001$	6.4 (1.5–22.3) $P = .088$	1.9 (0–19.5) $P = .718$	2.6 (0–32.5) $P = .634$	1.9 (0–19.5) $P = .718$	4.3 (0–82.4) $P = .490$
Klebsiella (n = 16)	0.6 (0–4.7) $P = .718$	23.7 (5.3–90.2) $P = .002$	6 (0–65.3) $P = .442$	8.4 (.1–108.3) $P = .405$	6 (0–65.3) $P = .442$	14 (.1–272.2) $P = .317$
Enterobacter (n = 6)	5.8 (.6–30.4) $P = .237$	5.1 (0–48) $P = .467$	259.8 (29.1–3325.3) $P < .001$	21.6 (.2–303.1) $P = .286$	15.4 (.1–185) $P = .317$	36 (.2–745.6) $P = .237$
Stenotrophomonas (n = 2)	4.2 (0–52.4) $P = .490$	13.3 (.1–177) $P = .317$	40.2 (.3–642.5) $P = .237$	470.3 (27.4–10281.7) $P = .002$	40.2 (.3–642.5) $P = .237$	94.1 (.6–2348.5) $P = .237$
Pseudomonas (n = 1)	6.9 (0–132.5) $P = .442$	22.1 (.1–441.3) $P = .286$	67.1 (.4–1529.3) $P = .237$	94.1 (.6–2348.5) $P = .237$	846.6 (36.6–137439.9) $P = .002$	157 (.9–5033.8) $P = .237$
Citrobacter (n = 2)	4.2 (0–52.4) $P = .490$	13.3 (.1–177) $P = .317$	40.2 (.3–642.5) $P = .237$	56.4 (.4–1017.7) $P = .237$	40.2 (.3–642.5) $P = .237$	94.1 (.6–2348.5) $P = .237$

"Normal" microbiota eliminates persistent VRE

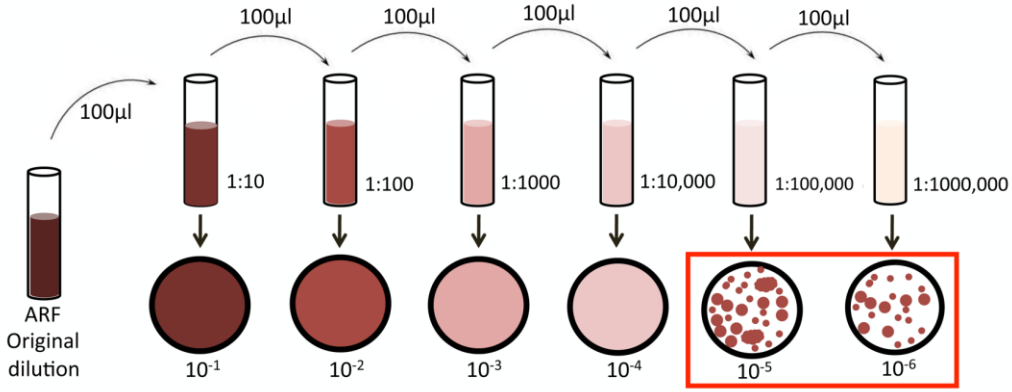


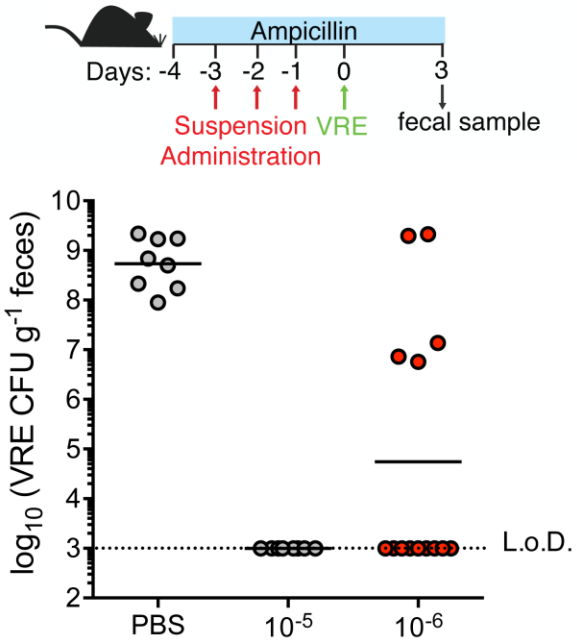


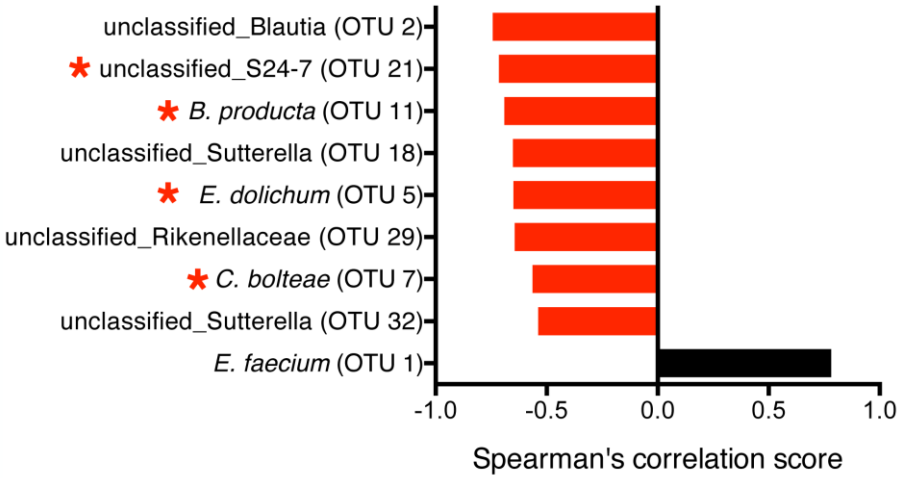


Ubeda et al. (2013) Infection and Immunity

Microbiota dilution facilitates identification of commensal taxa associated with colonization resistance.

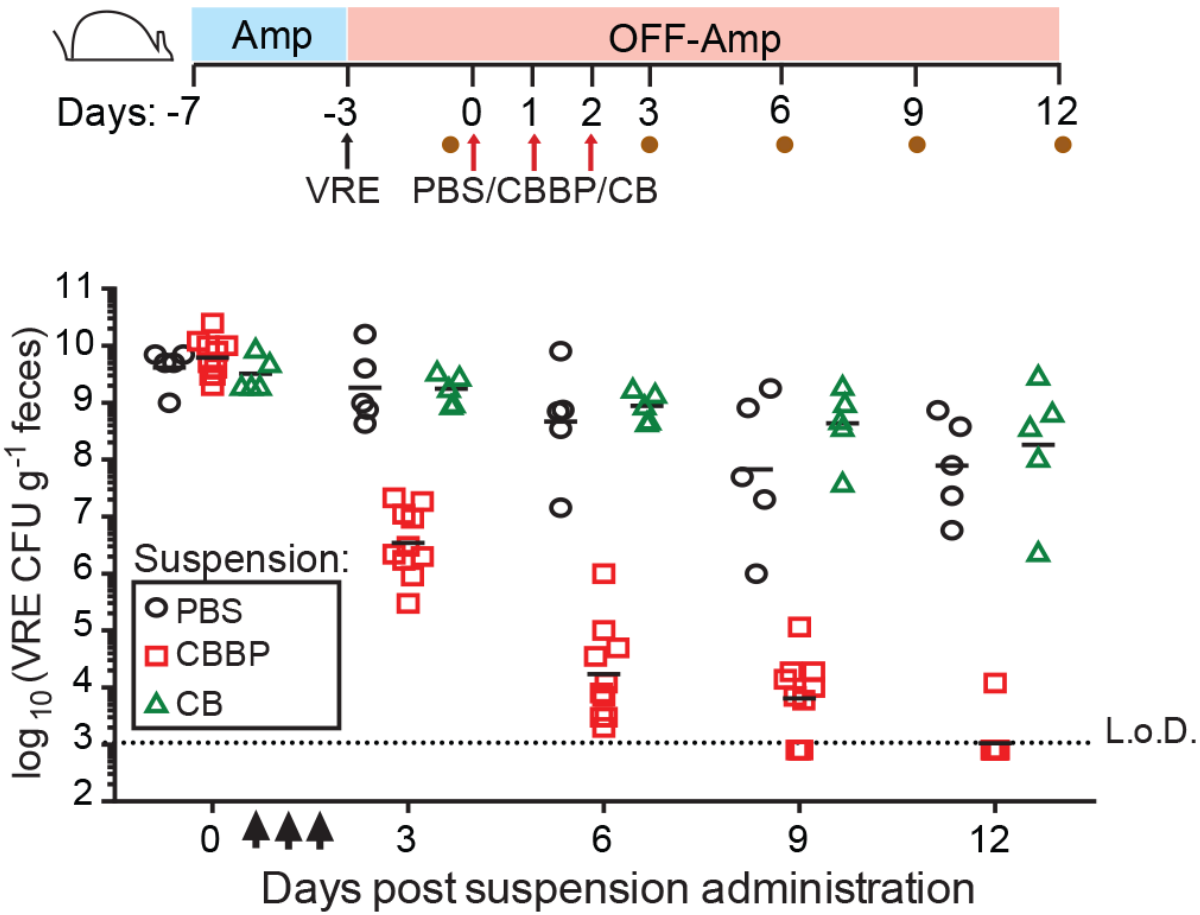






Caballero et al. 2017, Cell Host & Microbe 21(5):592-602

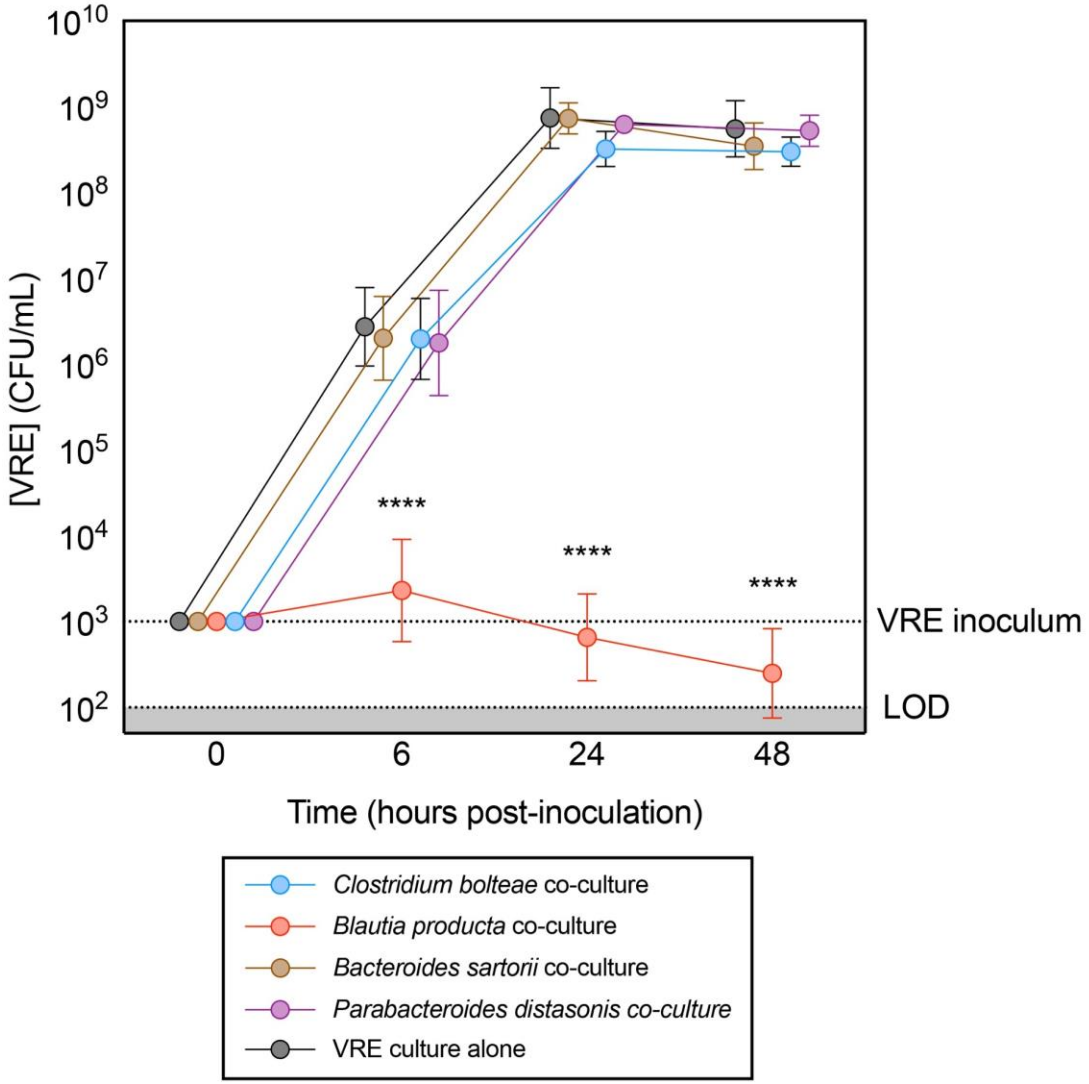
Four bacterial strains (CBBP) mediate colonization resistance against VRE.



CBBP
Clostridium bolteae
Bacteroides sartorii
Blautia producta
Parabacteroides distasonis

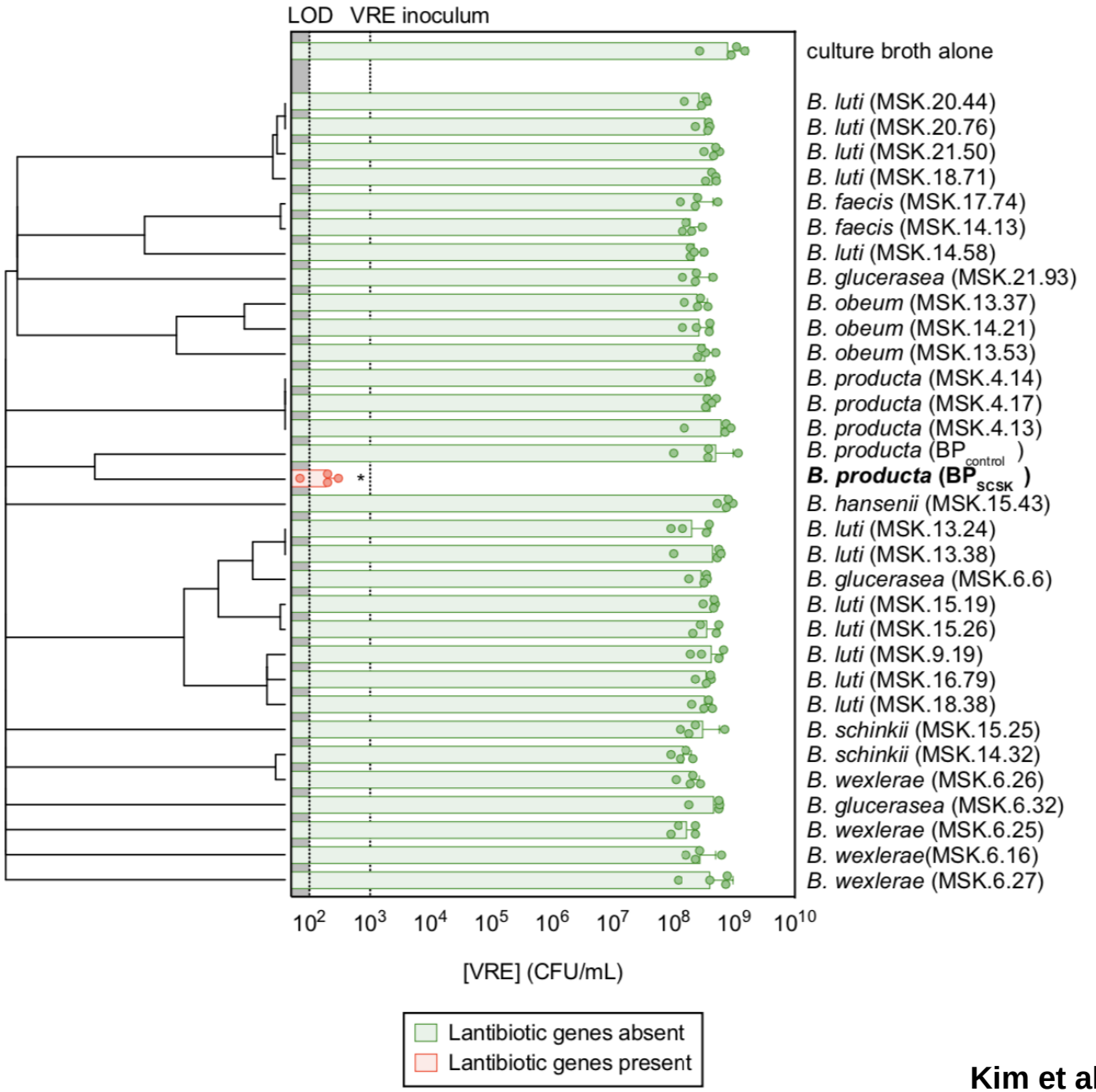
Caballero et al. 2017, Cell Host & Microbe 21(5):592-602

One member of CBBP, *Blautia producta*$_{SCSK}$, inhibits VRE growth *in vitro*.



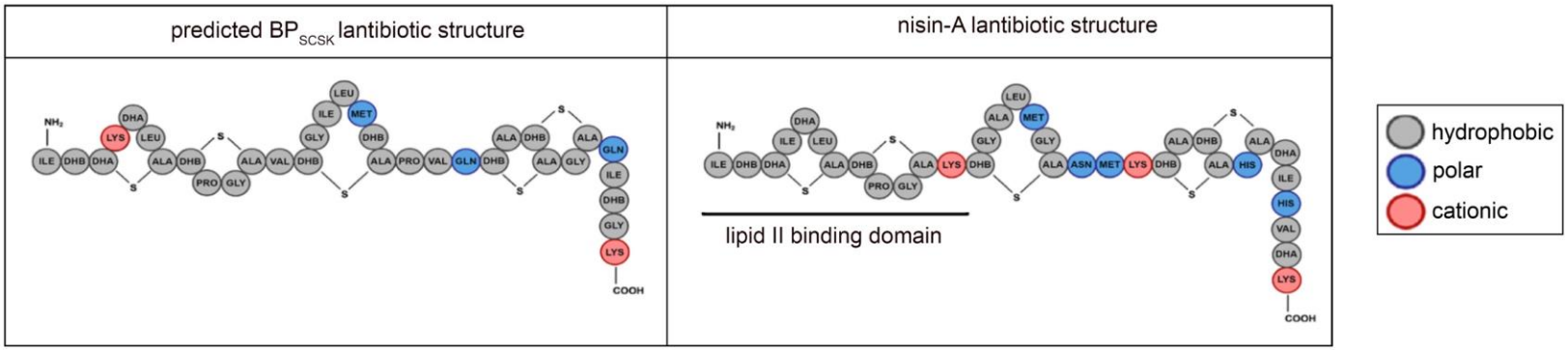
Kim et al. (2019) Nature 572:665-669

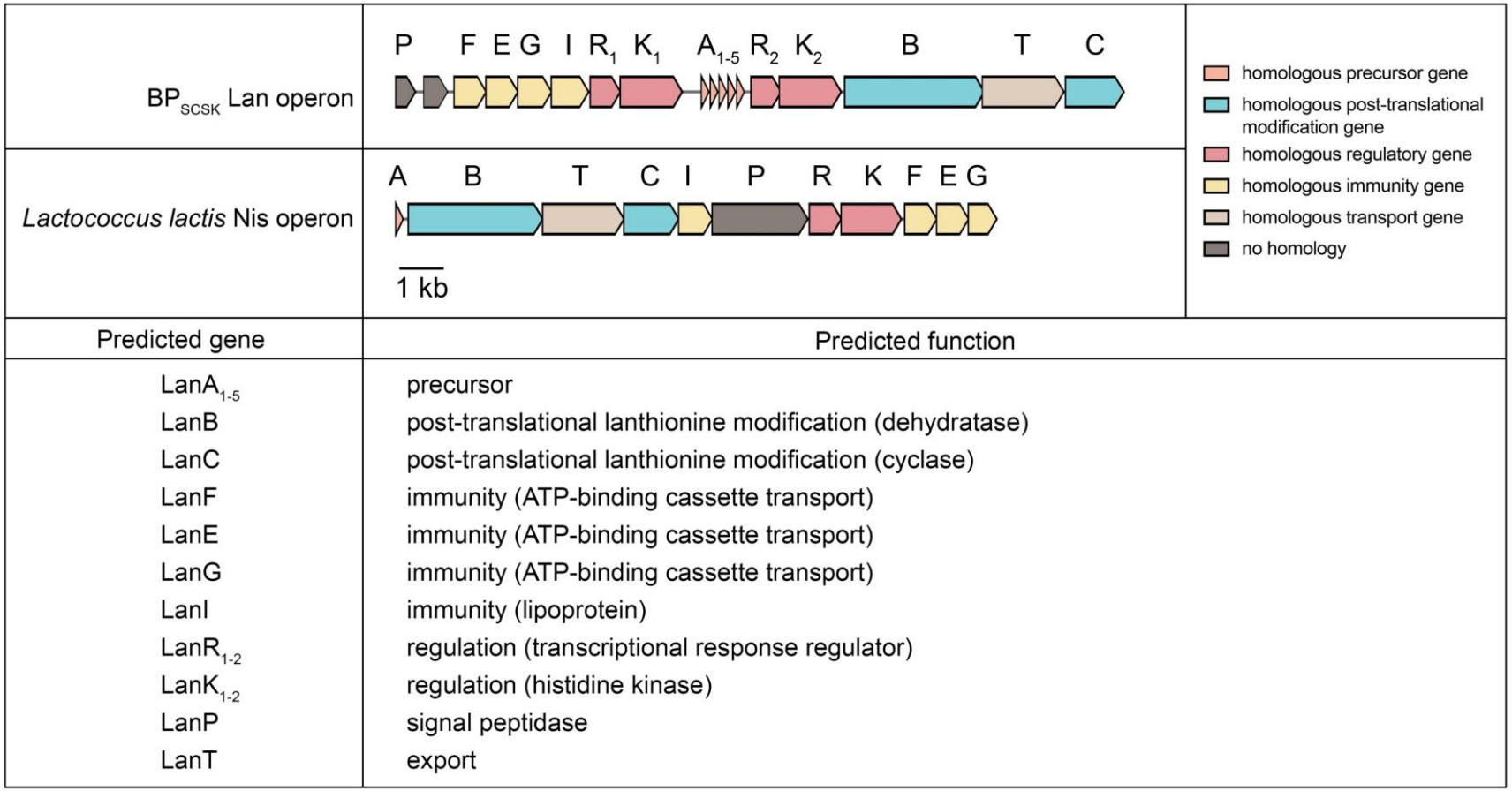
Most Blautia strains do not inhibit VRE.



Kim et al. (2019) Nature 572:665-669

Blautia producta$_{SCSK}$ encodes a complete lantibiotic operon.

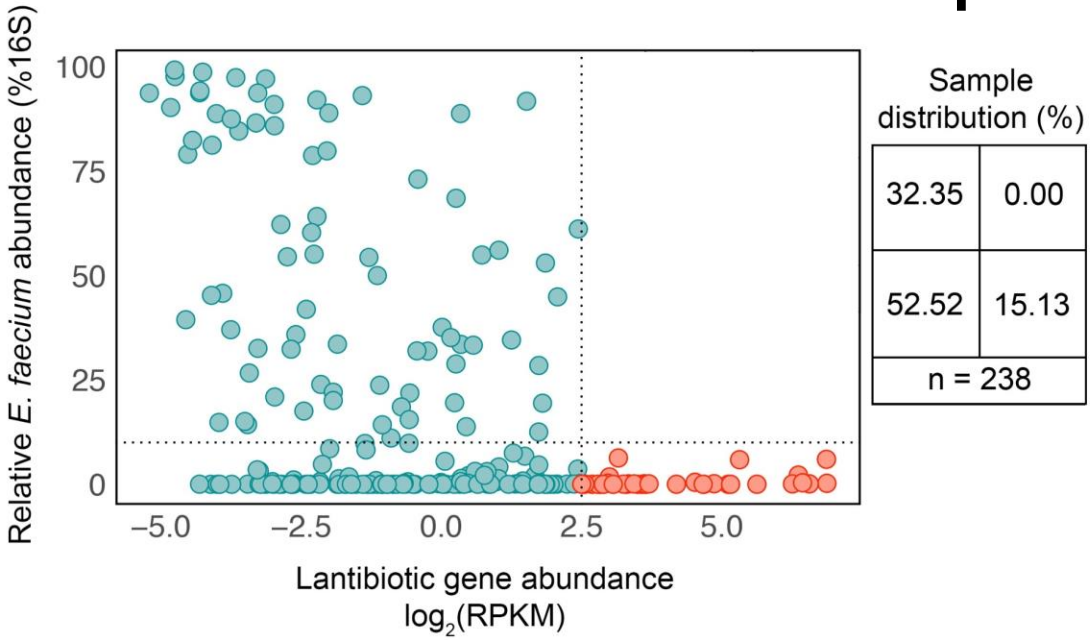


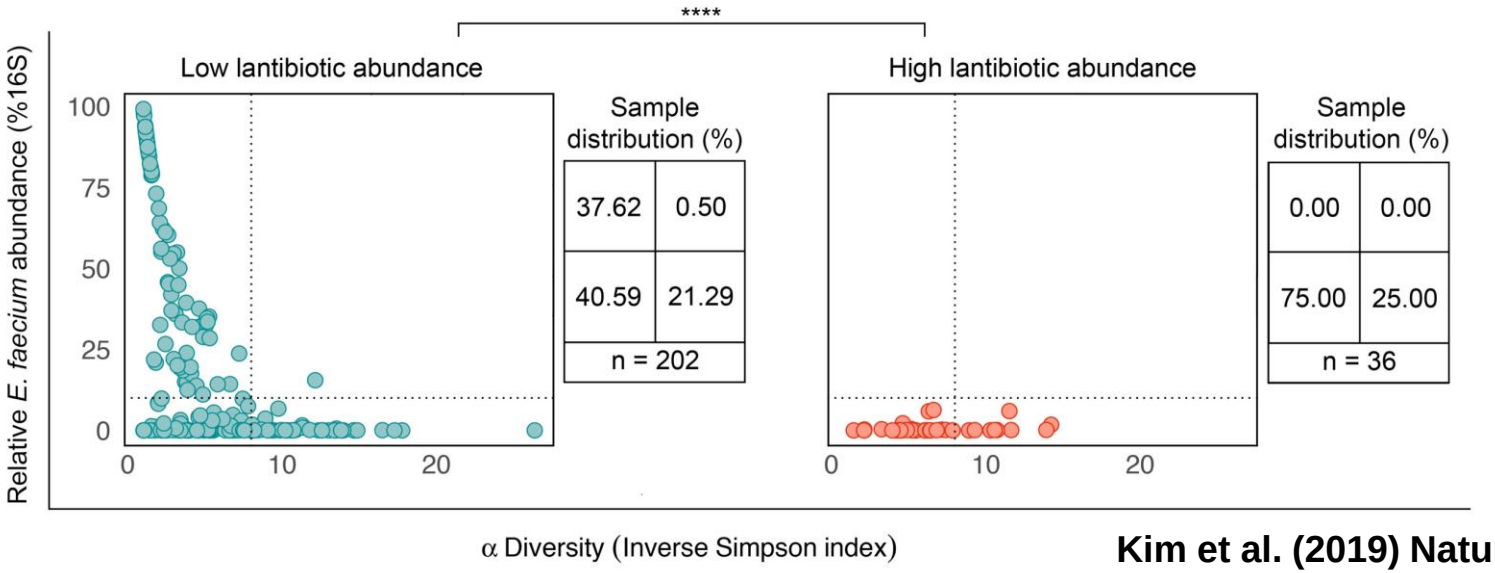


Predicted gene	Predicted function
LanA$_{1-5}$	precursor
LanB	post-translational lanthionine modification (dehydratase)
LanC	post-translational lanthionine modification (cyclase)
LanF	immunity (ATP-binding cassette transport)
LanE	immunity (ATP-binding cassette transport)
LanG	immunity (ATP-binding cassette transport)
LanI	immunity (lipoprotein)
LanR$_{1-2}$	regulation (transcriptional response regulator)
LanK$_{1-2}$	regulation (histidine kinase)
LanP	signal peptidase
LanT	export

Kim et al. (2019) Nature 572:665-669

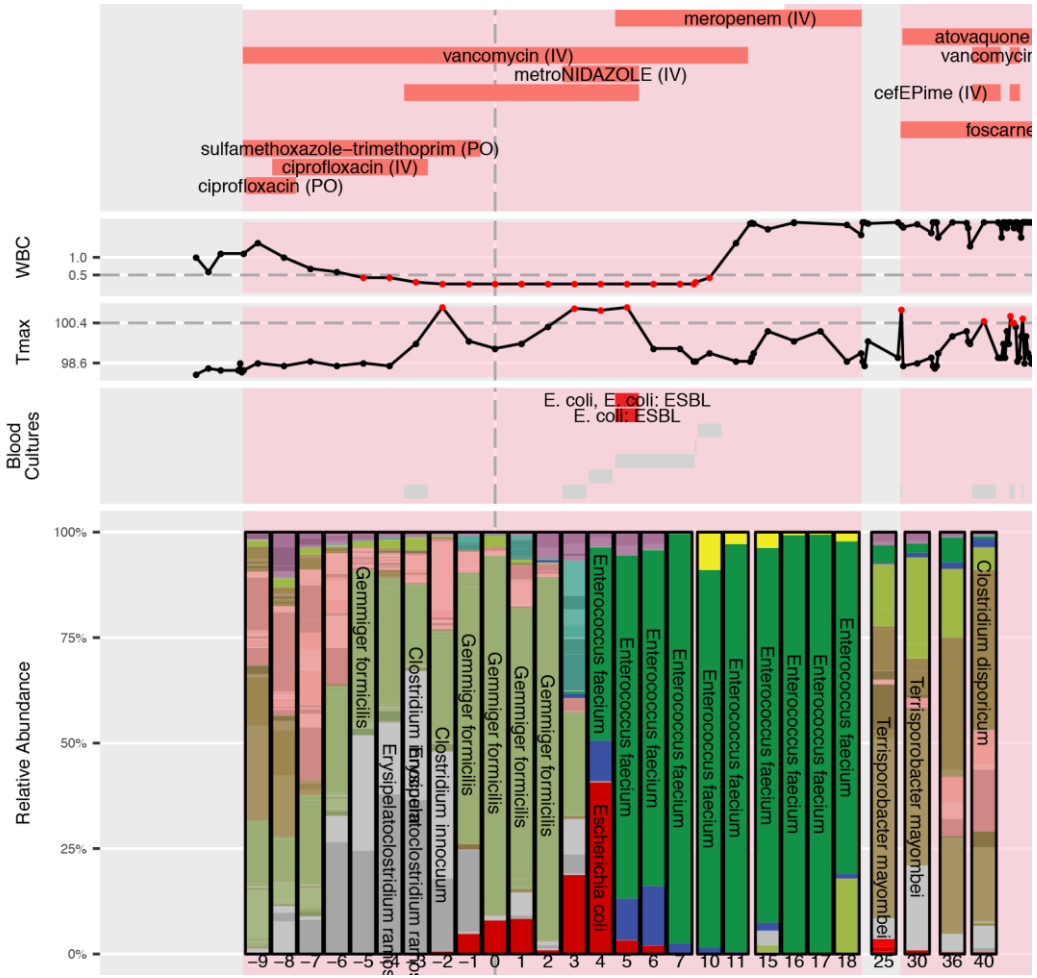
Lantibiotic gene abundance correlates inversely with VRE abundance in allo-HSCT patients.

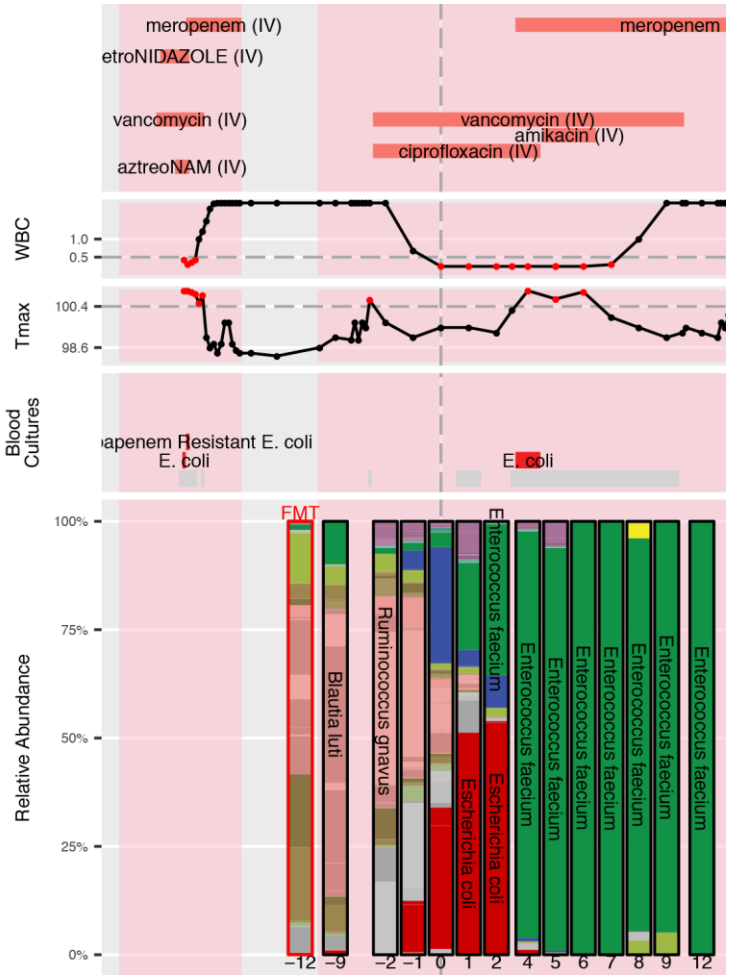




Kim et al. (2019) Nature 572:665-669

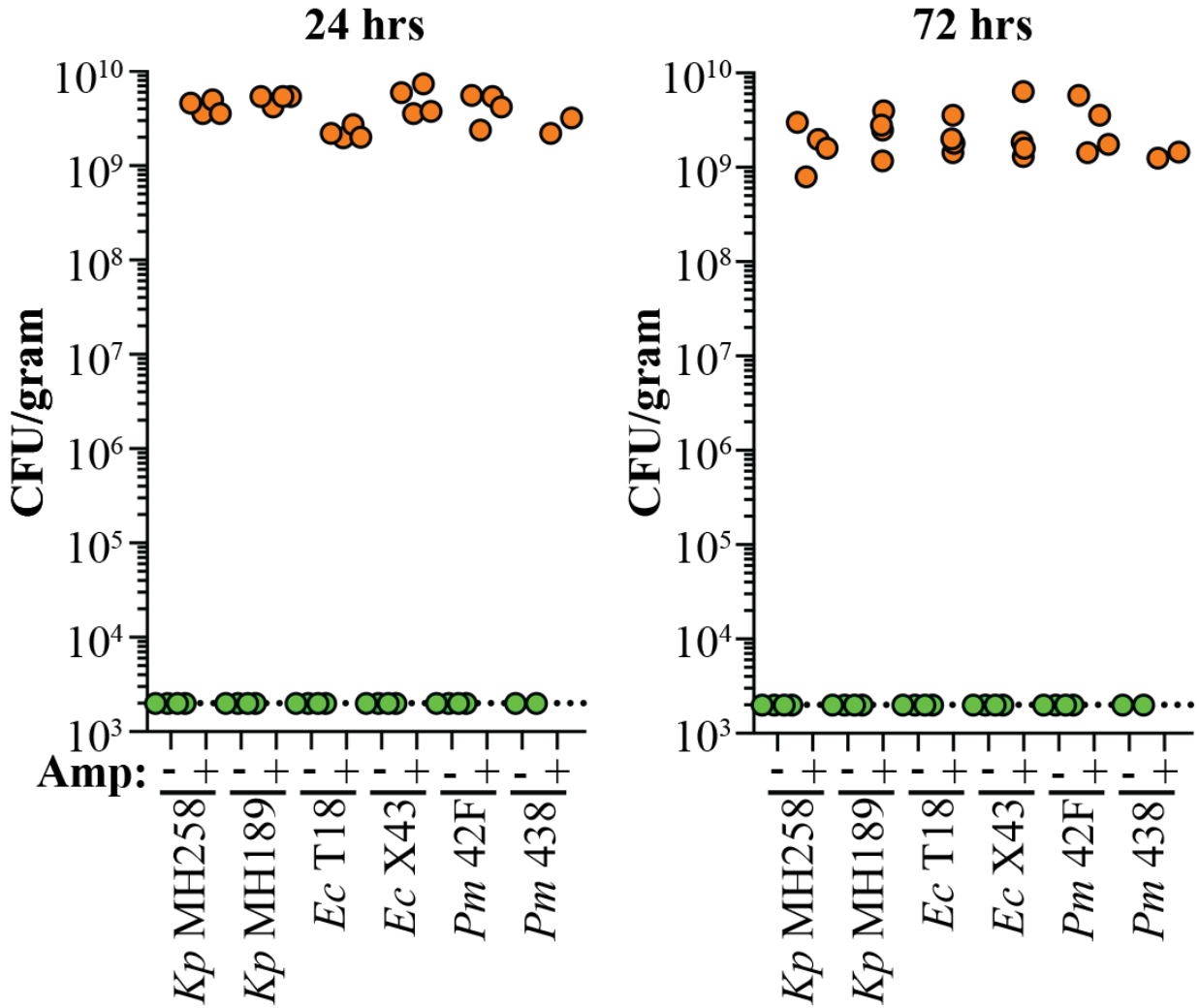
GNR Domination of the GI tract following allogeneic hematopoietic stem cell transplantation is associated with GNR bacteremia.





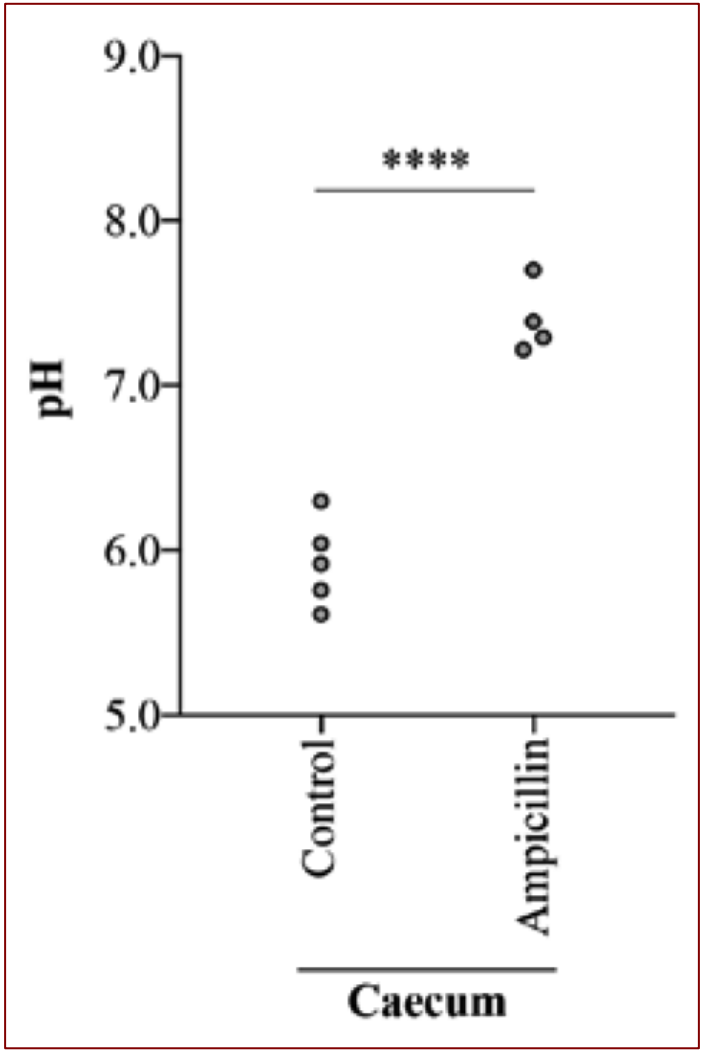
Ying Taur, MSKCC

Antibiotic treatment enables expansion of clinical *Enterobacteriaceae* isolates



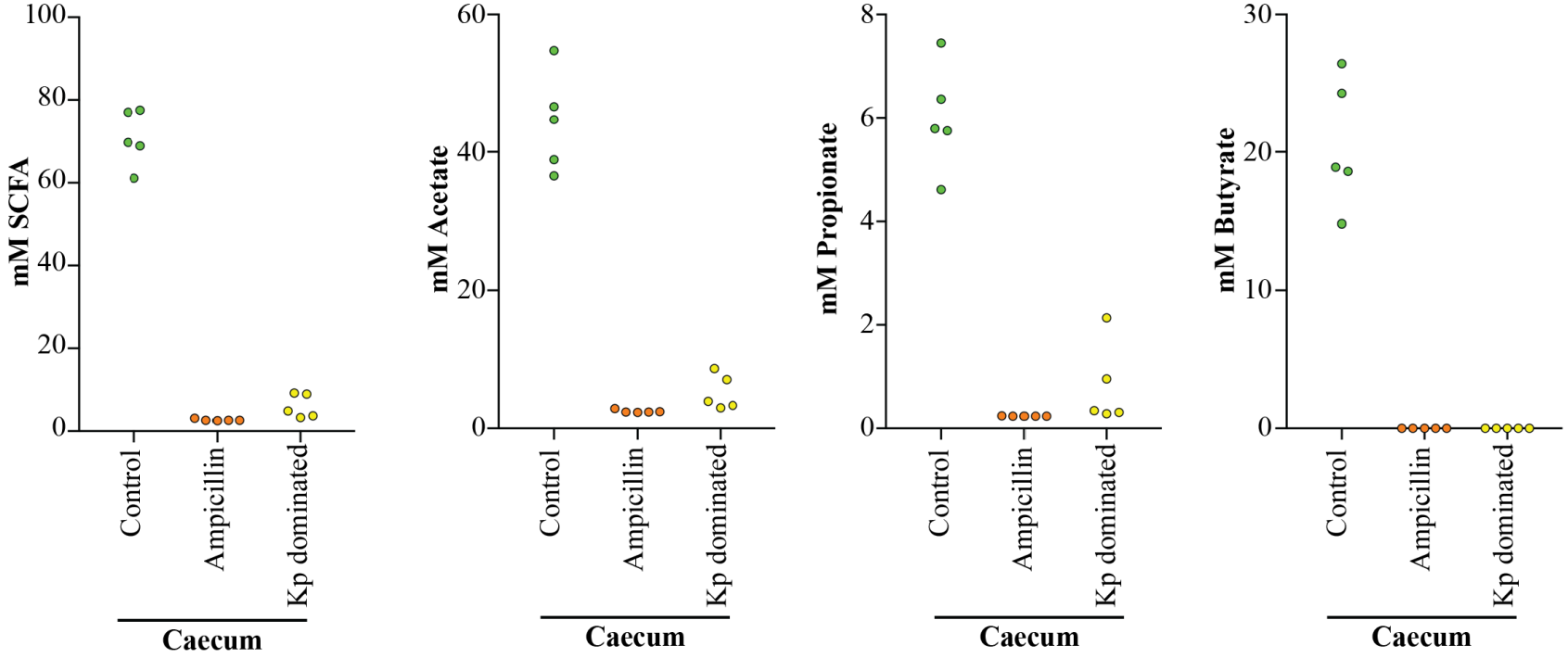
Sorbara et al. (2019) Journal of Experimental Medicine 216:84-98.

Antibiotic treatment neutralizes the normally acidic lower gastrointestinal tract.



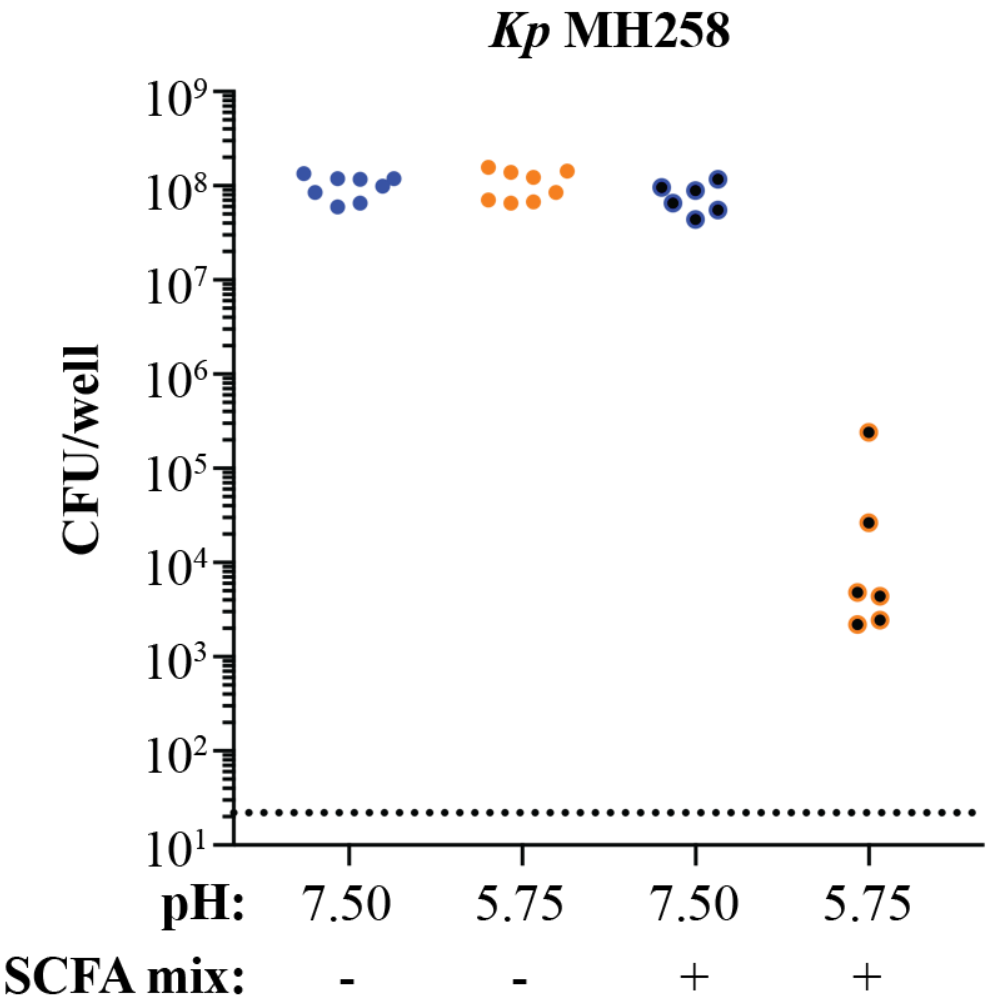
Sorbara et al. (2019) Journal of Experimental Medicine 216:84-98.

Ampicillin-treatment markedly reduces intestinal SCFA concentrations



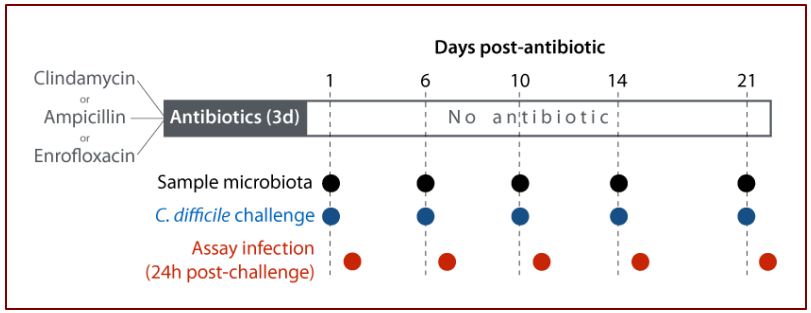
Sorbara et al. (2019) Journal of Experimental Medicine 216:84-98.

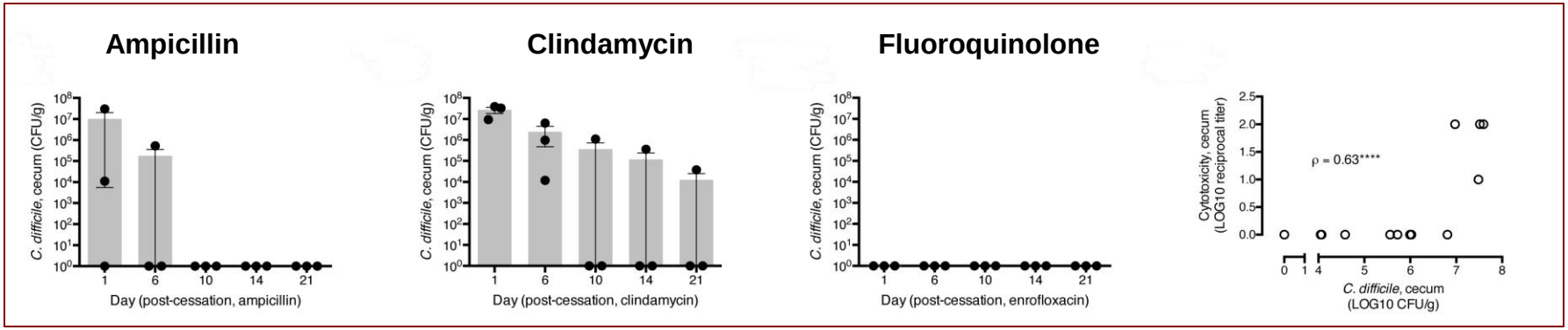
pH-dependent SCFA-mediated inhibition of Enterobacteriaceae growth

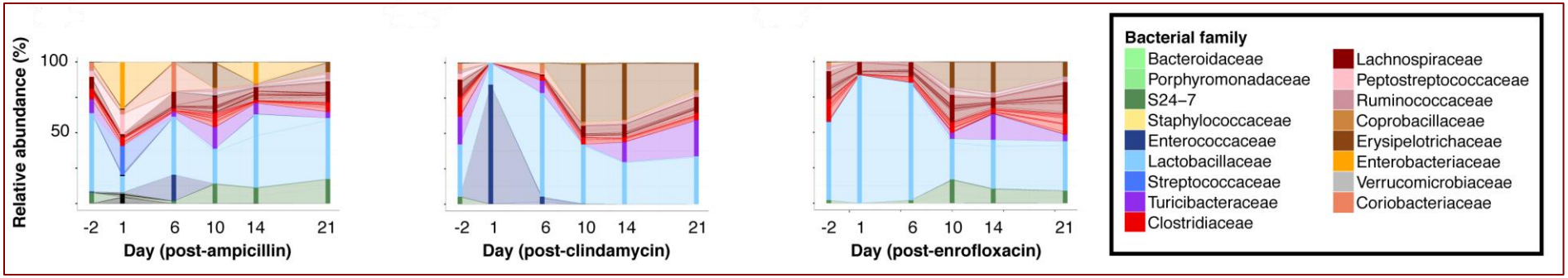


Sorbara et al. (2019) Journal of Experimental Medicine 216:84-98.

Different antibiotics: disparate duration of susceptibility to *Clostridium difficile* infection.

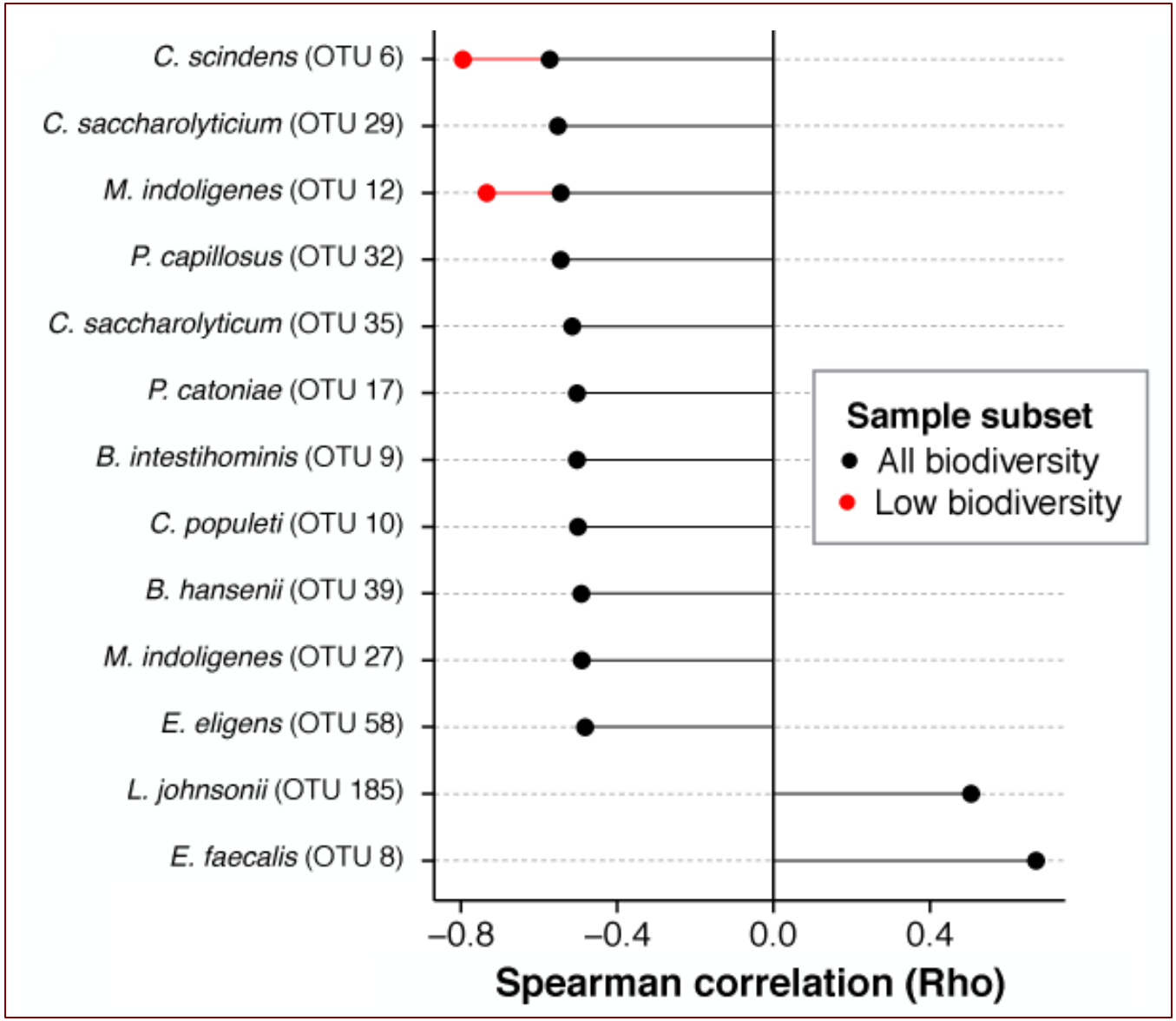






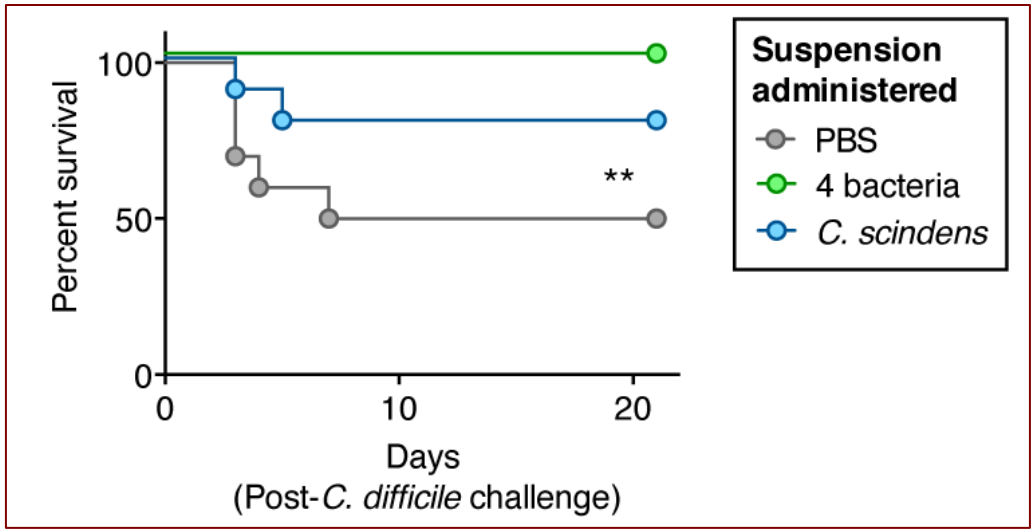
Buffie et al. (2015) Nature 517:205

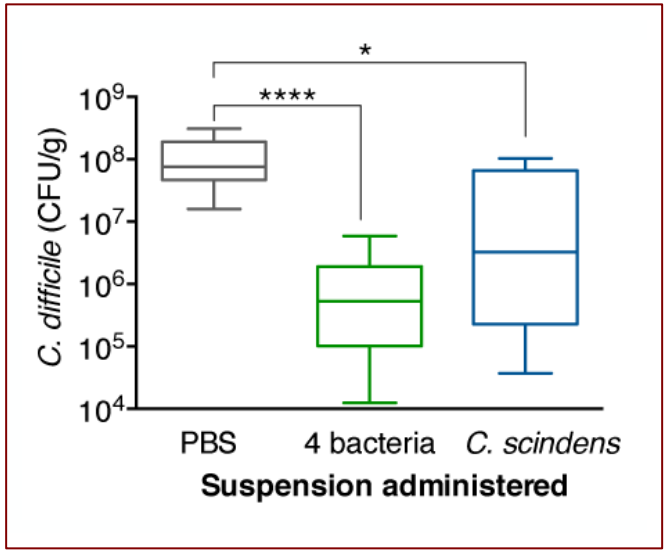
Correlating microbiota components & CDI resistance

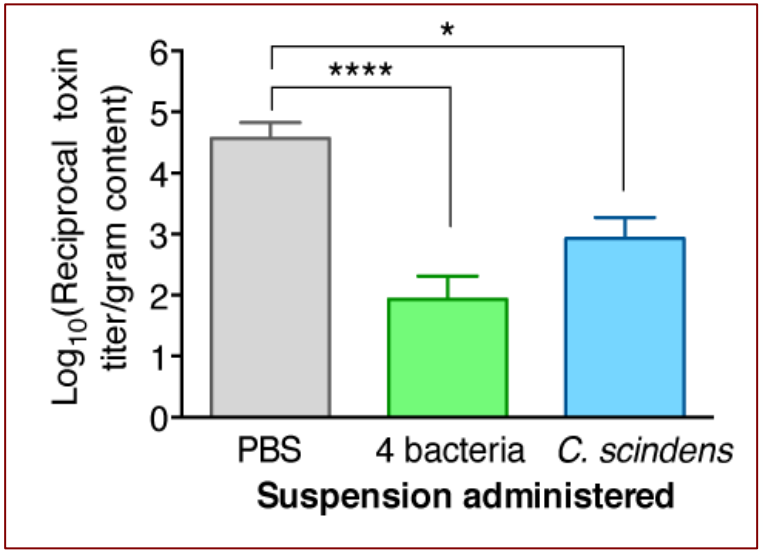


Buffie et al. (2015) Nature 517:205

Protection against *C. difficile* mediated by four commensal bacterial species: *B. intestihominis*, *Blautia hansenii*, *Pseudoflavonifractor capillosus* and *C. scindens*

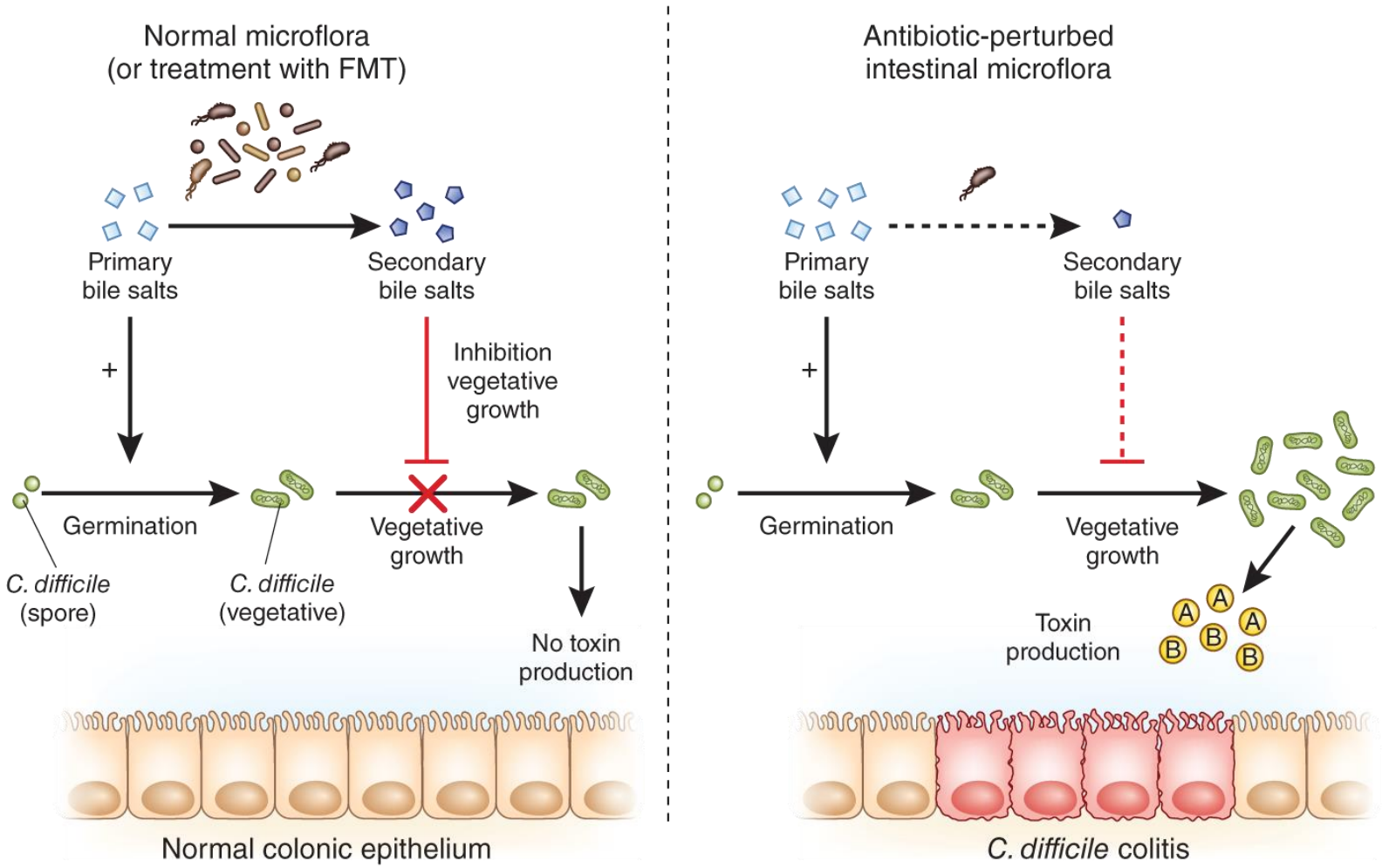






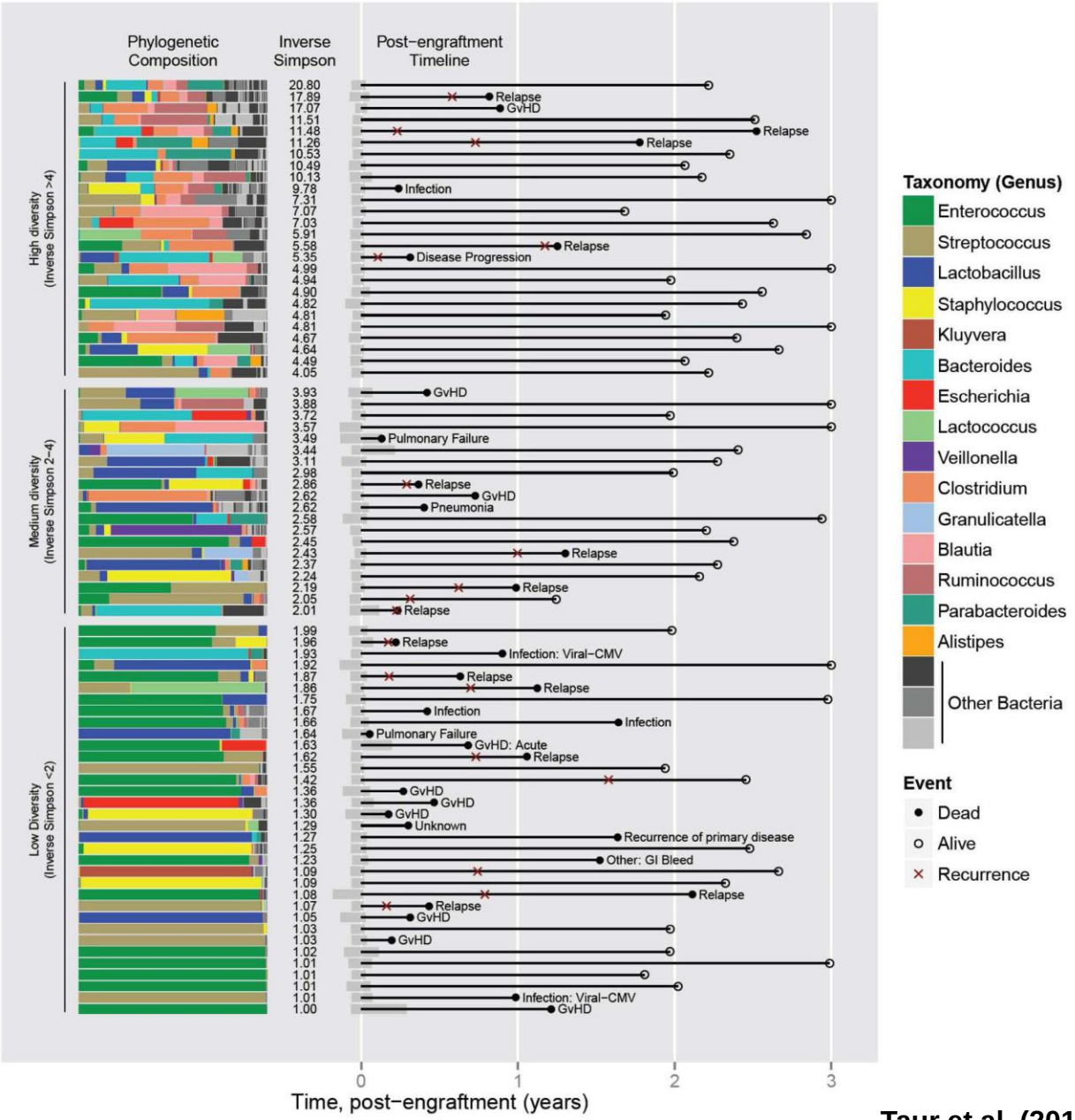
Buffie et al. (2015) Nature 517:205

Secondary bile salt-mediated inhibition of *Clostridium difficile* growth



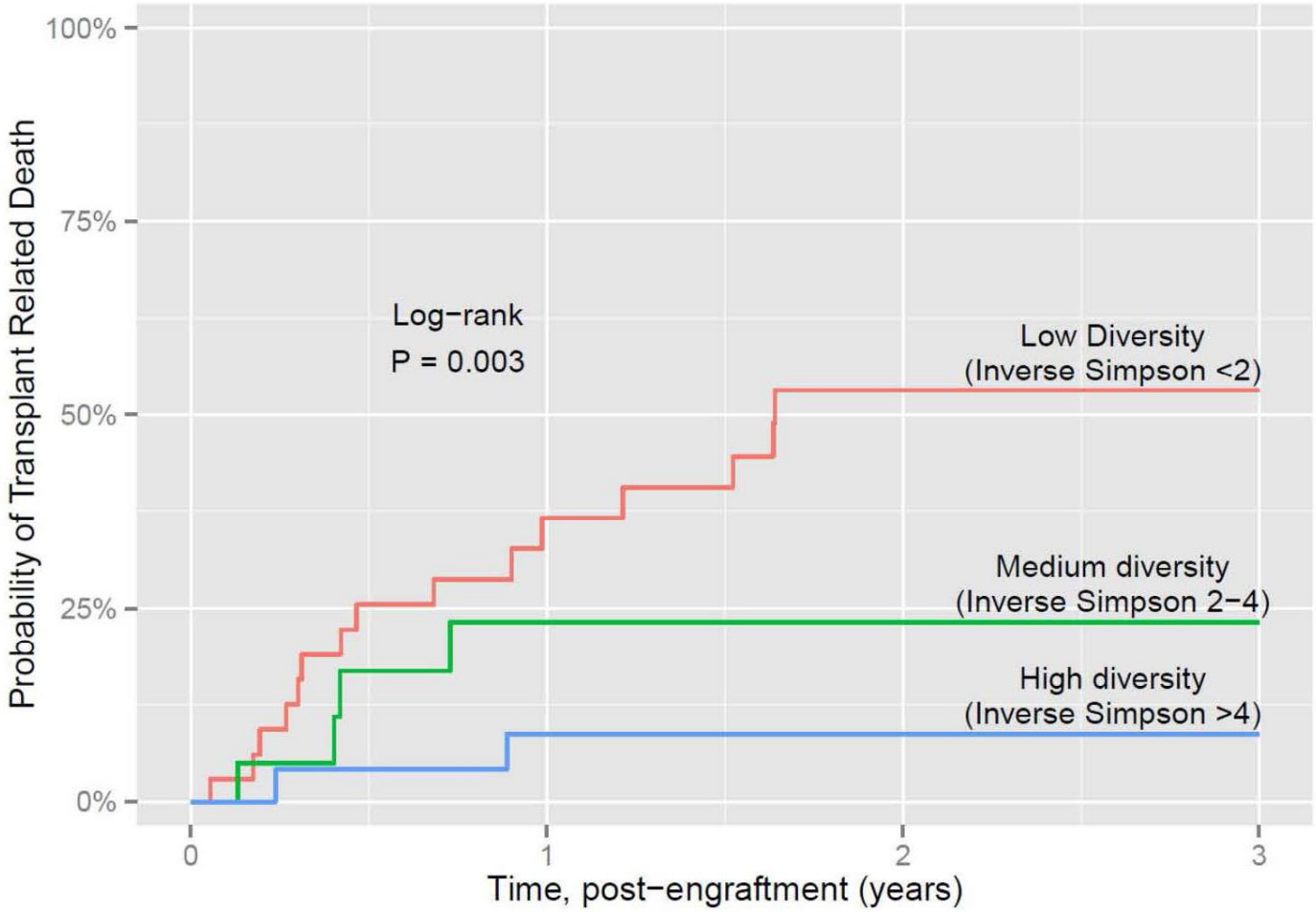
Taur & Pamer (2014) Nature Medicine

Allo-HSCT patients can be divided into low, intermediate and high microbiota diversity groups.



Taur et al. (2014) Blood 124:1174-82.

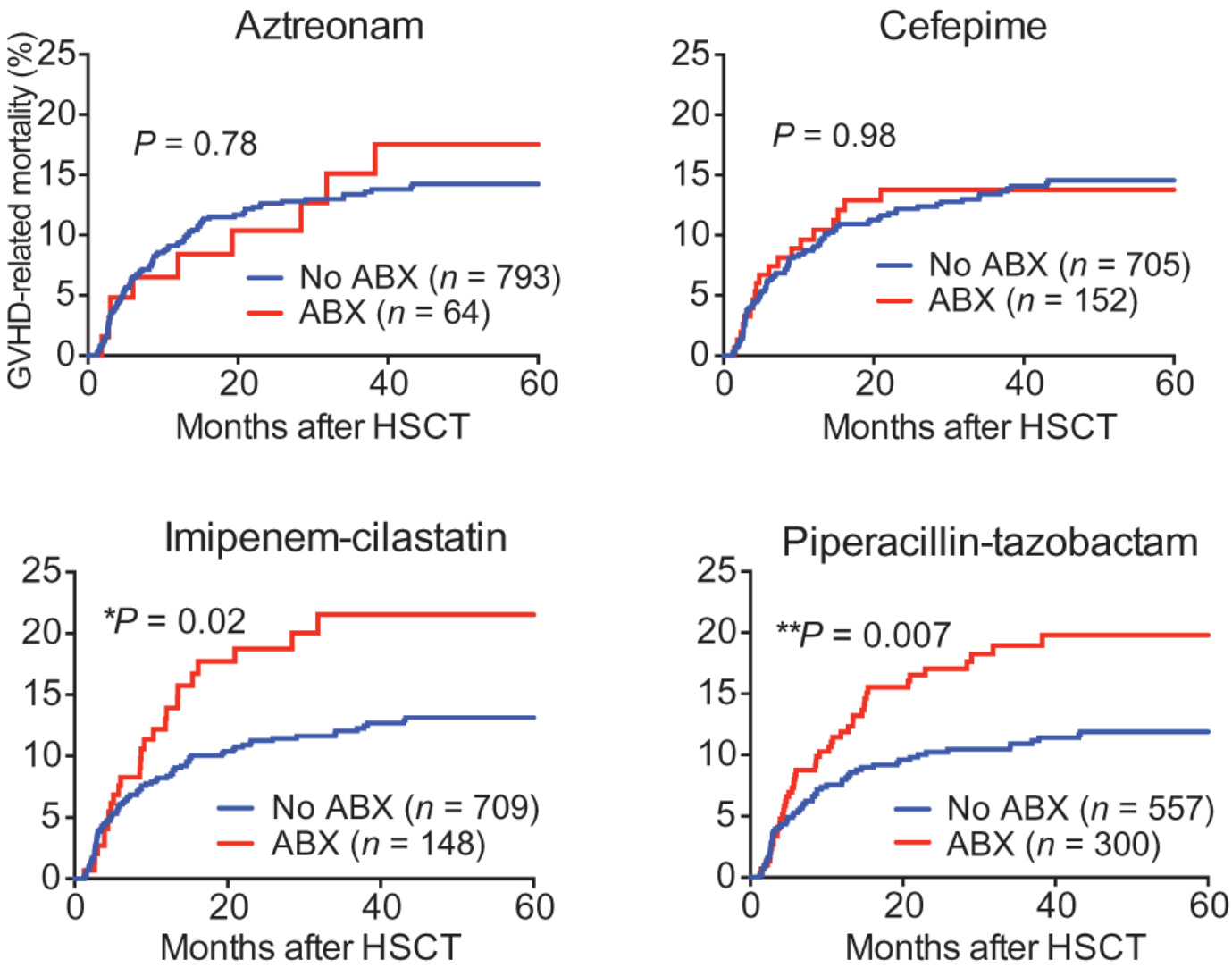
Transplant-related mortality is markedly reduced in patients with a diverse microbiota following engraftment



Number at Risk				
High diversity	26	21	17	4
Medium diversity	20	12	10	3
Low Diversity	34	17	7	2

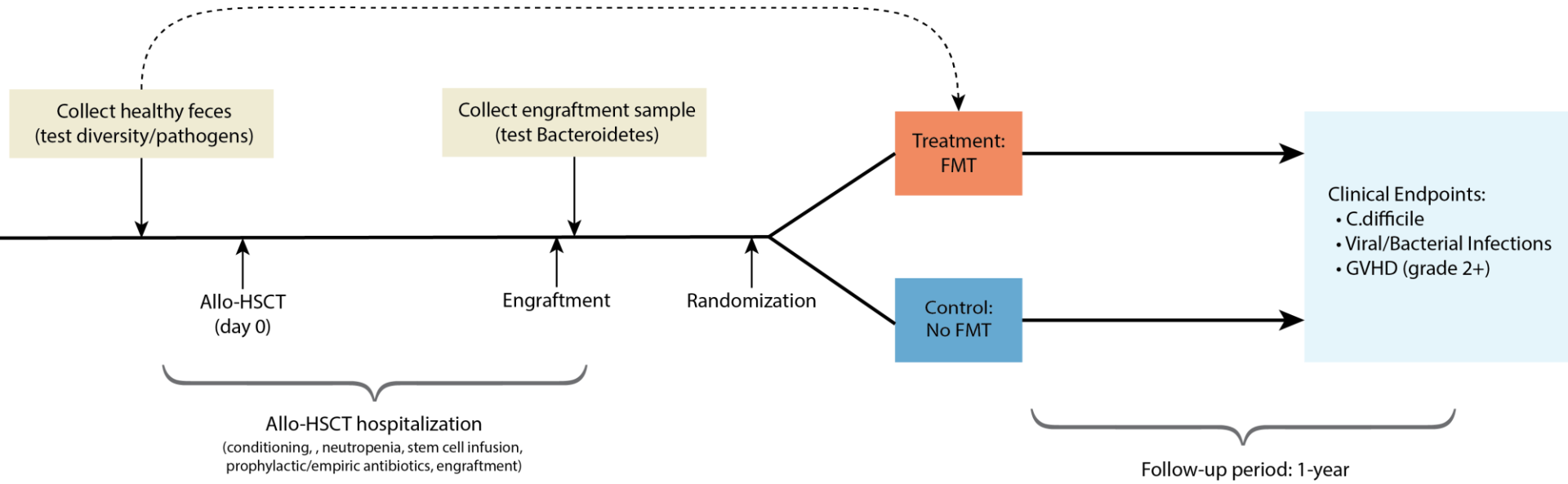
Taur et al. (2014) Blood 124:1174-82.

GVHD-related mortality is markedly increased by treatment with antibiotics that kill obligate anaerobes.



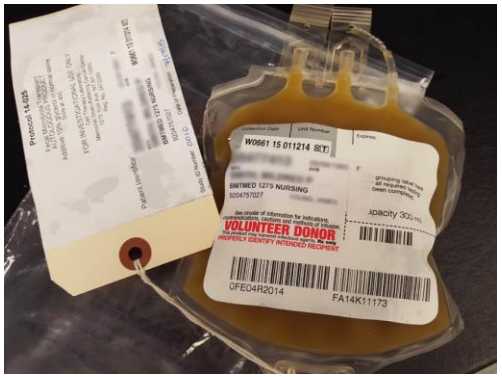
Shono et al. (2016) Science Translational Medicine 8:339a.

14-025: Randomized Trial of Auto-FMT in Allo-HSCT

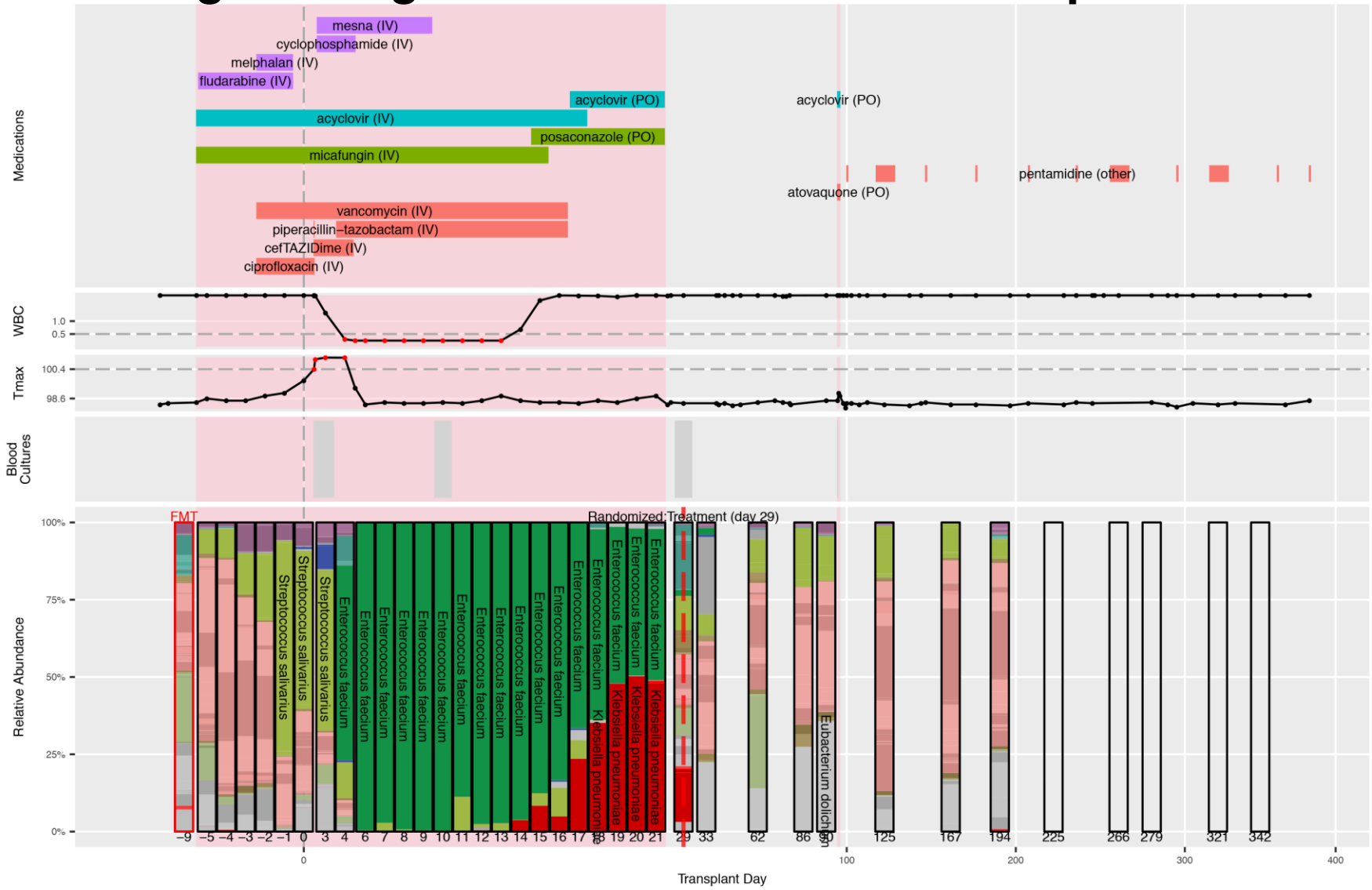


- **215 allo-HSCT patients enrolled (since Jan 2015)**
  - **31 patients currently**
  - **55 excluded, detectable Bacteroidetes at engraftment**
  - **61 excluded, other reasons (initial sample failed pathogen screen)**
  - **20 withdrew from study**

- **59 Randomized**
  - **29 Treatment (FMT)**
  - **30 Control**

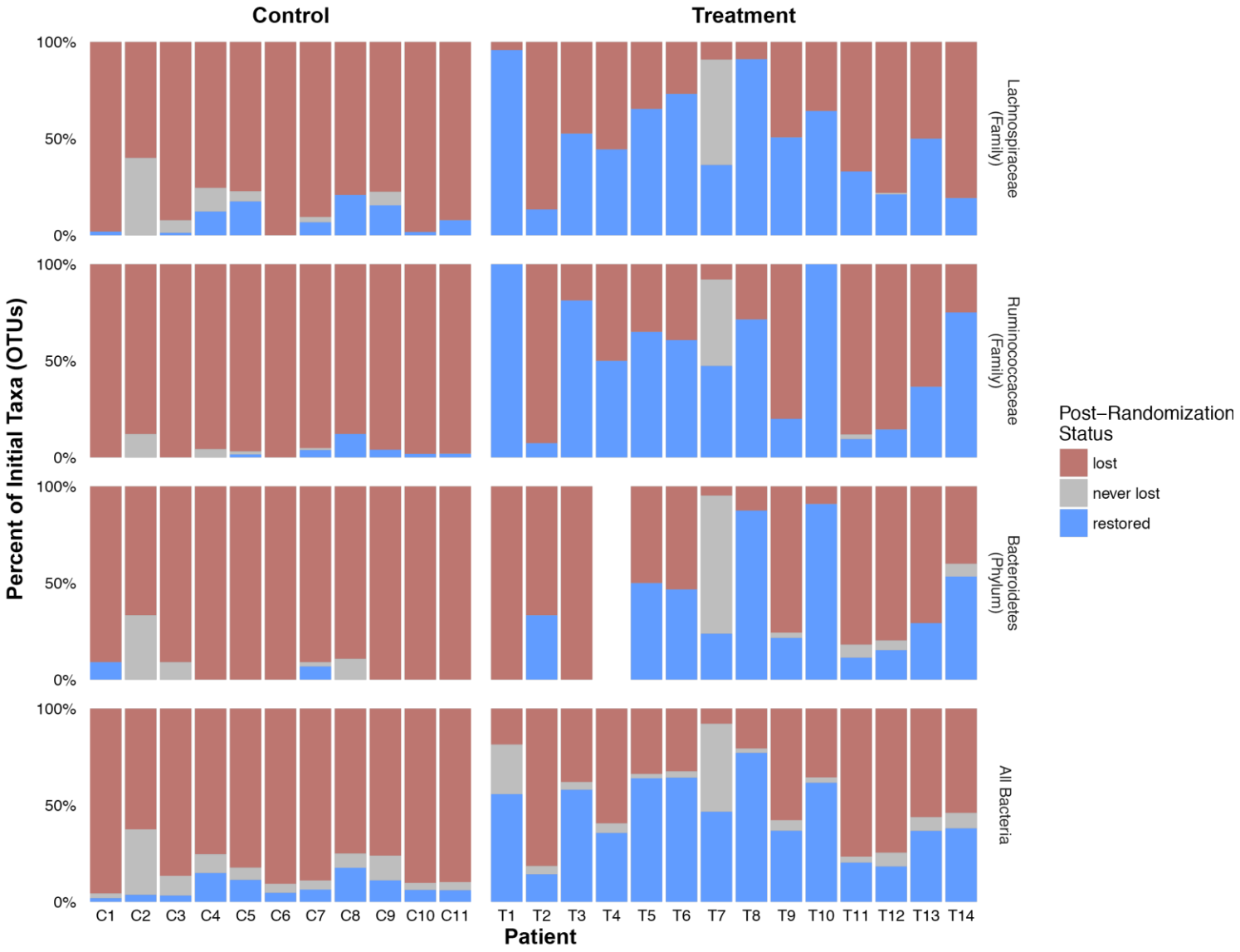


Rapid re-establishment of a diverse microbiota following autologous Fecal Microbiota Transplantation.



Taur et al., 2018 Science Translational Medicine.

Auto-FMT re-establishes pre-HCT commensal bacterial families.



Taur et al., 2018 Science Translational Medicine

Symbiotic Bacteria Strain Bank
(1,615 Bacterial Strains)

Ruminococcaceae (47)

[Clostridium] leptum
Flavonifractor plautii
Oscillibacter ruminantium
Ruminococcus albus
Ruminococcus bromii
Drancourtella massiliensis
Ruminococcus callidus
[Hungateiclostridium] straminisolvens
Agathobaculum butyriciproducens
Anaeromassilibacillus senegalensis
Pseudoflavonifractor phocaeensis
Anaerotruncus colihominis
Faecalibacterium prausnitzii

Clostridiaceae (3)

Lactonifactor longoviformis

Akkermansiaceae (4)

Akkermansia muciniphila

Bifidobacteriaceae (140)

Bifidobacterium adolescentis
Bifidobacterium animalis
Bifidobacterium bifidum
Bifidobacterium breve
Bifidobacterium catenulatum
Bifidobacterium faecale
Bifidobacterium longum
Bifidobacterium pseudocatenulatum

Lachnospiraceae (548)

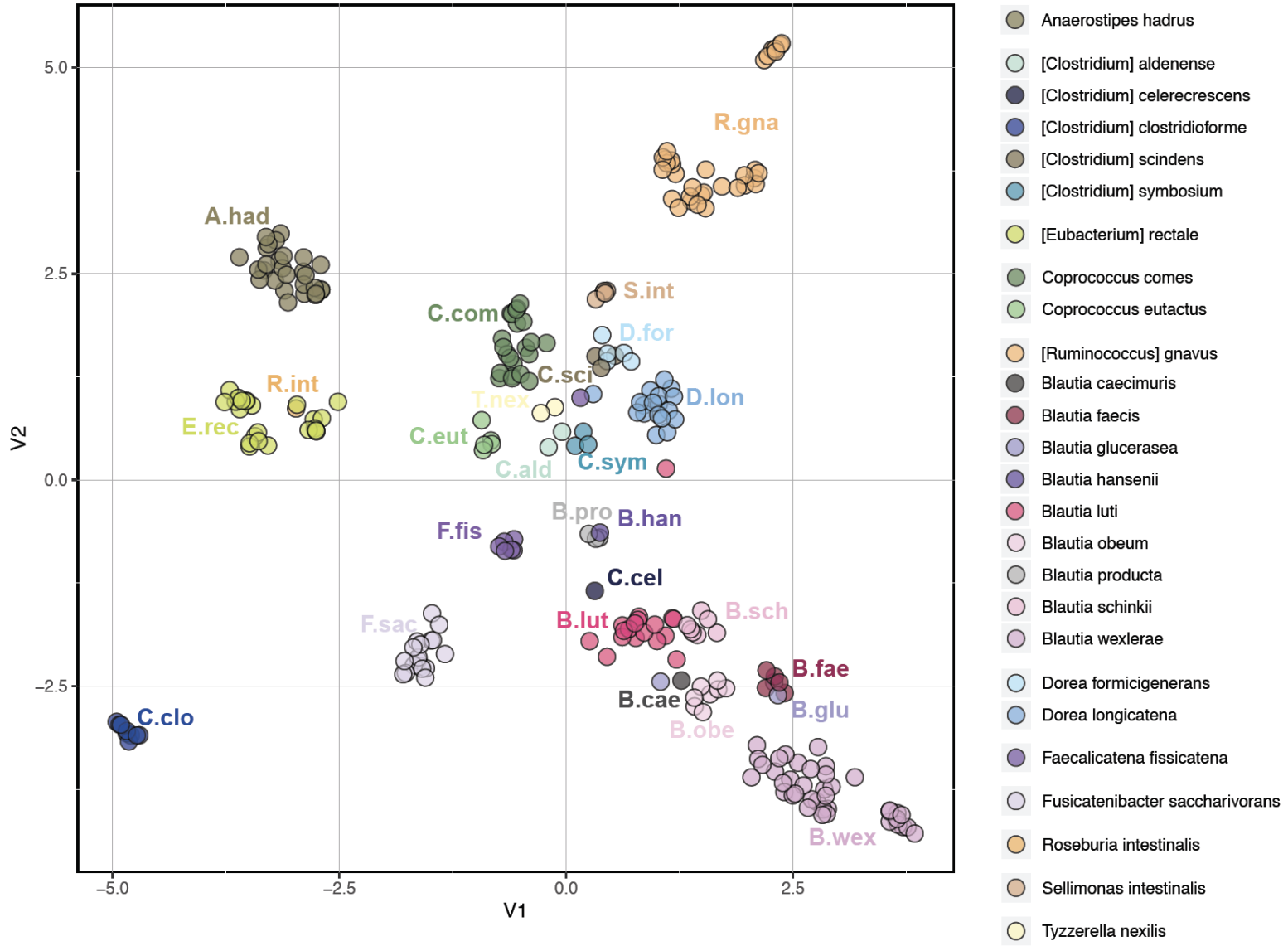
Lachnoclostridium scindens
Anaerobutyricum hallii
[Ruminococcus] torques
Anaerocolumna xylanovorans
Blautia faecicola
Blautia hominis
Blautia hansenii
Blautia producta
Lachnospira pectinoschiza
Dorea formicigenerans
Enterocloster bolteae
Faecalimonas umbilicate
Fusicatenibacter saccharivorans
Lachnoclostridium pacaense
Mediterraneibacter glycyrrhizinilyticus
Roseburia inulinivorans
Lachnoclostridium symbiosum
Enterocloster clostridioformis
[Eubacterium] siraeum
[Ruminococcus] lactaris
Anaerocolumna jejuensis
Bariatricus massiliensis
Blautia faecis
Blautia luti
Blautia marasmi
Blautia stercoris.
Coprococcus comes
Dorea longicatena
Enterocloster lavalensis
Frisingicoccus caecimuris
Lacrimispora amygdalina
Lacrimispora saccharolytica
Mediterraneibacter faecis
Mediterraneibacter gnavus
Enterocloster aldenensis
Faecalicatena fissicatena
[Eubacterium] rectale
Anaerostipes caccae
Anaerostipes hadrus
Blautia caecimuris
Blautia glucerasea
Blautia obeum
Blautia schinkii
Blautia wexlerae
Coprococcus eutactus
Enterocloster aldensis
Kineothrix alysoides
Merdimonas faecis
Roseburia hominis
Roseburia faecis
Roseburia intestinalis
Extibacter muris
Tyzzerella nexilis
Coprococcus catus

Bacteroidaceae (328)

Phocaeicola dorei
Phocaeicola vulgatus
Bacteroides cellulosilyticus
Bacteroides faecis
Bacteroides fragilis
Bacteroides koreensis
Bacteroides oleiciplenus
Bacteroides salyersiae
Bacteroides thetaiotaomicron
Bacteroides xylanisolvens
Phocaeicola paurosaccharolyticus
Bacteroides caccae
Bacteroides eggerthii
Bacteroides finegoldii
Bacteroides intestinalis
Bacteroides nordii
Bacteroides rodentium
Bacteroides stercoris
Bacteroides uniformis
Bacteroides ovatus

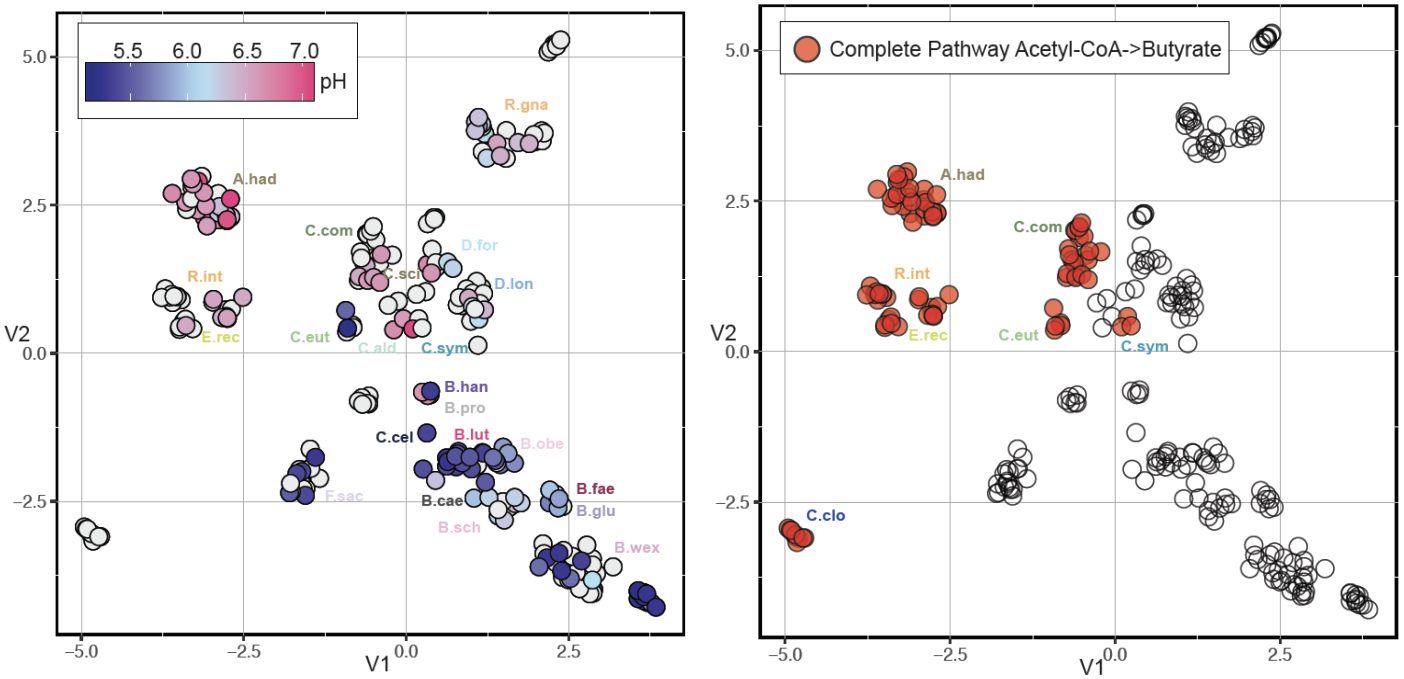
Thomas Moody, Matt Sorbara,
Eddi Lin, Claire Kohout,
Emily Waligurski

Clustering of Lachnospiraceae species/strains.



Sorbara et al. 2020 Cell Host & Microbe

Metabolite production by *Lachnospiraceae* isolates

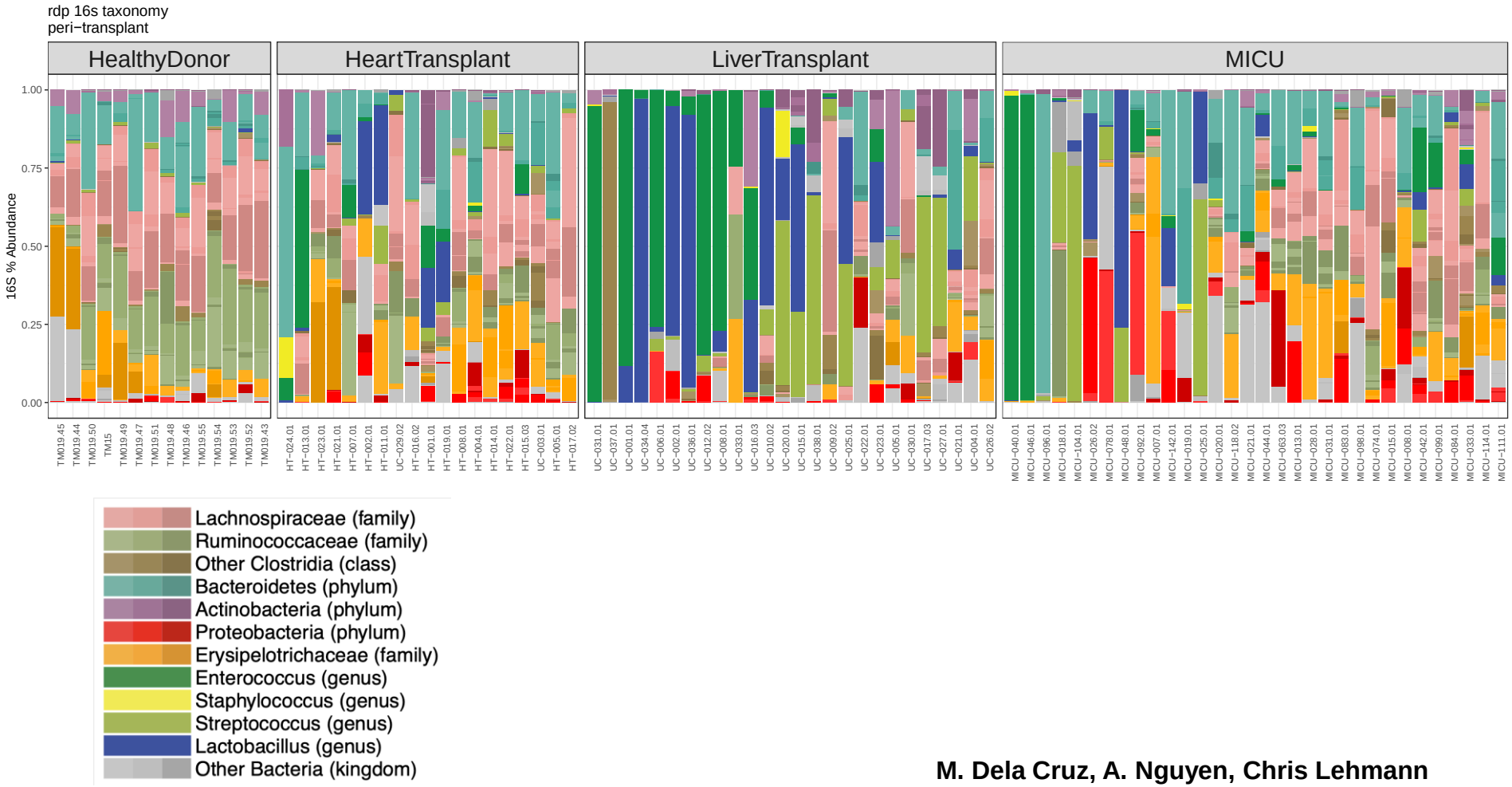


Sorbara et al. 2020 Cell Host & Microbe

Metabolite production by commensal bacterial strains.

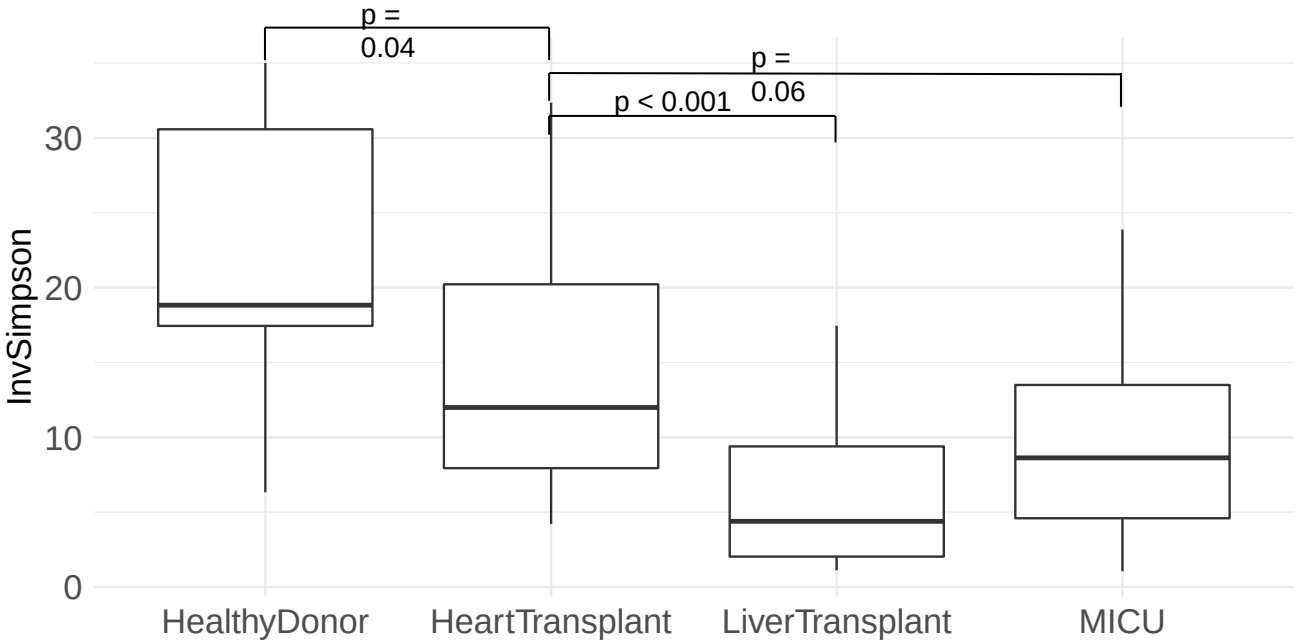
Chaubard, Sidebottom, Sorbara UChicago

Microbiota deficiencies in heart transplant, liver transplant and medical intensive care unit patients.



M. Dela Cruz, A. Nguyen, Chris Lehmann
Talia Baker, Matt Stutz, JP Kress. UChicago

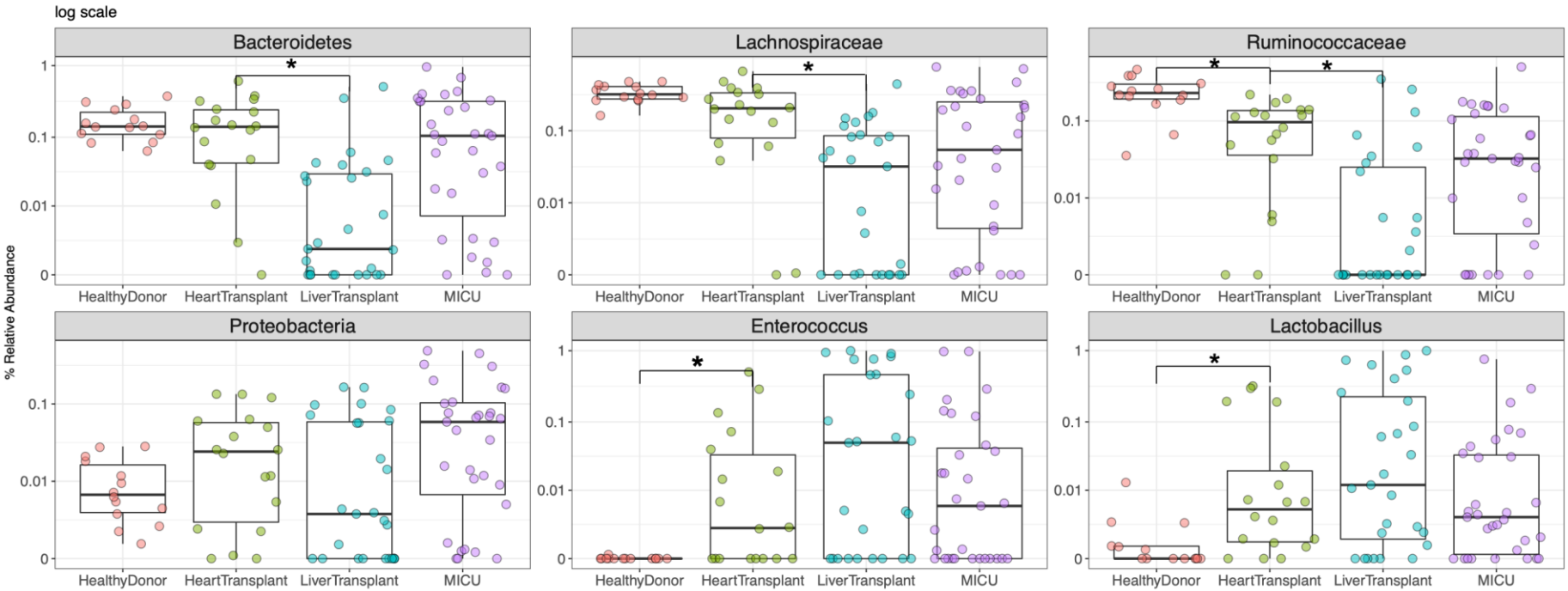
Reduced microbiota diversity in heart transplant, liver transplant and medical intensive care unit patients.



*The statistical comparisons shown here only include those between the HT and other cohorts.

Dela Cruz, Nguyen, Lehmann, Baker, Stutz, Patel, Kress. UChicago

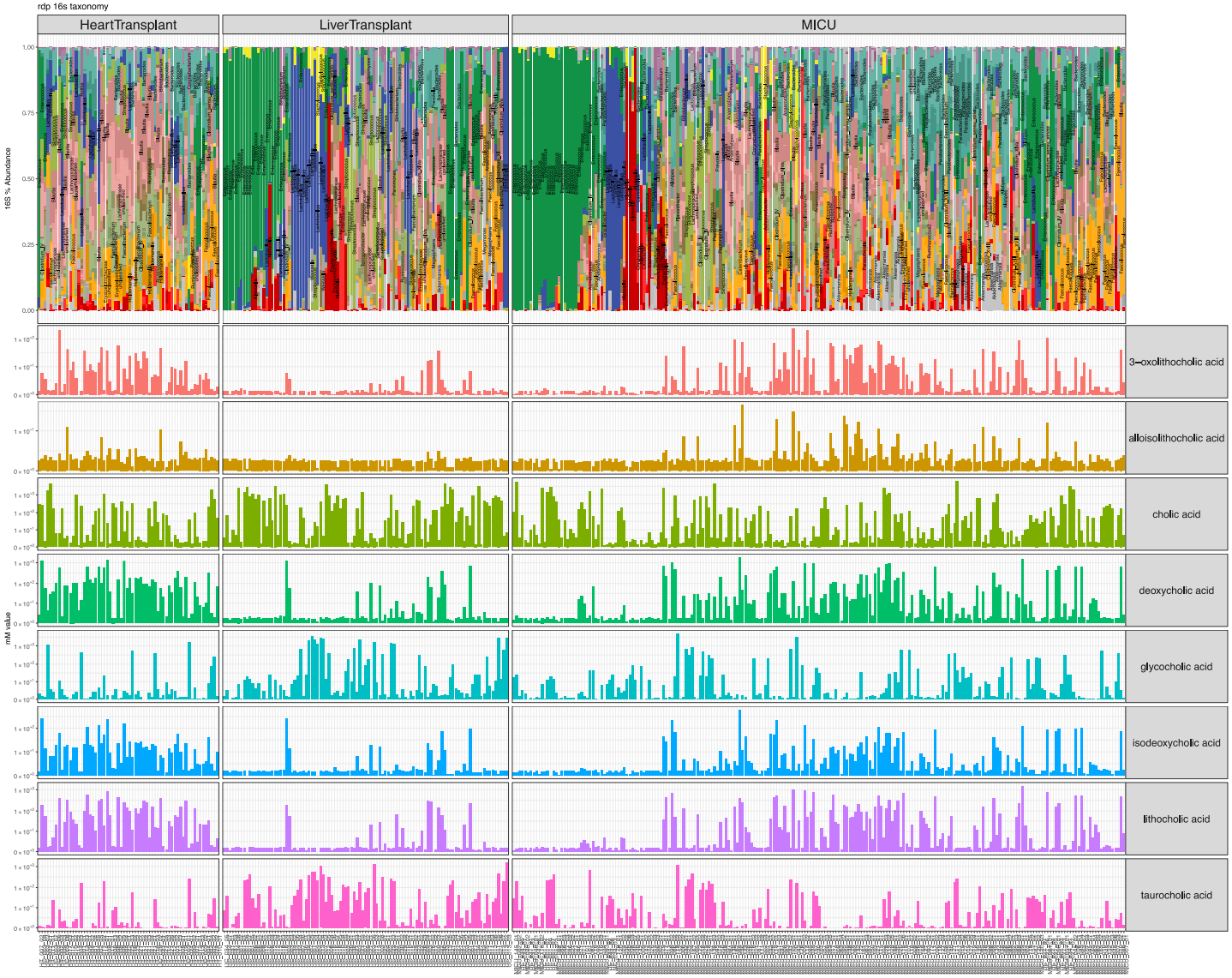
Reduced frequencies of beneficial symbiotic bacterial taxa in heart transplant, liver transplant and medical intensive care unit patients.



* = p<0.05

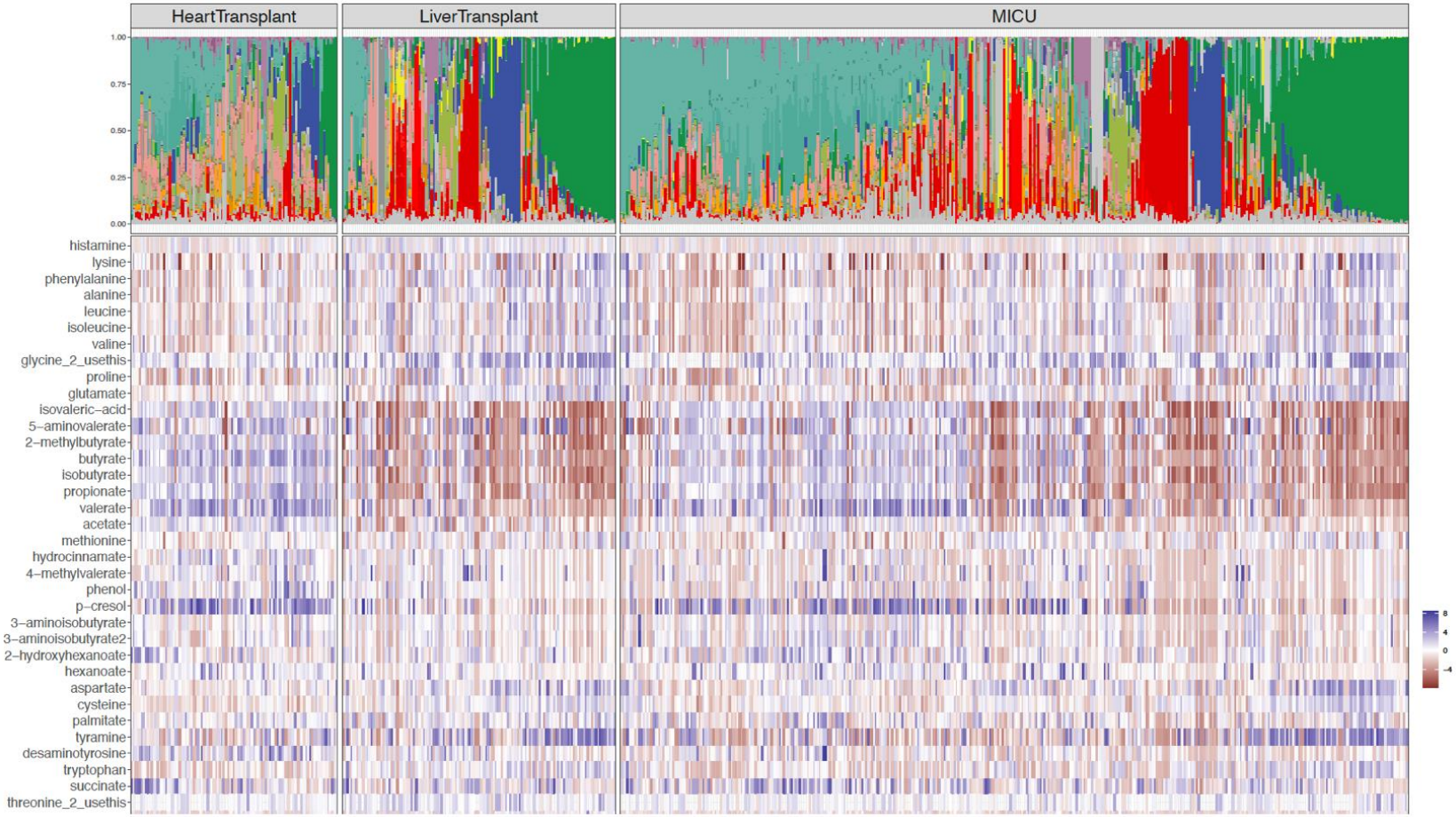
Dela Cruz, Nguyen, Lehmann, Baker, Stutz, Patel, Kress. UChicago

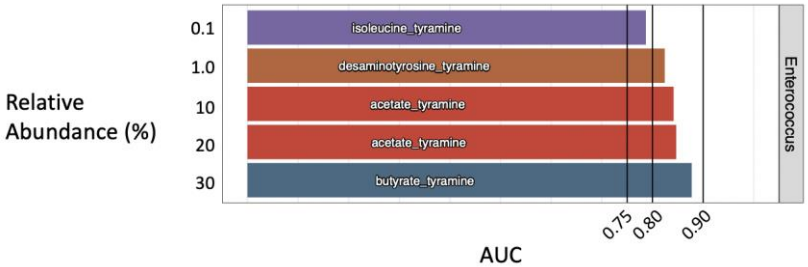
Reduced levels of secondary bile acids in hospitalized patients.



M. Dela Cruz, A. Nguyen, Chris Lehmann
Talia Baker, Matt Stutz, JP Kress. UChicago

Fecal metabolite ratios correlate with microbiota compositions.





Nicholas Dylla, UChicago

Microbiota-mediated defense against antibiotic-resistant bacterial infections.

Complex microbial networks in the gut provide colonization resistance; the indirect and direct mechanisms remain incompletely defined.

Bacterial populations that confer resistance can be defined by metagenomic analyses.

Commensal microbes inhibit antibiotic-resistant pathogens by secreting bacteriocins (e.g. lantibiotics), modifying bile acids and producing SCFAs.

Reconstitution of mucosal bacterial populations following antibiotic therapy using FMT or specific commensal microbes provides an alternative approach to treat and prevent infections in an era of decreasing antibiotic susceptibility.

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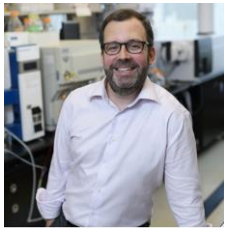
Manager

Memorial Sloan Kettering Cancer Center

Joao Xavier



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