Microbiome and Immunology

Interactions for Risk Assessors from 21st Century Science

SUNY ESF Environmental Risk Assessment (ENS 470)

Peg Coleman
April 19, 2018

COLEMAN SCIENTIFIC CONSULTING

Peg Coleman
Microbial Risk Assessor

COLEMAN SCIENTIFIC CONSULTING

Who we are

- ESF alumna (EFB) leading woman-owned small business specializing in **medical microbiology** and scientific support for **microbial risks**

What we provide

- Analysis and training about safety of exposures to bacteria in **air**, **foods**, **water**, and the **environment**

Value of our services

- **Enhance transparency** and give clients confidence to **separate facts** from **myths** about risk and health

http://www.colemanscientific.org/; http://www.sra.org/upstateny/

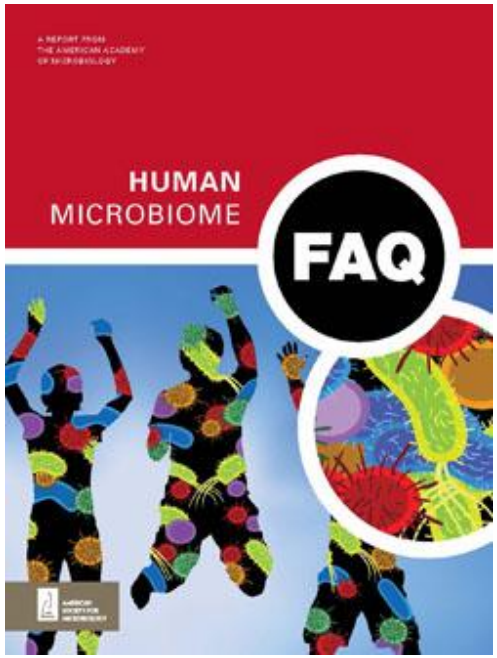
Microbiome and Immunology Lecture Outline
Environmental Risk Assessment

- Section 1: Microbes and the Human Microbiome Project
- Section 2: Interconnections with Microbial Risk Assessment
  - Exposure Assessment
  - Dose-Response Assessment
- Section 3: Interconnections with Immunology
  - Microimmunosome
  - Colonization Resistance
- Section 4: Future for Microbial Risk Analysis

SECTION 1: MICROBES & HUMAN MICROBIOME PROJECT

Microbes and Microbiota

- Microorganisms are too small to be seen with the naked eye and share our environments: air, water, soil, subways, AND in/on our bodies.
- Extremely diverse and represent the different kingdoms of life – animals, plants, fungi, protists and bacteria.
- Some microbes (pathogens) can make us sick, but relationships are complex ecologically.
- The natural microbes in our bodies form dense, diverse communities, our **microbiomes,** that do more **good** than harm and **benefit** our health.



Complex Relationships with Microbes:
Presence Alone Insufficient to Predict Risk or Health

- **Probiotics** Live microorganisms which, **when administered in adequate amounts**, confer a **health benefit** on the host (FAO/WHO, 2002)

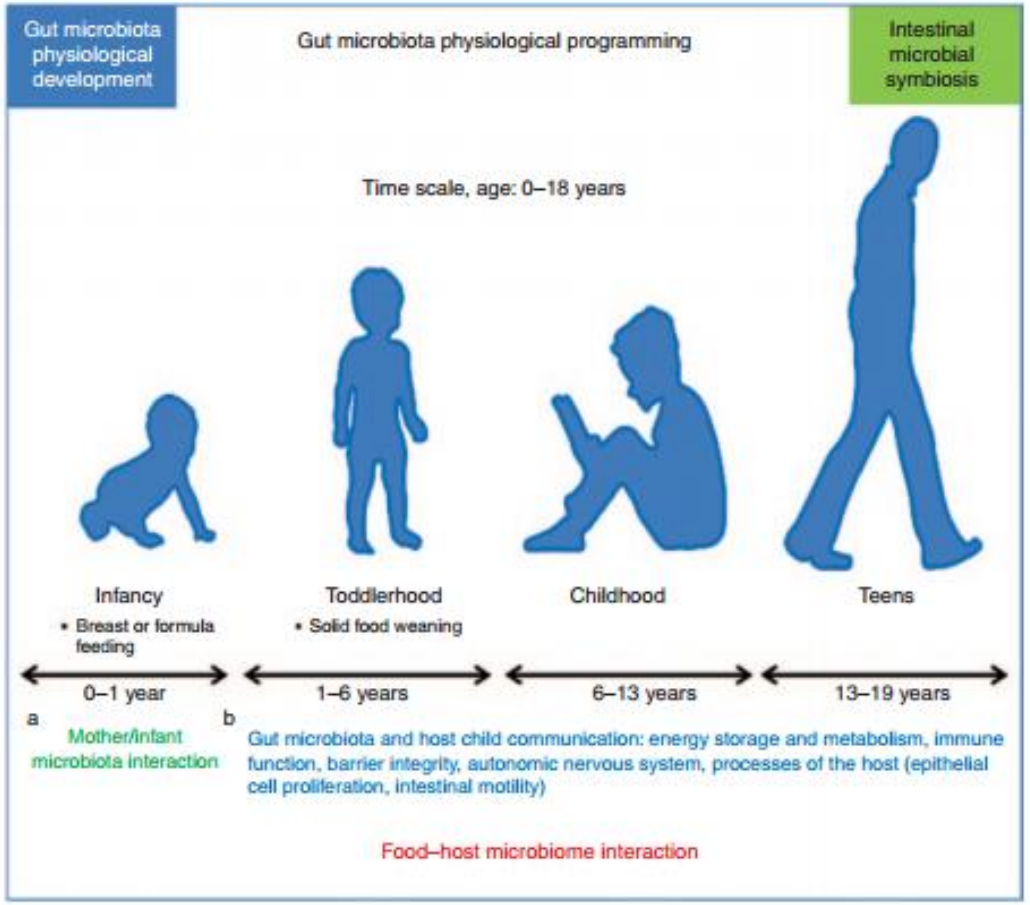
- **Pathogens** Live microorganism which, **when administered in adequate amounts**, **causes disease** in the host

- **Commensals** Live microorganisms which benefit by relationship with host but do not harm or provide known benefits to host
  - Commensal *Staphylococcus aureus* can become an opportunistic pathogen causing mastitis **at high doses**, e.g., above limit for toxin production **100,000 bacteria per mL/g food**

Dose Matters for both Health and Disease

Microbiota, Ecosystems, and Risk

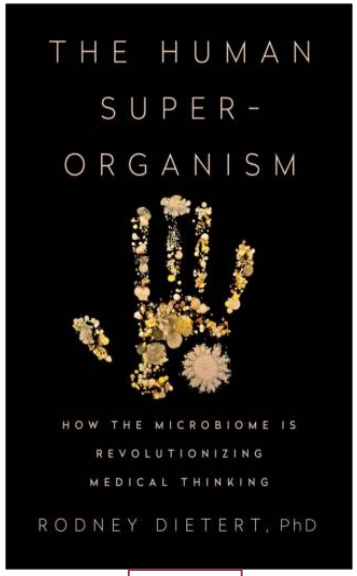
- As **expected for ecosystems,** microbial communities change over time and space (succession, competitive exclusion).
- **Predicting risk** of illness is **complex** and **uncertain** (e.g., age, doses of pathogens, environment, foods, health status, medications, nutrition, stress, water)
- **Perceptions of risk** in the media often **NOT** supported by **science** OR balanced
- **Perceptions of bacteria** as **germs to be eradicated** being replaced by awareness of fostering **symbiotic partnerships in health, ESPECIALLY FOR NEWBORNS.**
- In first decade of research, **knowledge of roles of microbiota of milks** is **advancing.**



Putignani, et. al., 2014, http://www.nature.com/pr/journal/v76/n1/pdf/pr201449a.pdf

Living in Microbial Ecosystems

- **Microbiota** are **symbiotic**, commensal or mutualistic partners, with few pathogens that may cause disease with disturbance of ecosystems (dysbiosis).



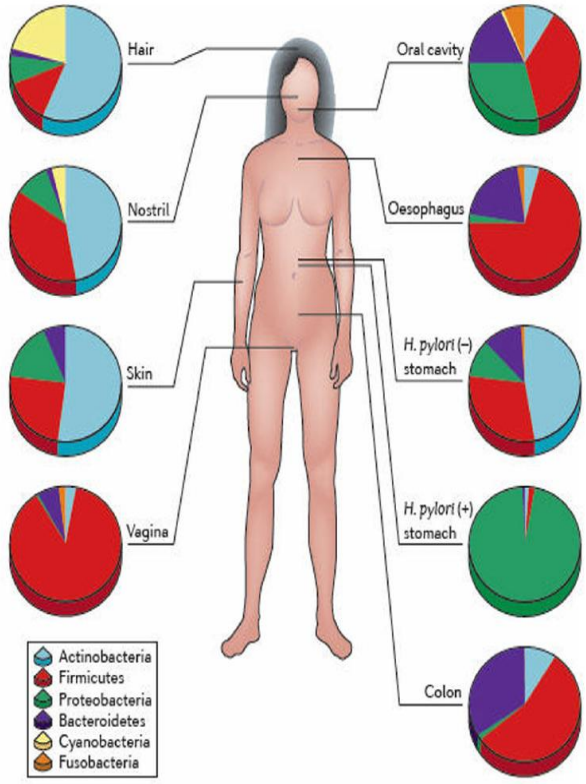
2016

- ***Homo sapiens*** + **microbiota** =
  human 'superorganism',
  holobiont, 'supraorganism'

- New medical landscape emerging in **21st century**, with **microbial ecology** challenging assumptions about health and disease.

Learnings about Human Microbiota

Human Microbiome Project (HMP):
nine body sites initially examined
(**NOT** breast)



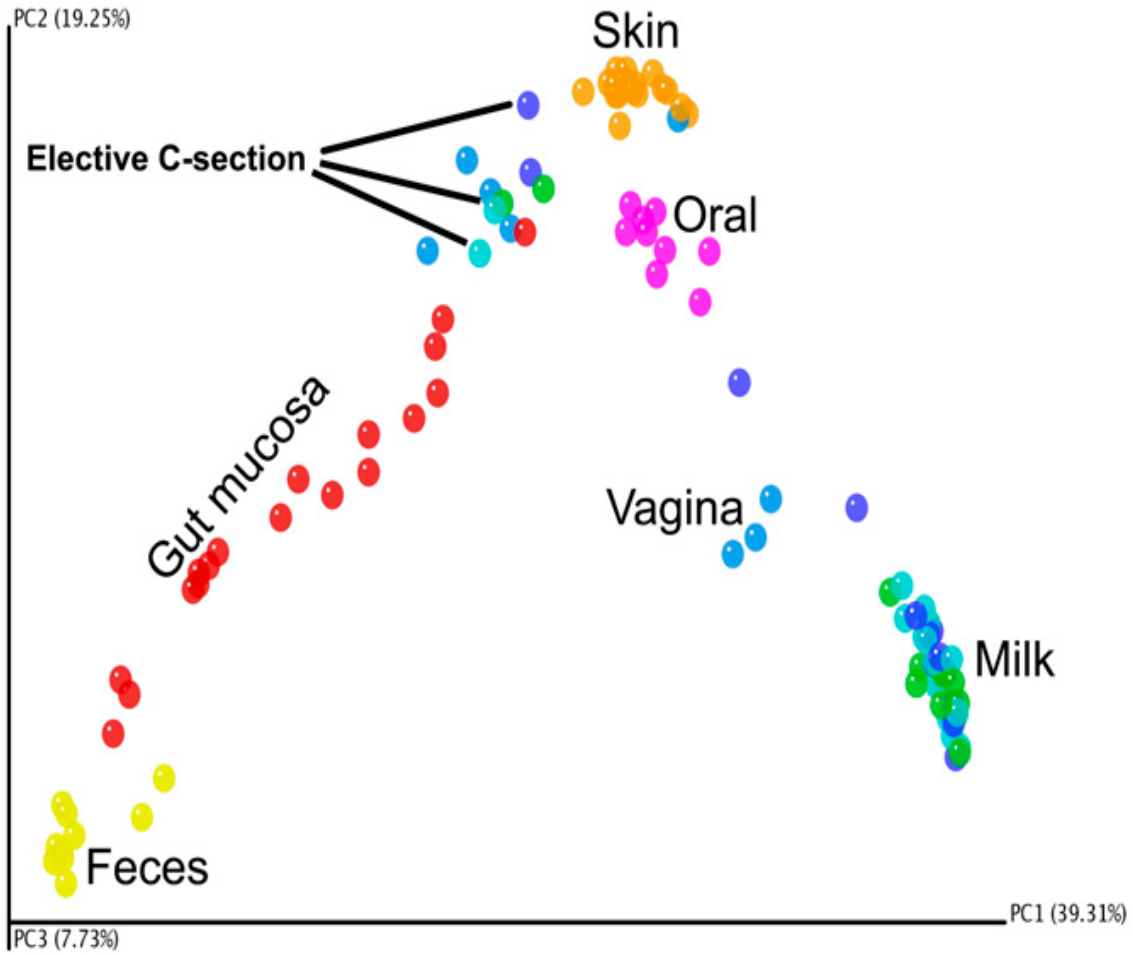
Cho and Blaser, 2012

Results in first decade continue to challenge established theories (dogma):

- **Breast tissue expected to be sterile**, now known to be **FALSE assumption!** (Urbaniak et al., 2014)
- **Milk microbiota NOT** contaminants! (Hunt et al., 2011; Rodriquez, 2014; Addis et al., 2016)

Relatedness of Human Microbiota

- **Not random associations**, some niches with ‘core’ groups present or dominant in most individuals
- **Gut microbiota** are more similar between individuals than to skin or oral or other microbiota of same individual
- Stability and resilience high, but can be disturbed.

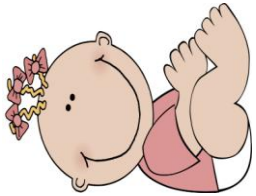


(Cabrera-Rubio et al., 2012)

How Do We Get Our Microbiome?

Birth

A newborn gets its microbes from its mother's birth canal, skin of its **mother** and other **caregivers**



Unpasteurized Breast Milk

Breast milk has been fine-tuned over millions of years to provide nutrients, vitamins, antibodies, **diverse microbes** to populate the baby's gut

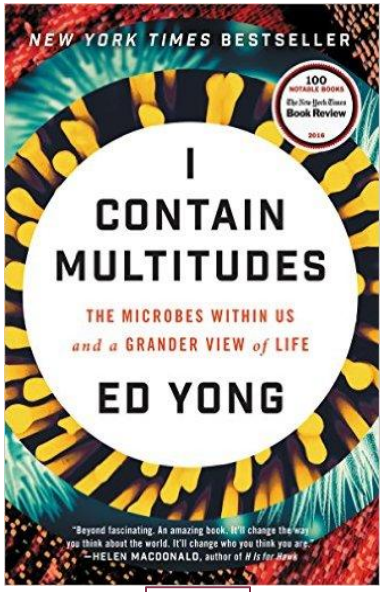
Environment

We continuously encounter bacteria everywhere, from air, water, people, pets, soil, plants, and **foods** daily

(http://academy.asm.org/index.php/faq-series/5122-humanmicrobiome)

Milk: A Mammalian Innovation

200 Million-Year-Old 'Superfood' (Yong, 2017)

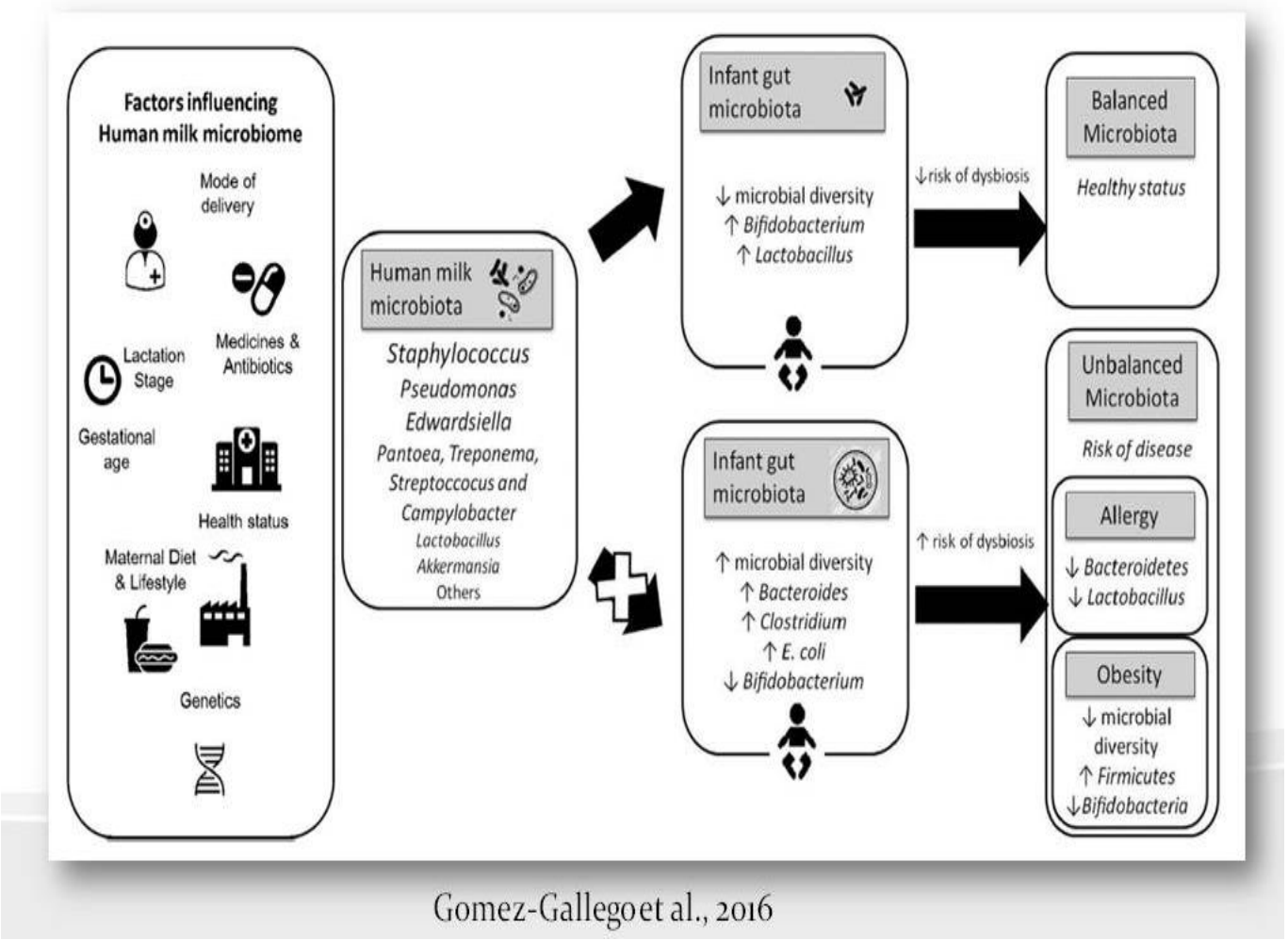


2017

Human milk

- 'Breast is best' more true than ever
- **Exclusive breastfeeding** with intact milk microbiota protects against common infections during infancy and lessens the **frequency** AND **severity** of infectious episodes (Ladomenou et al., 2010)
- Wet nursing ancient practice in many cultures (Code of Hammurabi from 2250 BC)
- Recent establishment of human donor milk banks (e.g., Human Milk Banking Association of North America, HMBANA; https://www.hmbana.org/about-hmbana) for care of at-risk infants (very low birth weight, premature, ill)
  - Early 20th century donor milk unprocessed, now **PASTEURIZED** due to potential presence of pathogens

Breastfeeding Seeds and Balances Infant Gut Microbiota



Gomez-Gallego et al., 2016

Milk Living Food with Dense Diverse Bacterial Communities Linked with Development of Healthy GI and Immune Systems in Offspring

Extensively-Studied Bacterium:
Still Learning about *Escherichia coli*

frontiers in Microbiology

Front Microbiol. 2018; 9: 498.
Published online 2018 Mar 20. doi: 10.3389/fmicb.2018.00498
PMCID: PMC5869251
PMID: 29616010

Outer Membrane Vesicles From Probiotic and Commensal *Escherichia coli* Activate NOD1-Mediated Immune Responses in Intestinal Epithelial Cells

María-Alexandra Cañas,[1,2,†] María-José Fábrega,[1,2,†] Rosa Giménez,[1,2] Josefa Badia,[1,2,‡] and Laura Baldomà[1,2,*‡]

World Journal of Gastroenterology

World J Gastroenterol. 2016 Jun 21; 22(23): 5415–5421.
Published online 2016 Jun 21. doi: 10.3748/wjg.v22.i23.5415
PMCID: PMC4910662
PMID: 27340358

Intestinal-borne dermatoses significantly improved by oral application of *Escherichia coli* Nissle 1917

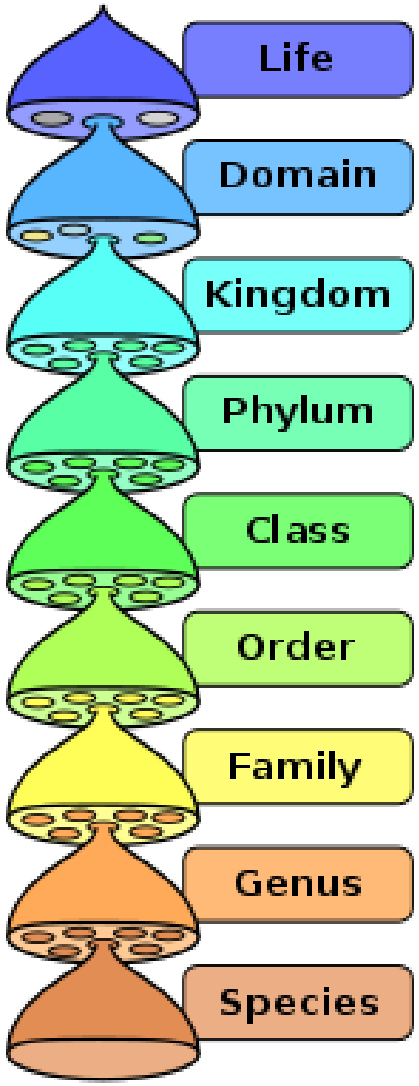
Elina Manzhalii, Daniel Hornuss, and Wolfgang Stremmel

- Most *E. coli* commensals, some probiotics, few deadly pathogens
- O157:H7 caused outbreaks of **bloody diarrhea** with fatal complications
- Tragically, **4 children died** in US fast food outbreak from undercooked hamburgers (Jack in the Box) in 1993
- Associated with recent produce outbreaks

AMERICAN SOCIETY FOR MICROBIOLOGY

Mneumonic for *E. coli* Taxonomic Ranks

First Letter	Mneumonic	Taxonomic Rank	*Escherichia coli*
D	**D**etermined	**D**omain	Bacteria
K	**K**ind	**K**ingdom	Bacteria
P	**P**eople	**P**hylum	Proteobacteria
C	**C**an	**C**lass	Gammaproteobacteria
O	**O**ften	**O**rder	Enterobacteriales
F	**F**ollow	**F**amily	Enterobacteriaceae
G	**G**hostly	**G**enus	*Escherichia*
S	**S**creams	**S**pecies	*E. coli*



https://www.mnemonic-device.com/biology/taxonomy/domain-kingdom-phylum-class-order-family-genus-species/

“Core” Breast Milk Microbiota Complex and Variable

Characterizing microbial communities in breast milk

Table 1. Genus assignments of the 9 OTUs identified in every sample ($n = 47$) and their relative abundance (%).

Core OTU Genera	Relative abundance of OTU in total community (%)
Staphylococcus	15.8
Streptococcus	8.2
Serratia	7.6
Pseudomonas	4.5
Corynebacterium	3.8
Ralstonia	3.7
Propionibacterium	3.6
Sphingomonas	2.4
Bradyrhizobiaceae	1.9
Sum of all “core” OTUs	51.5

doi:10.1371/journal.pone.0021313.t001

“Stacked bar charts” – just look at color patterns

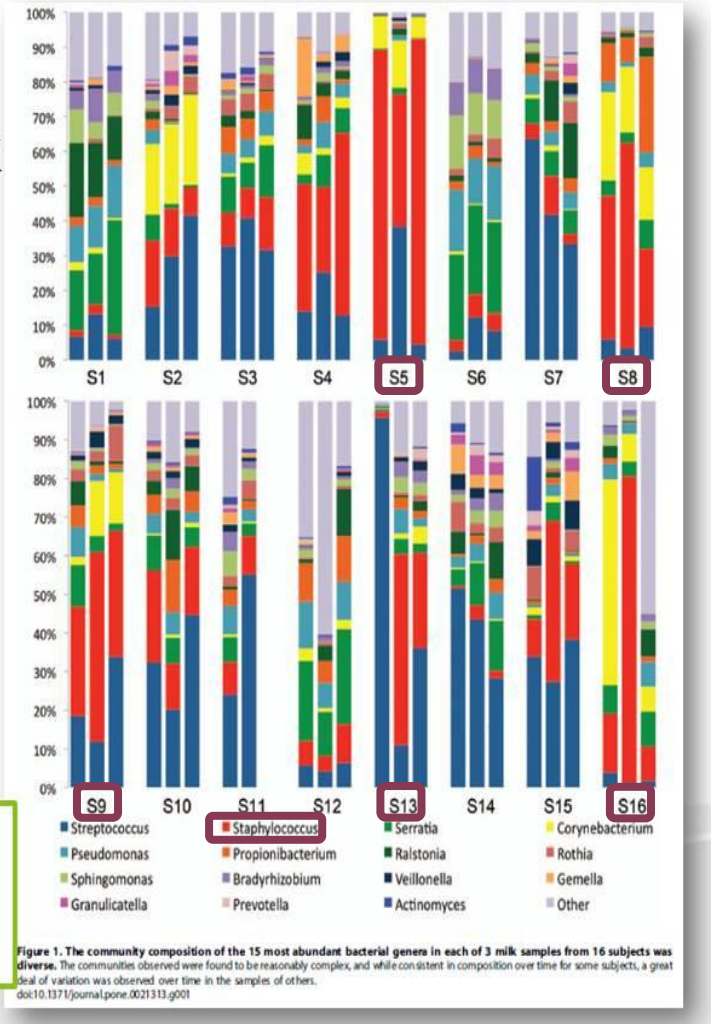
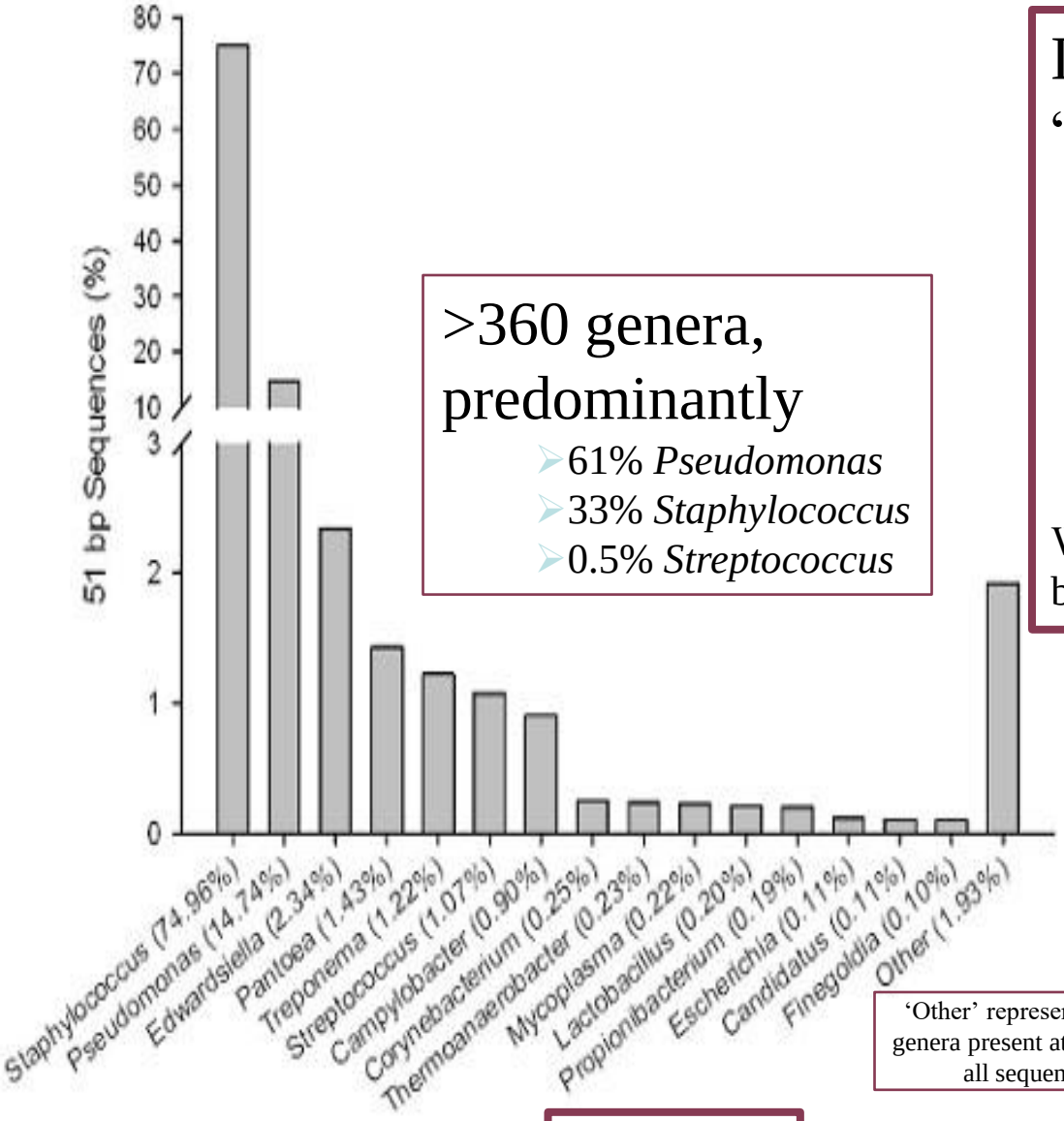


Figure 1. The community composition of the 15 most abundant bacterial genera in each of 3 milk samples from 16 subjects was diverse. The communities observed were found to be reasonably complex, and while consistent in composition over time for some subjects, a great deal of variation was observed over time in the samples of others.
doi:10.1371/journal.pone.0021313.g001

Shelley McGuire’s 2017 SRA webinar cited data above from Hunt et al. (2011, n=16)
Now more recent studies by **Ward et al., 2013; Jimenez et al., 2015; Cacho & Lawrence, 2017**

What Is Known About Abundance, Functions of Breast Milk Microbiota from Culture-Independent Methods? (2011-2015)



>360 genera, predominantly

- 61% *Pseudomonas*
- 33% *Staphylococcus*
- 0.5% *Streptococcus*

Ward et al., 2013

'Other' represent sum of genera present at <0.1% of all sequences

Later study compared healthy "core" microbiota

- *Staphylococcus*
- *Streptococcus*
- *Bacteriodes*
- *Faecalibacterium*
- *Ruminococcus*
- *Lactobacillus*
- *Propionibacterium*
- Fungal, protozoal, and viral sequences

With "Unhealthy Core" (mastitis) dominated by *Staphylococcus aureus*

Jimenez et al., 2015

GI Microbiome in Health

- **Food-Borne Microbes**: Shaping the **Host** Ecosystem (Jaykus et al., 2009)
  - Estimate of **10^{10} (10,000,000,000 or TEN BILLION!)** microorganisms as **daily dietary consumption**, most commensals and few pathogens (<0.1% abundance)
  - Most pass through the intestines into feces without attaching to (or infecting) any human cells

- Findings of **NIH Human Microbiome Project** http://commonfund.nih.gov/hmp/
  - GI microbiome includes diverse consortia of **>40,000** microorganisms
  - **10^{14} (100,000,000,000,000!)** microorganisms typically present in human colon
  - **Complex** spatial and temporal gradients
  - **NOT** simple well-mixed flasks of nutrient media

Some of My Best Friends are Germs

MICHAEL POLLAN

BOOKS | APPEARANCES | MEDIA | PRESS KIT | ARTICLES | NEWS | RESOURCES | ON TWITTER

Some of My Best Friends Are Germs

Michael Pollan
The New York Times Magazine, May 15, 2013

"Mother's milk, being the only mammalian food shaped by natural selection, is the Rosetta stone for all food," says Bruce German, a food scientist at the University of California, Davis, who researches milk. "And what it's telling us is that when natural selection creates a food, it is concerned not just with feeding the child but the child's gut bugs too."

Journalist Michael Pollan started thinking of himself as a 'superorganism' on March 7, 2013 when he received output from the citizen science project **American Gut Project**.

Recent Study of Gut Microbiota

Five Most Abundant Genera/Phyla Heat Map

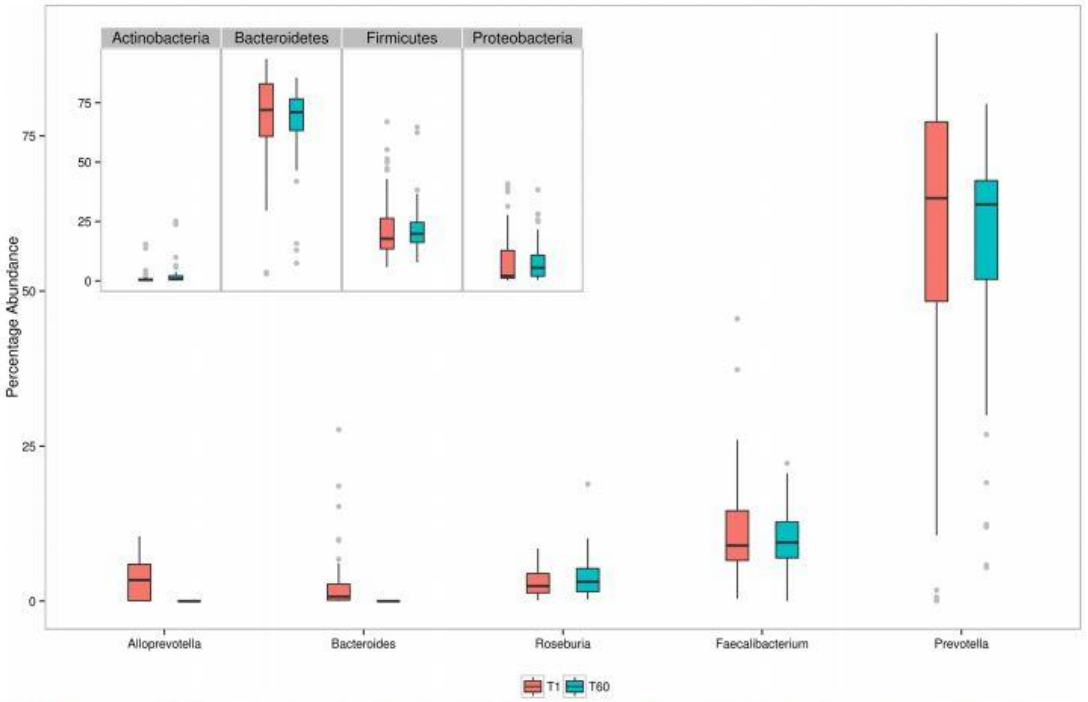


Fig 1. Box plots representing relative abundances of five most abundant bacterial genera at the two time points, i.e. IGM-T1 and IGM-T60. Inset indicates corresponding box plots at phylum level.

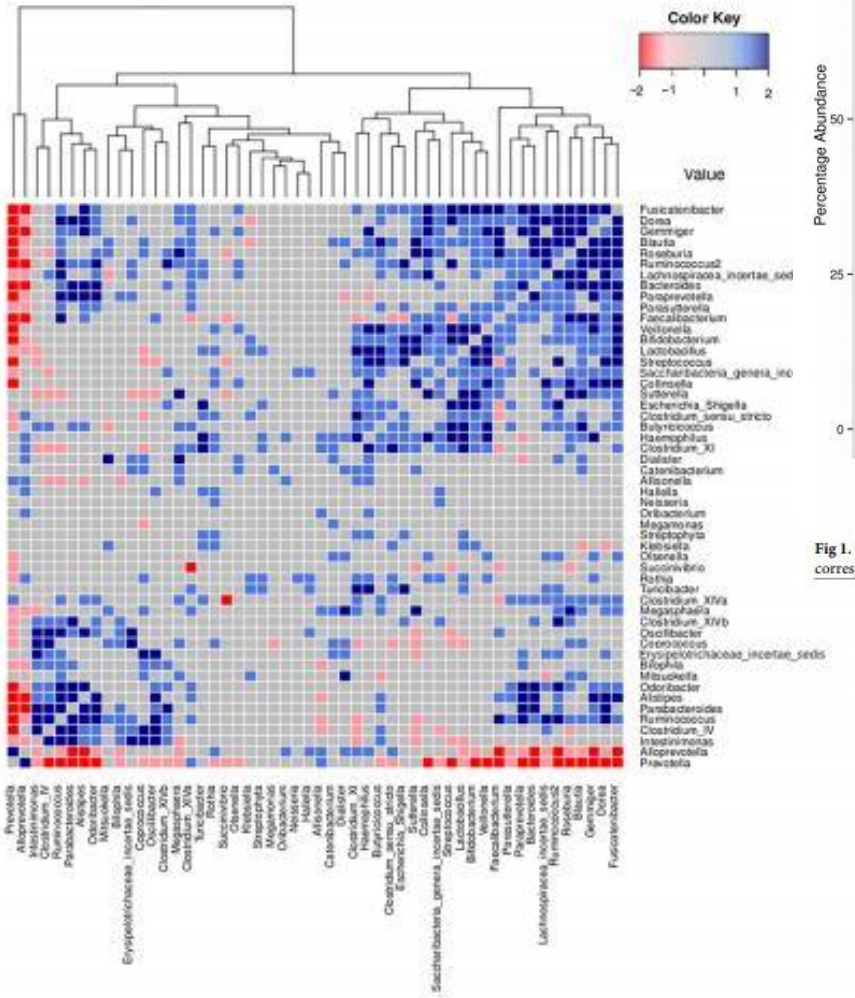


Fig 2. Heat map representing Pearson correlation values between various genera pairs. Positive or negative correlations (between a given pair of genera) identified at both time points (T1 and T60) are indicated as dark blue and dark red respectively. Interactions that are found at only 1 time point are depicted in respective lighter shades.

https://doi.org/10.1371/journal.pone.0195643.g002

Tandon D, Haque MM, R. S, Shaikh S, P. S, Dubey AK, et al. (2018) A snapshot of gut microbiota of an adult urban population from Western region of India. PLoS ONE 13(4): e0195643. https://doi.org/10.1371/journal.pone.0195643

Recent Study of Gut Microbiota:
Cluster Comparisons, Differentiating Genera

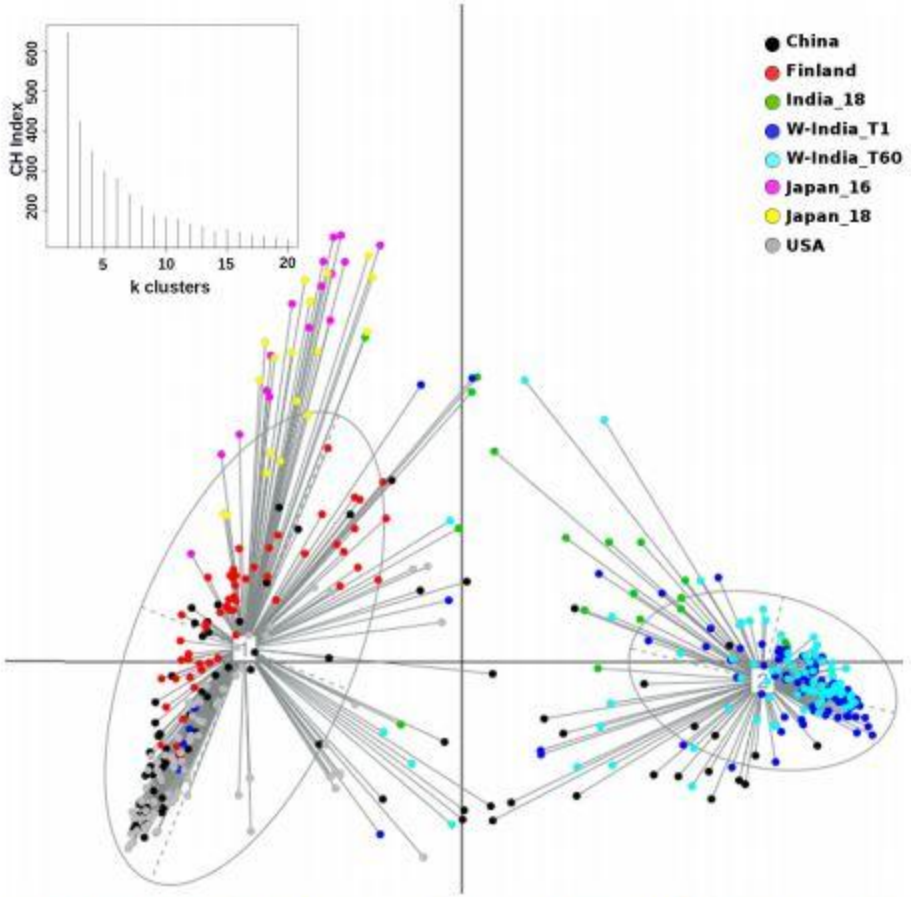


Fig 3. PCoA clustering of microbial abundance data based on Jensen Shannon divergence. Two distinct clusters were obtained. The bigger cluster corresponds to Cluster-1, while the smaller one depicts Cluster-2. The corresponding CH-index plot is depicted as inset within the main figure.

https://doi.org/10.1371/journal.pone.0195643.g003

PLOS ONE

Table 1. List of differential genera.

List of Differentiating Genera	Cluster_1a		
Bacteroides	*43*		
Roseburia	*3.6*		
Alistipes	*3*		
Parabacteroides	*2.2*		
Ruminococccus	*0.6*		
Gemmiger	*0.5*		
Oscillibacter	*0.5*		
Odoribacter	*0.3*		
Ruminococcus2	*0.2*		
Bifidobacterium	0		
Faecalibacterium	8.7		
Blautia	1.1		
Lachnospiracea incertae sedis	1.1		
Dorea	0.3	*1.7*	0.2
Streptococcus	0.1	*1.6*	0.2
Butyricicoccus	0.1	*0.3*	0.1
Prevotella	0	0.1	*64.4*
Alloprevotella	0	0	*2.6*
Sutterella	0	0	*0.5*
Dialister	0	0	*0.5*
Catenibacterium	0	0	*0.2*

https://doi.org/10.1371/journal.pone.0195643.t001

Recent Study of Gut Microbiota:
Diversity Indices, Differential Functionality

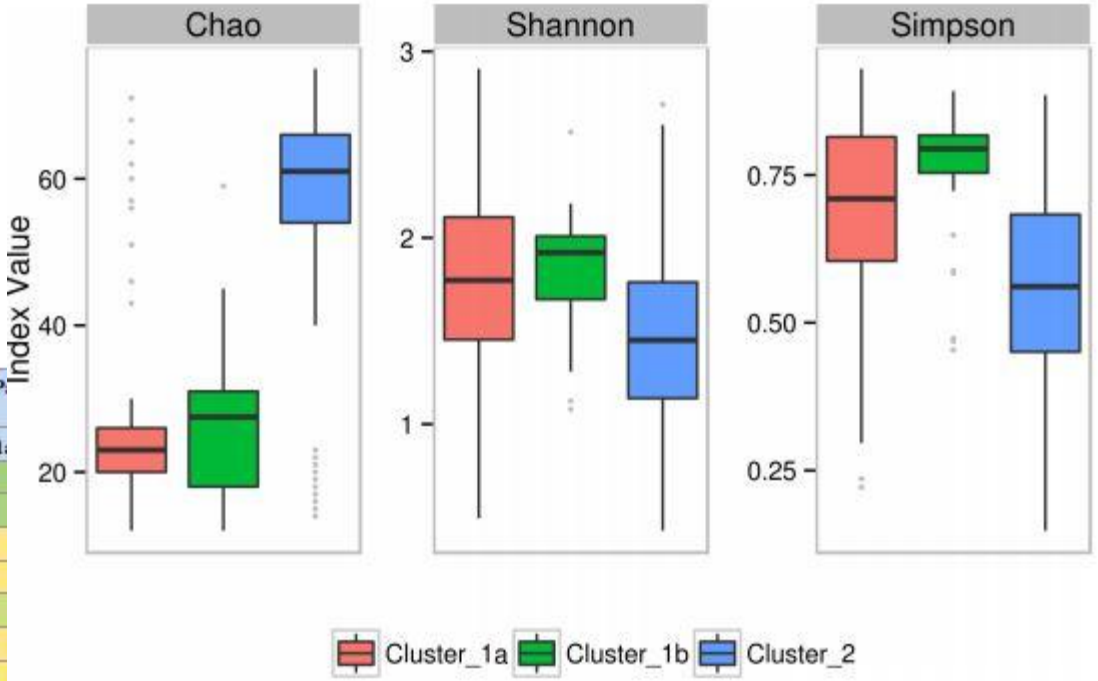


Fig 7. Box plots representing comparison between the diversity indices (Chao, Shannon and Simpson) for Cluster-1a, Cluster-1b and Cluster-2.

https://doi.org/10.1371/journal.pone.0195643.g007

Table 2. List of differentially-abundant pathway classes.

Pathway classes that significantly differentiate between three clusters	(Median) P		
	Cluster 1		
Folding sorting and degradation	16059		
Membrane transport	11291		
Immune diseases	2366		
Signaling molecules and interaction	1572		
Cell motility	4583		
Glycan biosynthesis and metabolism	28951		
Lipid metabolism	11971		
Metabolism of terpenoids and polyketides	17471		
Infectious diseases Bacterial	1178		
Metabolism of other amino acids	9603		
Energy metabolism	46982	57159	33639
Cell growth and death	7081	8942	5820
Replication and repair	30060	36443	25571
Translation	21133	24844	18784
Transcription	2715	3342	2544
Metabolism of cofactors and vitamins	40900	50412	49879
Carbohydrate metabolism	104526	117542	151564
Nucleotide metabolism	40603	48813	67972
Amino acid metabolism	34599	38770	77688
Xenobiotics biodegradation and metabolism	3971	4869	23764
Biosynthesis of other secondary metabolites	454	543	4512
Chemical structure transformation maps	3	2	176

List of differentially abundant pathway-classes (identified between Cluster-1a, Cluster-1b and Cluster-2). Significantly different pathway classes were identified using Kruskal-Wallis rank sum test (with Benjamini-Hochberg corrected p-values < 0.001 at a False Discovery Rate of 0.0001) coupled with a bootstrap approach. Pathway-classes with significantly different median abundances in at least 99% of iterations are shown in this table. The last column titled 'Vikodak Pathway Exclusion Cut-off (PEC) threshold' indicates the PEC value thresholds at which the pathway-class was reported by Vikodak. Green indicates presence and red indicates absence.

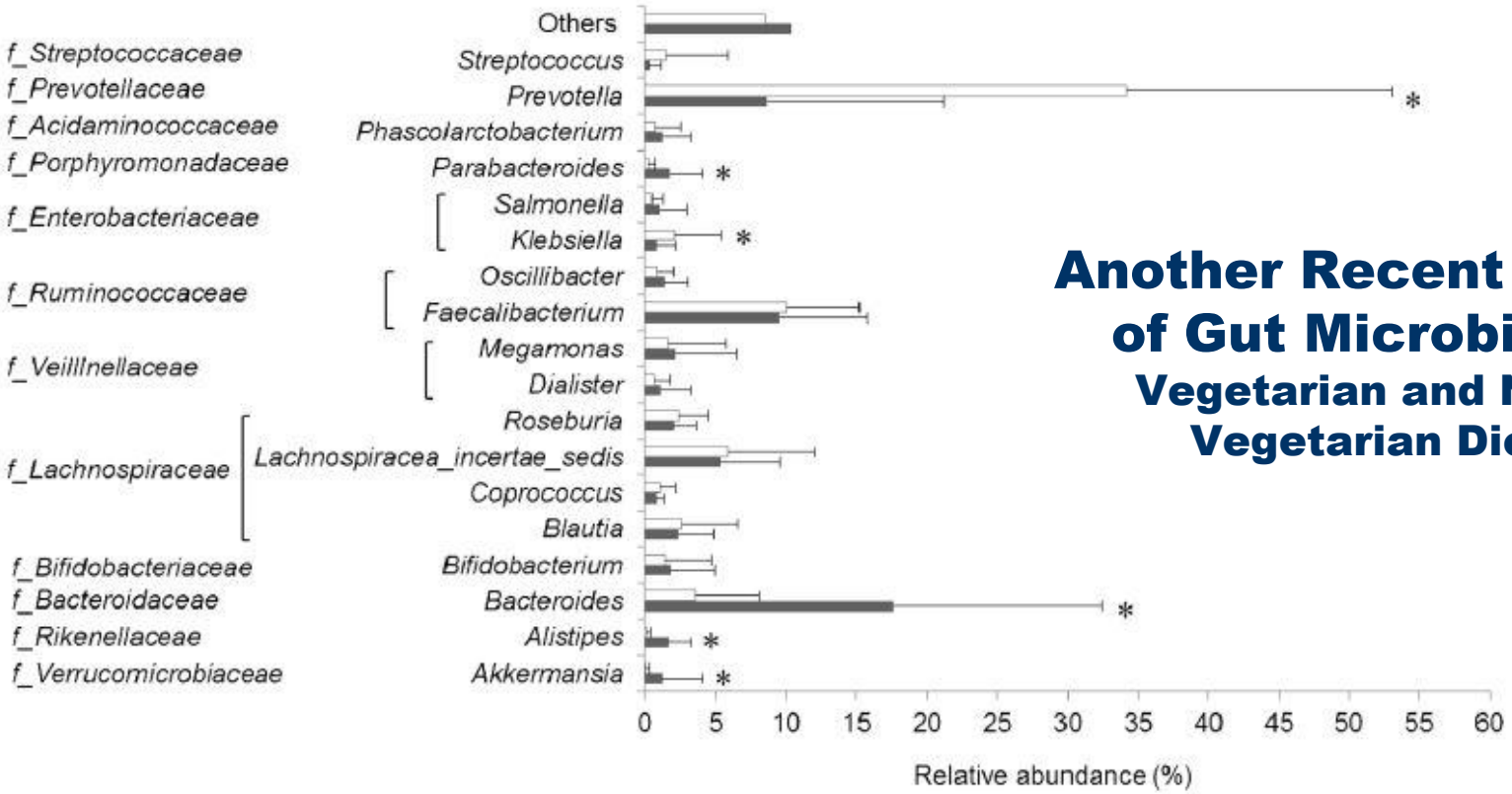
https://doi.org/10.1371/journal.pone.0195643.t002

J. Microbiol. Biotechnol. (2016), 26(10), 1723–1735
http://dx.doi.org/10.4014/jmb.1603.03057

jmb

Microbial Community of Healthy Thai Vegetarians and Non-Vegetarians, Their Core Gut Microbiota, and Pathogen Risk

Supatjaree Ruengsomwong[1,2§], Orawan La-ongkham[1], Jiahui Jiang[3], Bhusita Wannissorn[3], Jiro Nakayama[3], and Sunee Nitisinprasert[1,2§]



Another Recent Study of Gut Microbiota:
Vegetarian and Non-Vegetarian Diet

Fig. 3. Abundance of gut microbiota at the family and genus levels detected in both vegetarian and non-vegetarian groups. Abundance of the families and genera with >1% relative abundance from the vegetarian group (white bar) and non-vegetarian group (dark gray bar). An asterisk indicates genera with significant difference ($p < 0.05$).

Table 2. List of bacterial species of Thai vegetarians and non-vegetarians having ≥90% prevalence.

Non-vegetarians		Vegetarians	
Species	Prevalence (%)	Species	Prevalence (%)
Faecalibacterium prausnitzii	100.0	*Faecalibacterium prausnitzii*	100.0
Gemmiger formicilis	100.0	*Gemmiger formicilis*	100.0
Parabacteroides distasonis	100.0	*Roseburia inulinivorans*	97.2
Escherichia hermannii	97.2	*Blautia wexlerae*	97.2
Roseburia inulinivorans	97.2	*Ruminococcus obeum*	97.2
Ruminococcus obeum	97.2	*Prevotella copri*	94.4
Escherichia coli	94.4	*Eubacterium eligens*	94.4
Collinsella aerofaciens	94.4	*Dorea longicatena*	94.4
Blautia wexlerae	94.4	*Eubacterium rectale*	91.7
Klebsiella pneumoniae	94.4	*Klebsiella pneumoniae*	91.7
Ruminococcus torques	94.4	*Clostridium nexile*	91.7
Dorea longicatena	94.4		
Parabacteroides merdae	94.4		
Clostridium clostridioforme	94.4		

Another Recent Study of Gut Microbiota: Vegetarian and Non-Vegetarian Diet

Abundance/prevalence of opportunistic pathogens could be linked with **increased** or **decreased** risk of infection in healthy people!

OPPORTUNSTIC Bacterial pathogens	Non-vegetarians		Vegetarians		
	Relative abundance (%)	Prevalence (%)	Relative abundance (%)	Prevalence (%)	*p* Value
Escherichia coli	1.526	94.4	0.652	86.1	0.092
Escherichia hermannii	4.703[a]	97.2	0.716	86.1	0.032
Klebsiella pneumoniae	0.793	94.4	2.170[b]	91.7	0.032
Bilophila wadsworthia	0.166	86.1	0.014	30.6	<0.01

[a]Non-vegetarian group was significantly higher than the vegetarian group.

[b]Vegetarian group was significantly higher than the non-vegetarian group.

Living in Microbial Ecosystems

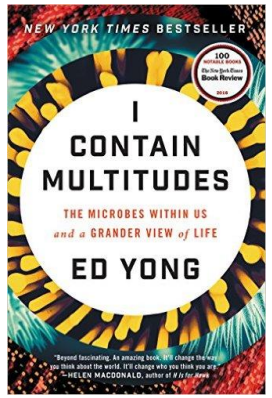
- Genomic methods challenge or falsify many assumptions of 20th century science

- Earth's ecosystems are full of **'superorganisms'** containing '**Multitudes**' of microbiota.

  - Human Genome Project began in 1990 and was completed 25 years ago (see https://unlockinglifescode.org/timeline?tid=4)

  - Human Microbiome Project began in 2007 and work is ongoing

- Unified Microbiome Initiative beginning in 2015 to study earth's diverse and connected microbial ecosystems



Section 1 Summary

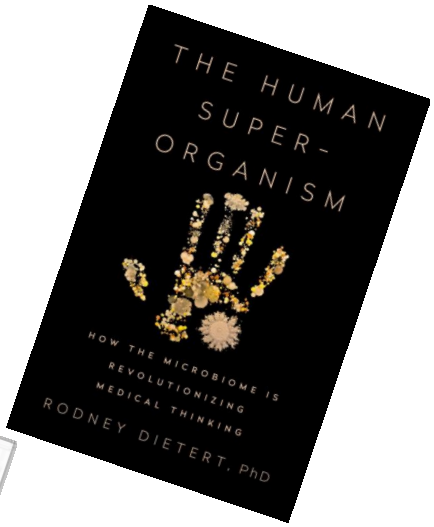
1. Microbes are our partners in health and disease
2. *Homo sapiens* plus the dense, diverse microbial communities of our microbiomes function as ‘superorganisms’ or holobionts
3. Succession of ‘core’ gut microbiota begins at birth and continues as a dynamic ecosystem though major life stages
4. Microbiota of human gut and foods are interrelated; both may contribute to health (and disease)

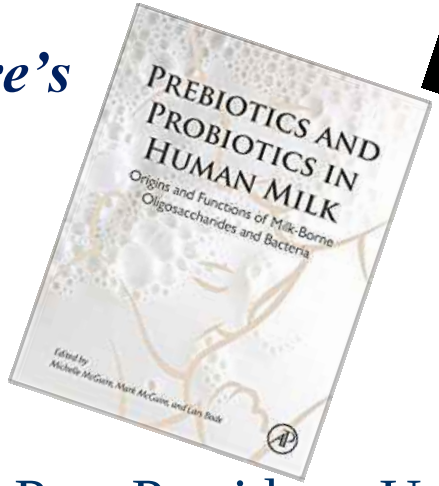
SECTION 2: INTERACTIONS WITH RISK ASSESSMENT

SRA Advancing the Science Webinar Series for 2017

Microbiota Informing Next-Generation Risks & Benefits

slide sets posted at http://www.sra.org/upstateny/





1. Rodney Dietert (Cornell University), ***Protecting the Human Superorganism*** (January 24)

2. Michelle McGuire (Washington State University), ***Human Milk: Mother Nature's Prototypical Probiotic Food*** (March 21)

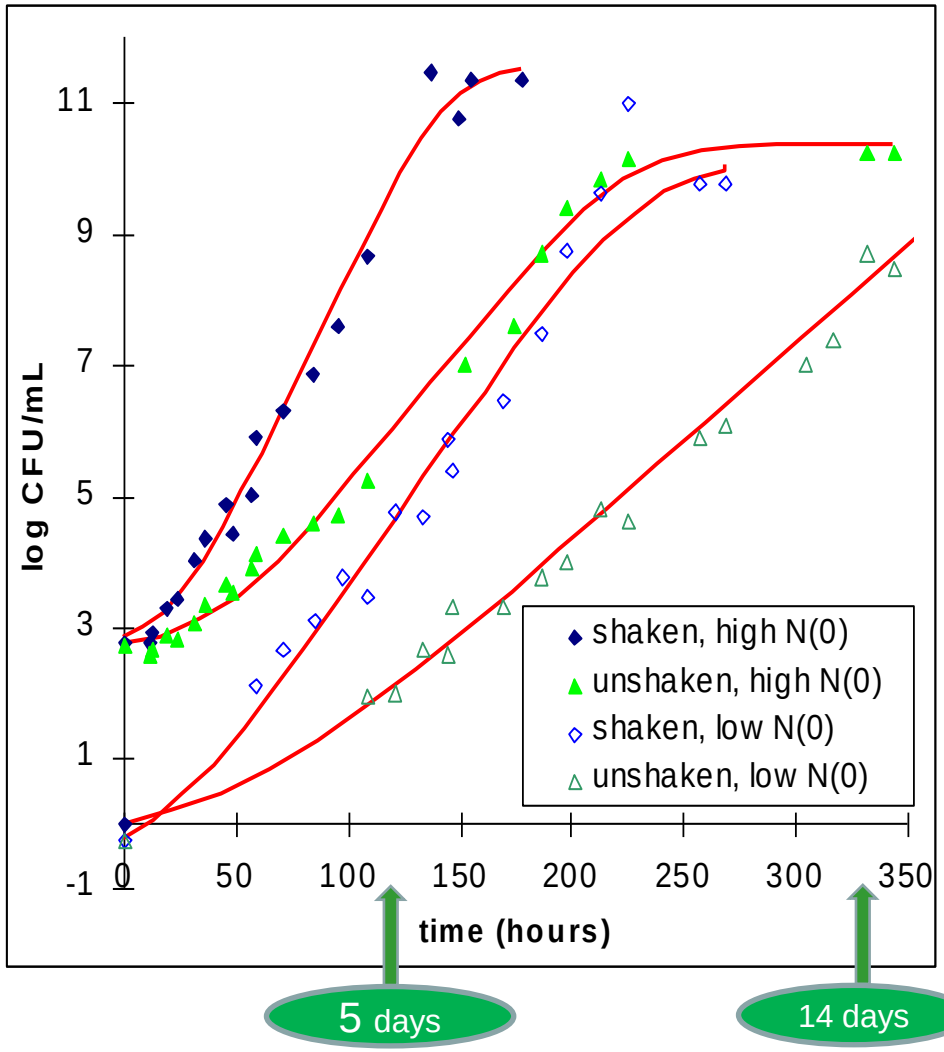
3. Mark McGuire (University of Idaho), ***Bovine Milk Microbiota*** (**May 23**)

4. Warner North & Peg Coleman (SRA Past-President, Upstate NY SRA President), ***Preparing to Deliberate Evidence for Benefits AND Risks*** (**August 28**)

Questions for Exposure Assessment

- Is a pathogen **detected** in milk?
- **How many** if detected?
  - ➢ Density (counts per serving) for positives
- Does pathogen **grow** (or survive) in milk?
  - ➢If yes, **how fast** (or how long)?
  - ➢Depends on **temperature** AND **milk microbiota**!
- **How many** pathogens (AND beneficial microbiota) are in **simulated serving** **(DOSE ingested)** at consumption?

E. coli O157:H7 Growth at Sub-optimal Conditions



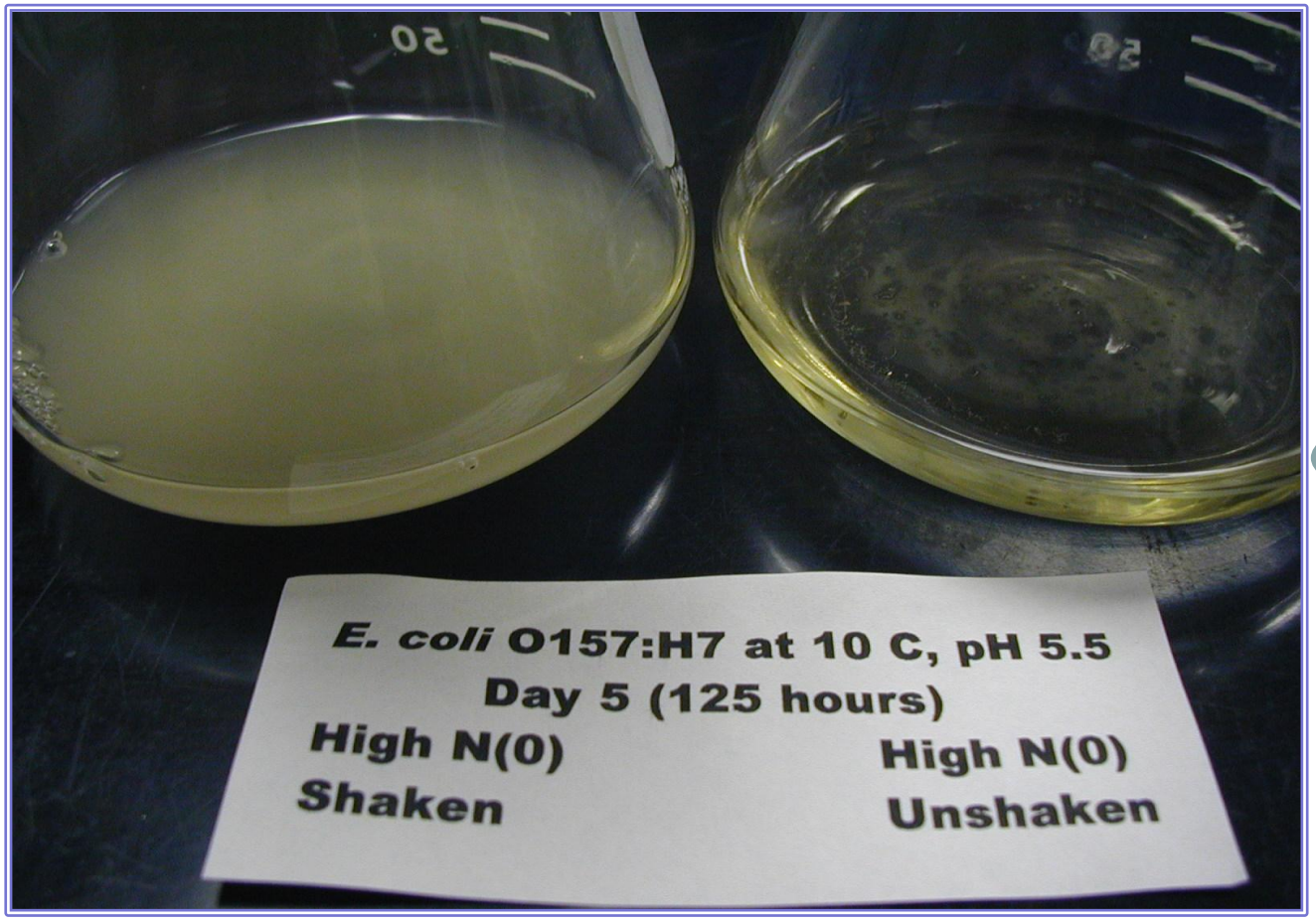
- **Refrigeration temperature**
  (upper limit for US survey, 50° F or 10° C; differences in growth at human body temperature or surface temperature in hot sun)
- **Low initial counts**
  (N0=1 bacterium/mL versus high counts N0=1,000 or more bacteria/mL)
- **No shaking**
  (like milk bottle in refrigerator versus culture flask on rotating shaker 24-7)

(Coleman et al., 2003)

Sub-optimal Conditions Limit Pathogen Growth

Shaken Flasks, Visible Growth (cloudy), Unshaken Flasks, No Visible Growth (clear) after 5 days refrigeration

Optimal
(with shaking)



Sub-Optimal
(no shaking)

Beyond Laboratory Flasks: Microbiota

A dense ecological community of **commensal**, **symbiotic** and potentially **pathogenic** microbes that literally share our body space

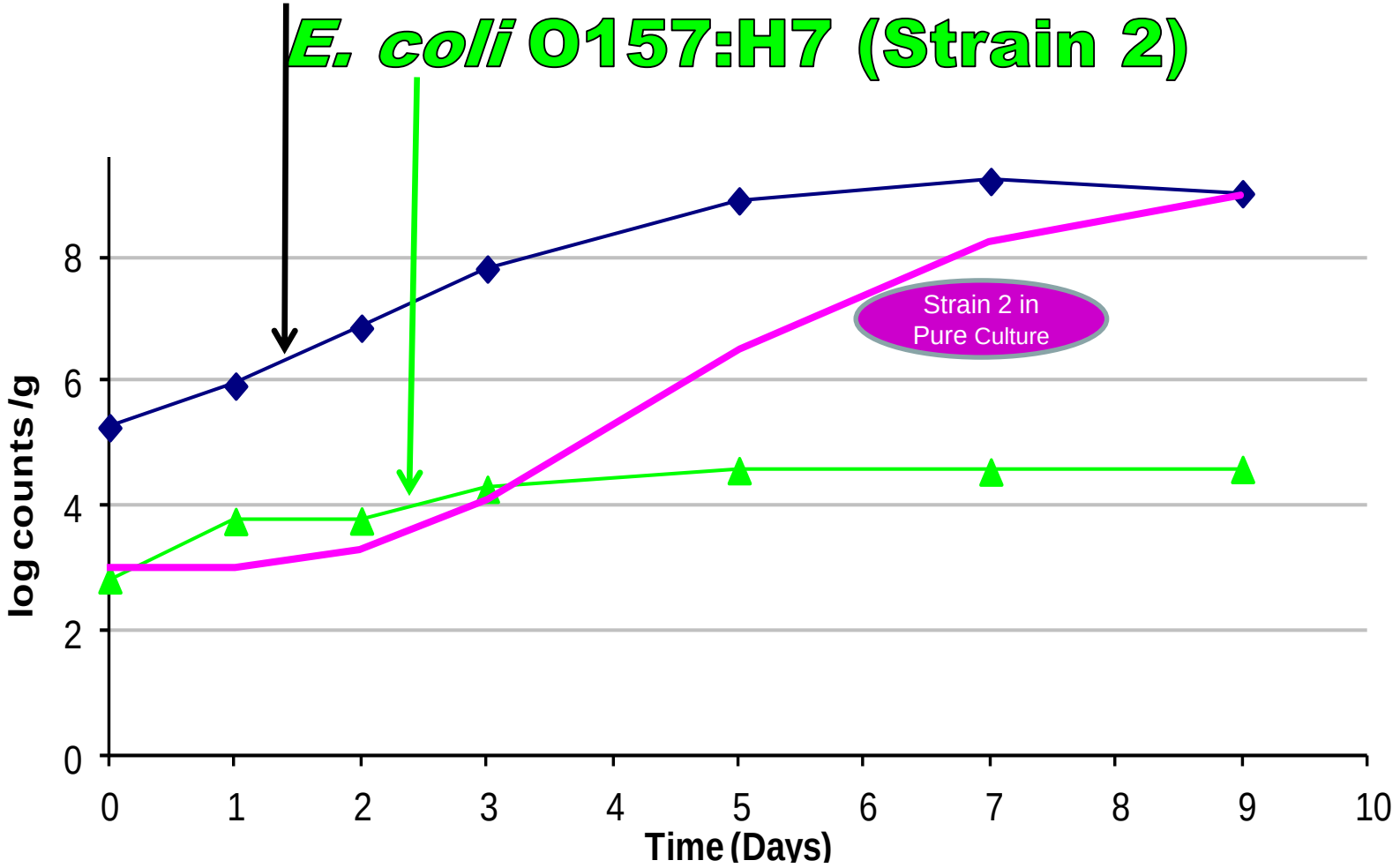
Commensal - relationship between two organisms where one organism gets food or other benefit from the other organism without helping or hurting it.

Symbiosis - relationship between two different organisms where there might be benefit for both (mutualism), benefit for one and harm for the other (parasitism), or neutral benefit/harm (commensalism).

Pathogenic - relationship between two different organisms where one is capable of causing disease in the other.

(Lederberg & McCray, 2001)

Microbiota of Ground Beef Inhibits *E. coli* O157:H7 (Strain 2)

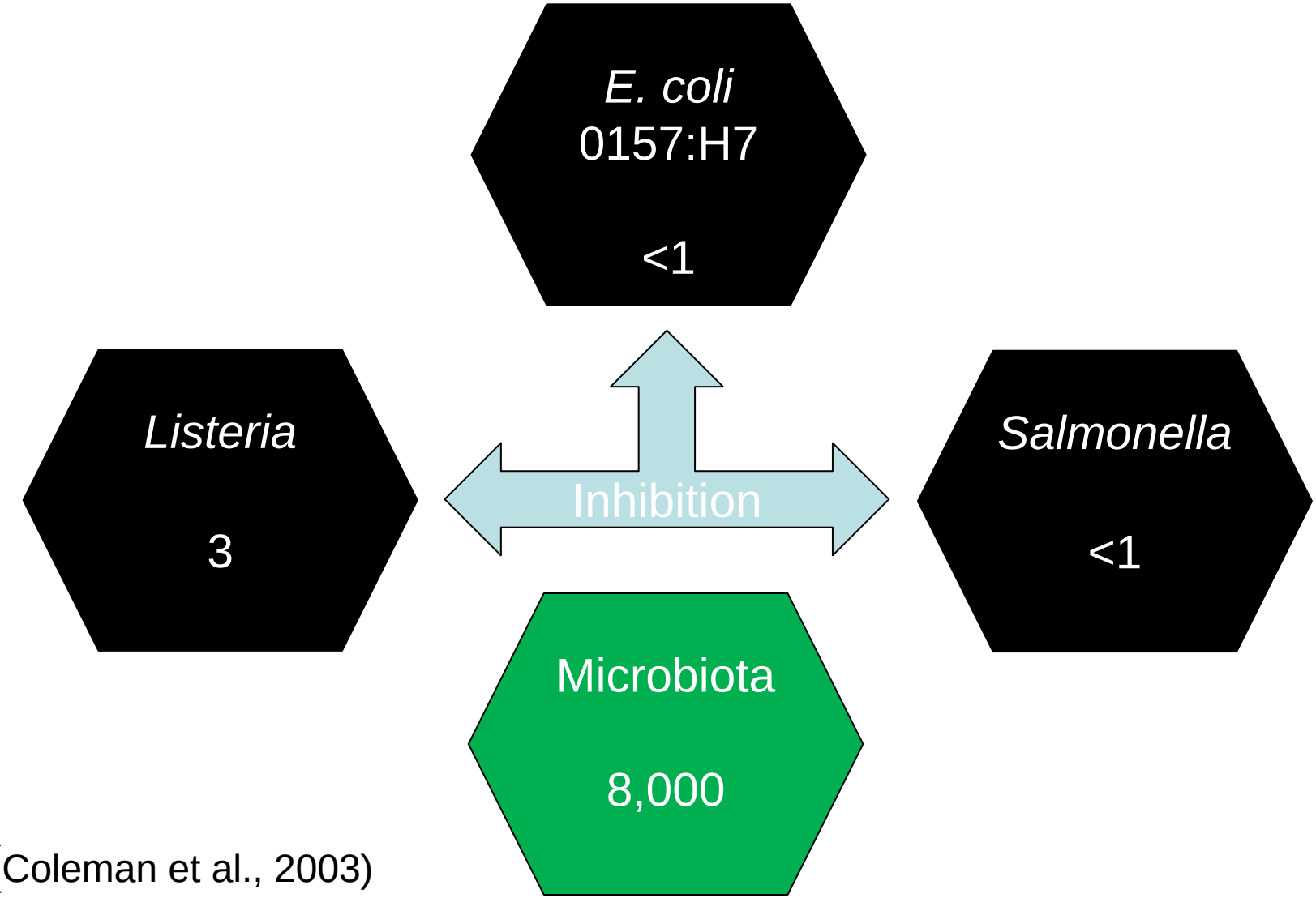


Ground beef **microbiota** measured by **total plate count**, predominated at refrigeration temperatures by non-pathogenic ***Pseudomonas*** spp., inhibits Strain 2 growth. Optimal pathogen growth in pure culture flasks in **pink** (Pathogen Modeling Program).

(Tamplin, 2001)

Microbial Ecology

Dominance of Ground Beef Microbiota over Pathogens (counts/mL when detected)

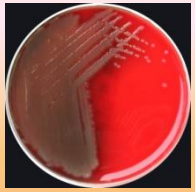


(Coleman et al., 2003)

Another Ecological Advantage:

Microbiota Grows **Faster** than **Pathogen**

Pseudomonas



36° F	0.09/hr
39° F	0.11/hr
50° F	0.24/hr

***Escherichia coli* O157:H7**

36° F	no growth
39° F	no growth
50° F	0.03/hr

Pseudomonads grow at the lowest temperature,
while pathogen does not grow at all

Exposure Assessment Issues for Foods

- **Optimal growth conditions** in laboratory experiments **unrealistic** for non-sterile foods (sub-optimal growth)
- Microbial growth depends on
  - How many **pathogens** present in foods (typically 1, 10, or <100, **not thousands or more**)
  - How many **competing microbes** present in foods (**tens of thousands or more in microbiota of foods**)
  - **Nature of food** (solid or unshaken liquids) and its **temperature**

REALITY CHECK: growth models should adjust for realistic, sub-optimal conditions, including inhibitory effects of microbiota

Microbiota Out-Competes Pathogens

Dairy Study	Numbers of Raw Milk Positives (range; mean; median) in CFU/mL				
	Standard Plate Count	***Listeria monocytogenes***	**STEC/VTEC**	***Salmonella spp.***	***Staphylococcus aureus***
D'Amico et al., 2008 Farmsted dairies N=62	62	3	0	0	17
	(10 to 10^5; **$4.9x10^4$**; $7.0 x10^2$)	<1	Non-detectable	Non-detectable	Unspecified; 250; <1
	Total Viable Count	***Listeria monocytogenes***	**STEC/VTEC**	***Salmonella spp.***	***Bacillus cereus***
Jackson et al., 2012 Commercial dairy silos N=184	184	23	30	5 - 33	4
	$7x10^2$ to $5x10^5$ **$4.2 x10^4$** -	<0.006 to 29 0.65 0.12	<0.006 to 1.1 0.19 0.26	<0.006 to 60 0.75 0.12	3 to 93 0.75 0.12

Temperature (°F)	***Pseudomonas* spp.**	***Listeria monocytogenes***	***Escherichia coli* O157:H7**	***Salmonella spp.***	**Campylobacter spp.**
36	0.09	No Growth	No Growth	No Growth	No Growth
39	0.11	0.01	No Growth	No Growth	No Growth
50	0.24	0.07	0.07	0.02	No Growth

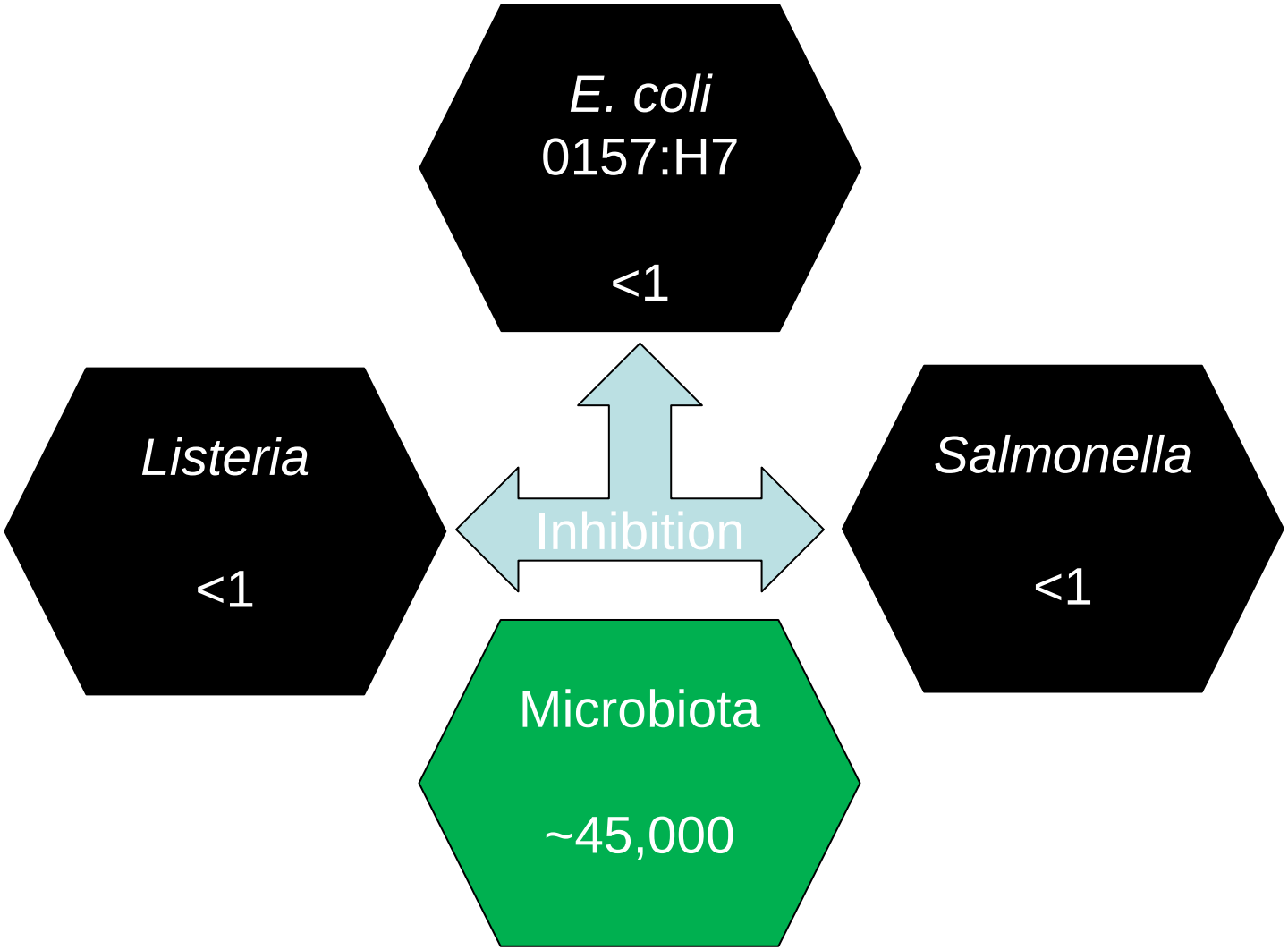
Optimal growth rates of non-pathogen and pathogens (Coleman et al., 2003)

Dominant microbe in Standard plate counts/ total viable counts of milk, non-pathogen ***Pseudomonas*** spp., grows optimally at low temperatures, outcompeting less adapted pathogens at refrigeration temperatures.

Microbial Ecology

Dominance of Milk Microbiota over Pathogens

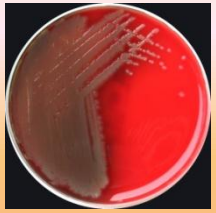
(counts/mL when detected)



Ecological Advantage:

Microbiota Grows **Faster** than **Pathogens**

Pseudomonas

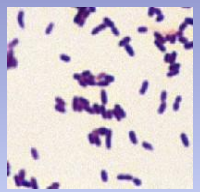


36° F 0.09/hr

39° F 0.11/hr

50° F 0.24/hr

Listeria monocytogenes



36° F no growth

39° F 0.01/hr

50° F 0.07/hr

Pseudomonads grow at the lowest temperature studied
where pathogens studied do not grow at all

Incorrect Assumptions about Pathogen Growth:
Listeria grows FASTER without Competition of Milk Microbiota

At refrigeration temperatures of 41-43 ˚ (F):

•*Listeria* growth rate assumed by FDA/FSIS for **both** **pasteurized** & **unpasteurized** milk

- 0.257 cfu/g/day

•*Listeria* growth rate **increased** with **increasing pasteurization temperature** for 25 seconds in recent university study

- 0.503 cfu/mL/day for milk treated at 162˚ (F)
- 0.562 cfu/mL/day for milk treated at 180˚ (F)

•Higher temperature also significantly **decreased** the time before *Listeria* growth began (shorter lag) and **increased** maximal growth (higher maximal density, N_{max}), causing **higher growth** of the pathogen at the higher pasteurization temperature

Exposure is **underestimated** for **PASTEURIZED milk** in FDA/FSIS assessment!

(FDA/FSIS, 2003; Stasiewicz et al, 2014)

Evidence-Based Policies for Listeriosis?

Some governments regulate Ready-to-Eat Foods that:

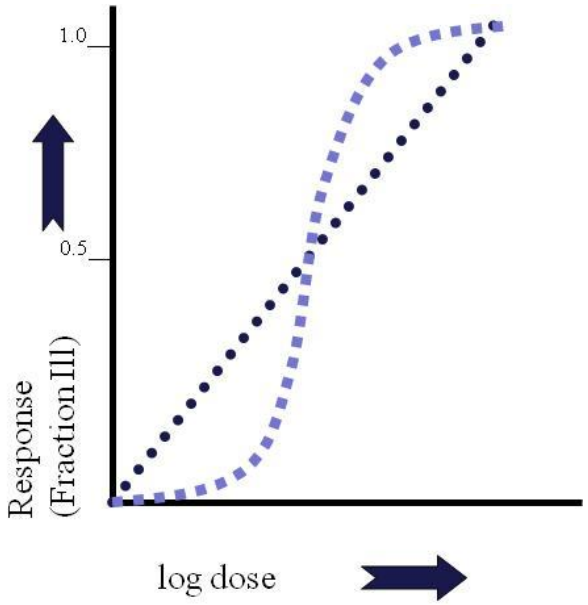
support growth as unsafe **(adulterated)** if **1 bacteria or colony forming unit** (CFU)/mL *Listeria* is detected.

do not support growth as **adulterated** only if **≥100 CFU**/mL *Listeria* is detected.

- No evidence for growth**/no growth** of pathogens in raw milk at normal levels (typically 1 to 10 CFU/mL)
- If no growth for *Listeria,* **raw milks <100 CFU/mL** could be considered **unadulterated** (acceptable or tolerable or 'safe')

Assumptions and Science for Microbial Dose-Response Assessment

- **Increasing the pathogen dose generally increases**
  - Likelihood, severity, and duration of illness
- **Increasing the pathogen dose may decrease**
  - Incubation period, fraction with asymptomatic illness, time to morbidity or mortality



- **Exposure ≠ illness (or mortality!)**
  - Healthy superorganism defends against many pathogen exposures
- **Low doses may not cause illness**
  - Innate defenses (including **gut microbiota** exerting **colonization resistance**) prevent adherence and growth of low doses of pathogens
- **Low-dose linearity and no threshold assumptions not feasible**

Exposures frequent and asymptomatic for farm families including children, even healthy six-month old baby positive for O157:H7 (Wilson et al., 1996; Karmali et al., 1996; Haack et al., 2003)

Evidence for Thresholds for Human Illness

Healthy people have **innate resistance** to many pathogens **particularly at low doses**.

Salmonellosis cases observed at doses greater than 10^9 or **1,000,000,000** ingested bacteria for *Salmonella* Pullorum

Coleman & Marks, 2000; Coleman et al., 2017

Listeriosis cases not simulated at doses less than 10^4 or **4,000** ingested bacteria for *Listeria monocytogenes*

FDA, 2008

Tularemia cases observed at doses greater than 10^6 or **1 million** ingested bacteria (*Francisella tularensis*)

Thran, 2015

Traditional Dose-Response Assessment for Quantitative Microbial Risk Assessment (QMRA)

Pathogen

- Characterize doses causing no response, asymptomatic infection, illness, or fatalities

Host

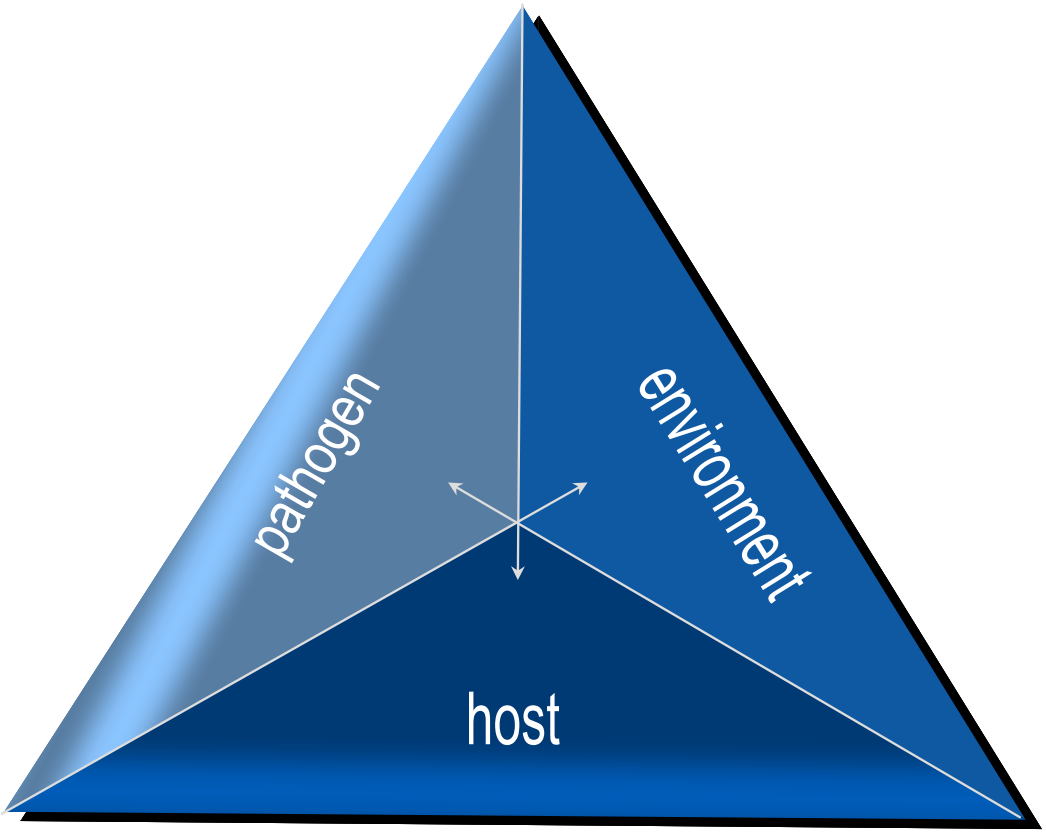
- Characterize dose-response relationships for populations at risk

Environment

- Characterize conditions causing disease

Interactions

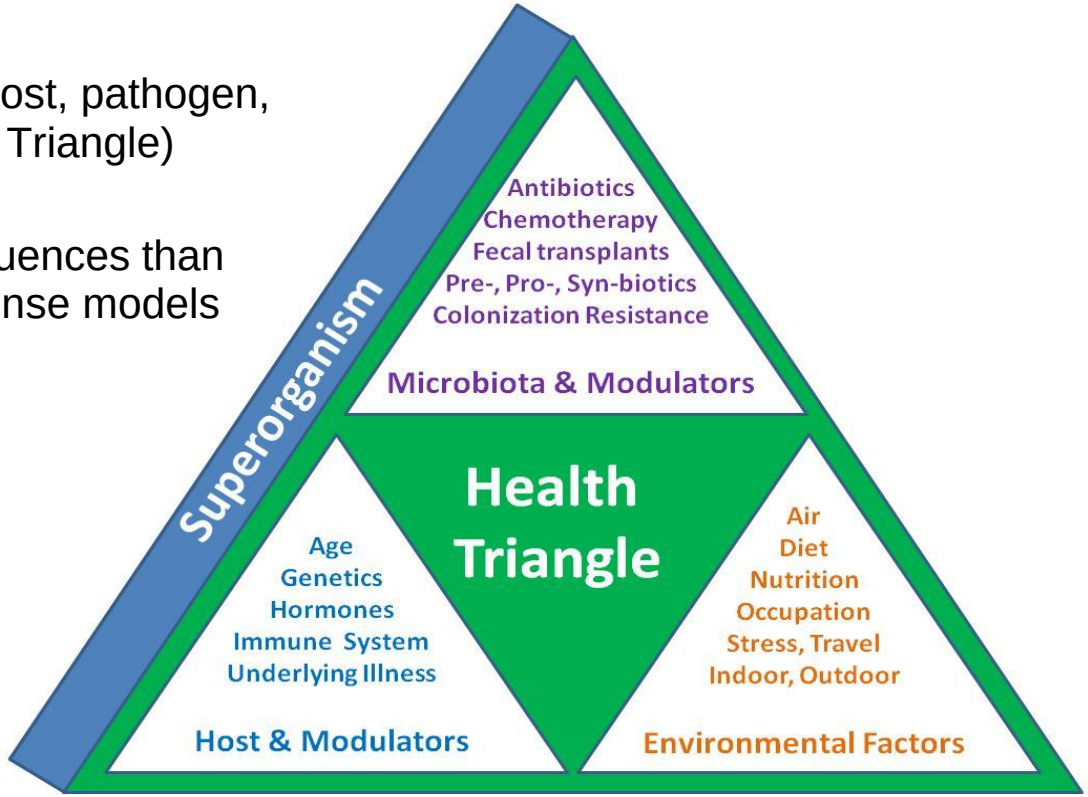
- Characterize conditions favoring sporadic disease and outbreaks



Disease Triangle

Evolution of Dose-Response Assessment in 21st Century

- Acknowledgement of ecosystem effects, superorganism and modulators
- More complex than interactions of host, pathogen, and environmental factors (Disease Triangle)
- Wider context for environmental influences than considered for microbial dose-response models
  - Age
  - Diet
  - Drugs
  - Exercise
  - Immune status
  - Indoor and outdoor environments
  - Occupation
  - Stress
  - Travel



Risks AND Benefits for Vulnerable Population

Human Milk Banks

provide **pasteurized** human donor milk to hospitalized preterm infants and sick/high risk infants

Holder pasteurization (heating to 62.5° C for 30 minutes) is required due to **perception**: possible presence of potential pathogens perceived as **'risky'**

Yet Loss of Benefits for Pasteurized Milks in Clinical Studies around the World!

- **Squires, 2017** : 302 low birth weight infants (US, WA)
- **Cossey** et al., **2013** : 303 very low birth weight infants (Belgium)
- **Strand** et al., **2012** : 335 infants and toddlers (Nepal)
- **Montjaux-Regis** et al., **2011** : 55 premature infants (France)
- **Schanler** et al., **2005** : 243 extremely low birth weight infants (US, TX)
- **Narayanan** et al., **1984** : 226 high risk, low birth weight infants (India)

Synbiotic Benefits Neonates

GUT MICROBIOTA FOR HEALTH
Public information service from European Society of Neurogastroenterology and Motility

http://www.gutmicrobiotaforhealth.com/en/research-practice/

Live *Lactobacillus* probiotic with prebiotic nutrients for optimal growth in GI tract

Panigrahi P, Parida S, Nanda NC, et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature. 2017. doi: 10.1038/nature23480.

Conclusion about Pathogen Presence in Breast Milk and Value for Prediction

‘Results of initial milk cultures **do not predict subsequent culture results**. Random milk cultures, even if obtained at any time during hospitalization, **are not predictive of infection** in premature infants. The sporadic nature of the appearance of certain isolates, however, suggests common exposure of both mother and infant. Routine milk cultures **do not provide sufficient data to be useful in clinical management**.’

Schanler et al., 2011

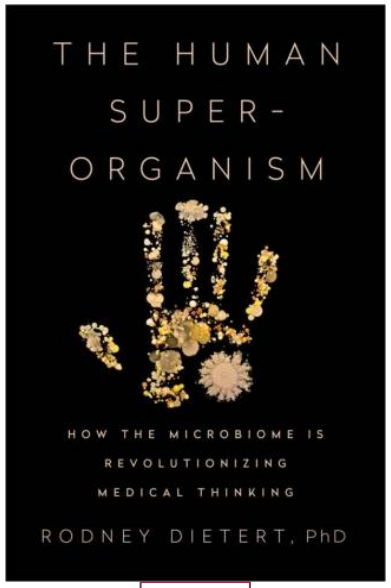
Section 2 Summary

1. Natural microbiota of foods (e.g., breast milk) competes with pathogens, suppresses or eliminates pathogen growth, and reduces pathogen survival under certain conditions
2. Gut microbiota competes with pathogens for space to adhere in the gut and for resources limiting growth important in simulating exposure assessment
3. Advancing knowledge of the gut microbiota challenges common simplifying assumptions for modeling pathogen dose-response relationships

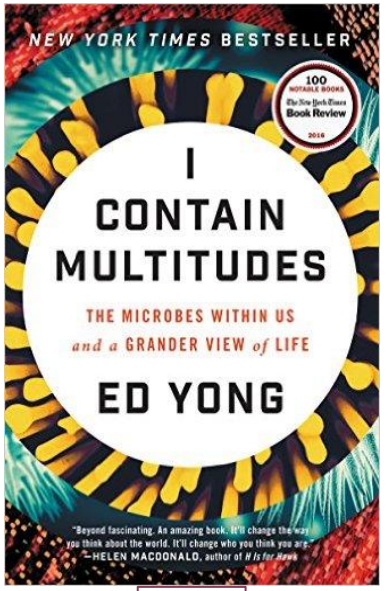
SECTION 3: INTERACTIONS WITH IMMUNOLOGY

Immunology in 21st Century

- **Classical portrait:**
  - Surveillance and destruction of pathogens
- **Emerging insights for 'superorganisms':**
  - 'Microimmunosome' includes dense and diverse microbiota that synergistically and cooperatively protects against pathogens
  - Joint management of our relationships with our resident microbes, particularly at mucosal epithelia
    - Thousands of commensals (Firmicutes, Bacteriodetes, Actinobacteria, Proteobacteria phyla) contribute to mucosal immune homeostasis in the gut
  - Alliances not fixed, but change with context
    - commensals can express mutualism or pathogenicity under certain conditions



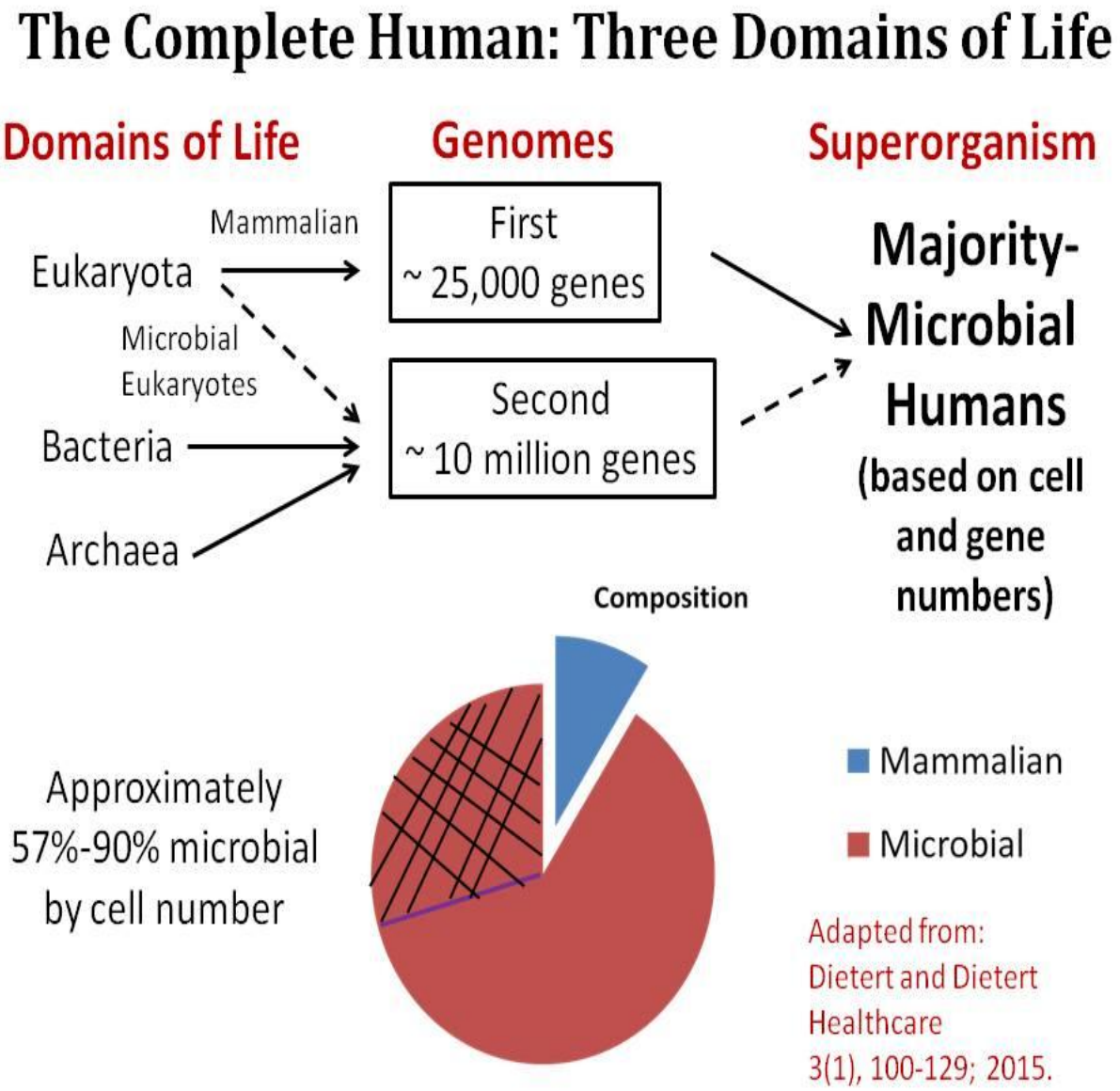
2016



2016

Learnings on Human Superorganism

Rod Dietert, 2017 SRA webinar



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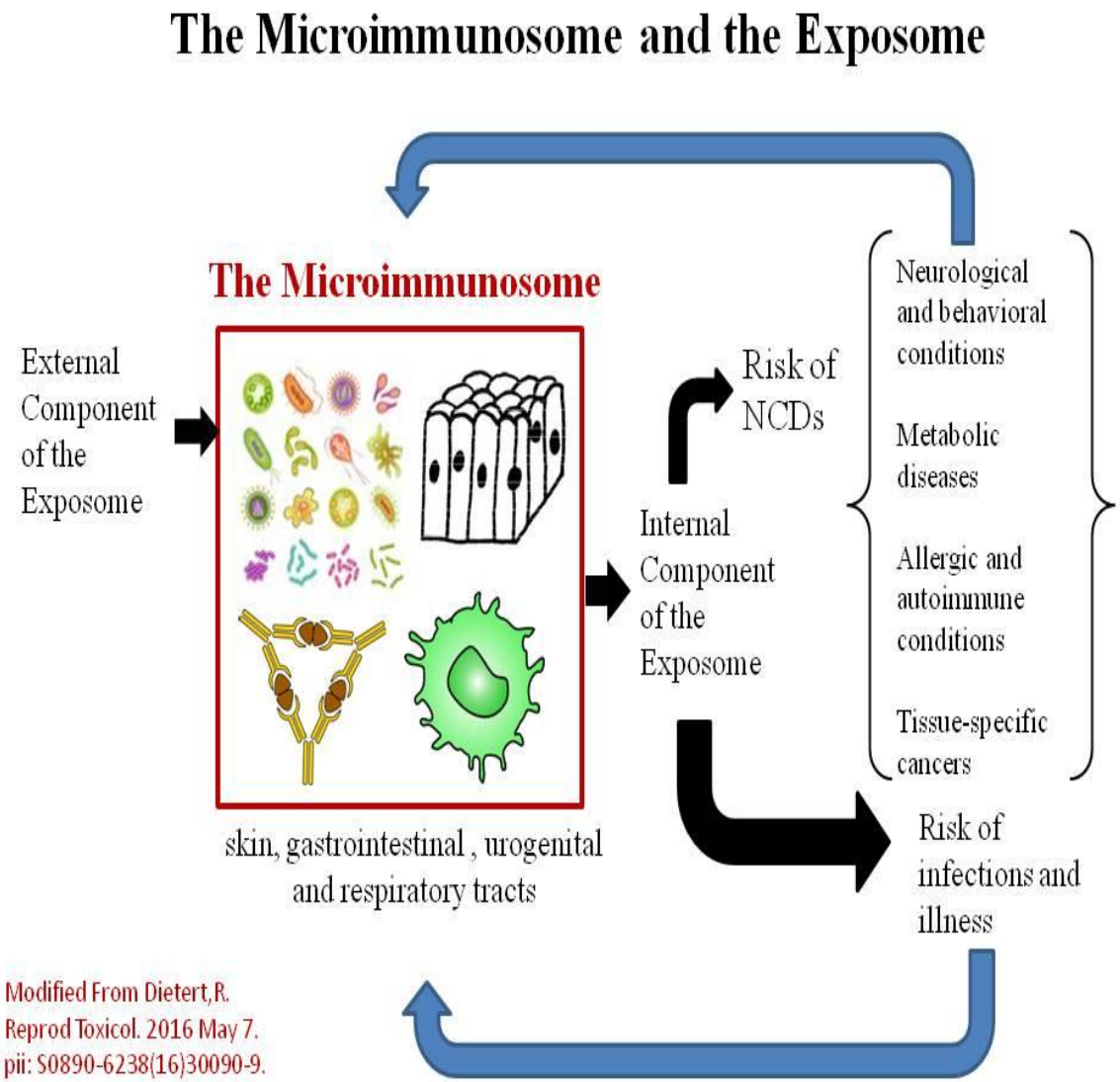


Updated Glossary for Risk Assessors
(Coleman et al., 2018)

- ***Adaptive (acquired) immune system*: Host defenses produced in response to invasion** by specific infectious agents involving humoral immunity with antibodies formed by B-lymphocytes and cell-mediated immunity through T-lymphocytes and activated macrophages.

- ***Innate immune system*:** host defenses always present and effective against low doses of most infectious agents, including: **physical barriers** (e.g., skin and mucous membranes, intestinal barrier function); **complement** and other proteins that mark invaders for phagocytic removal; **natural killer cells**; **phagocytic cells** (macrophages and monocytes, neutrophils); pattern recognition proteins including **Toll Like Receptors** that bind pathogen-/microbe-associated molecular patterns (flagellin, peptidoglycans, lipopolysaccharides) for removal/tolerance; and **washing and enzymatic actions of bodily secretions** (e.g., tears, saliva, gastric juice, bile).

  High doses of pathogens can overwhelm the innate immune system and cause disease in healthy and dysbiotic hosts.

Updated Glossary for Risk Assessors

Evolution Fueling 'Microbiome Revolution'

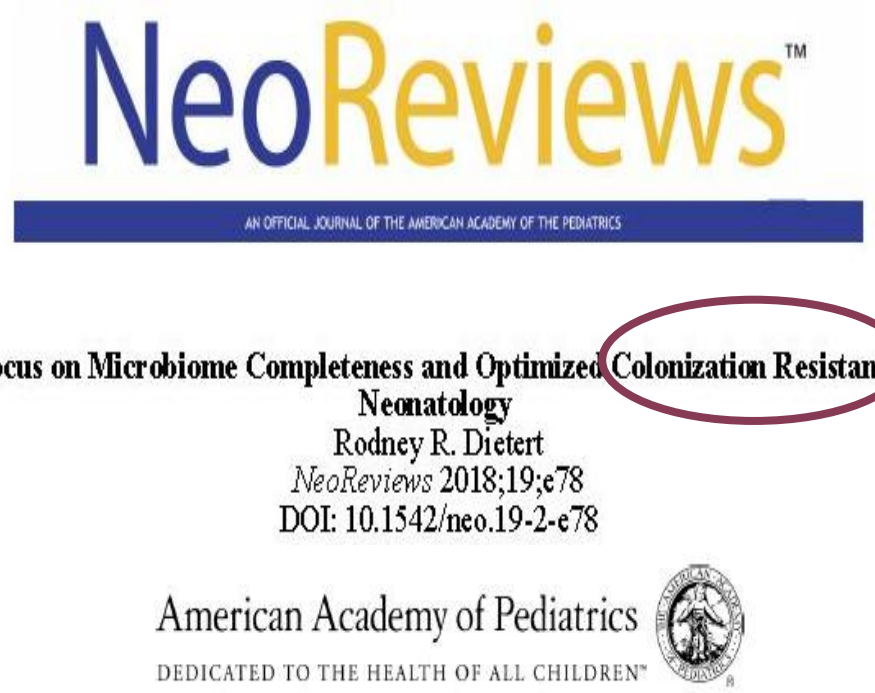
- antibiotic-induced susceptibility in mice treated prior to challenge with doses of *Salmonella* (Bohnhoff et al., 1954; Endt et al., 2010)
- ***colonization resistance*** – protection of hosts with healthy microflora/microbiota against pathogens, with dose- and time-dependencies (Van der Waaij et al., 1971; Brugiroux et al., 2016)
- Human Microbiome Project and Unified Microbiome Initiative beginning in 2007 and 2015, respectively, to study earth's diverse and connected microbial ecosystems
- ***superorganism*** – a hybrid consortium of human and microbial communities that together, synergistically and cooperatively, regulate health and disease (Turnbaugh et al., 2007; Dietert, 2016)

From Coleman et al, October 2018 issue of *Risk Analysis*

Colonization Resistance

Bibliography Available

- Microbiota of healthy people can effectively **inhibit colonization** and **overgrowth** by invading pathogens. First observed in 1954 and termed **colonization resistance** in 1971, current methods of the 21st century are revealing mechanisms.
  - associated with a **stable and diverse gut microbiota** that do not trigger inflammation (**homeostatis**)
  - involves specific interactions between the **immune system** and the **microbiota**



Dysbiosis

- Refers to **microbial imbalance** resulting from a change in the number or types of bacteria on or inside the body.

- Is most prominent in the digestive tract or on the skin, but can also occur on any exposed surface or mucous membrane.

- May have a role in illnesses such as inflammatory bowel disease, chronic fatigue syndrome, obesity, or certain cancers. One cause of dysbiosis is **antibiotic treatment**.

(Glossary of the Gut Microbiome Compiled by The American College of Gastroenterology World Digestive Health Day | May 29, 2014)

Colonization Resistance

Bibliography Available

- Microbiota of healthy people can effectively **inhibit colonization** and **overgrowth** by invading pathogens. This phenomenon was first documented in **1954** and termed **colonization resistance** in **1971**.
- **Colonization Resistance**
  - is associated with a **stable and diverse gut microbiota** that do not trigger inflammation (**homeostatis**)
  - involves specific interactions between the **immune system** and the **microbiota**
  - is characterized mechanistically by rapidly expanding body of evidence

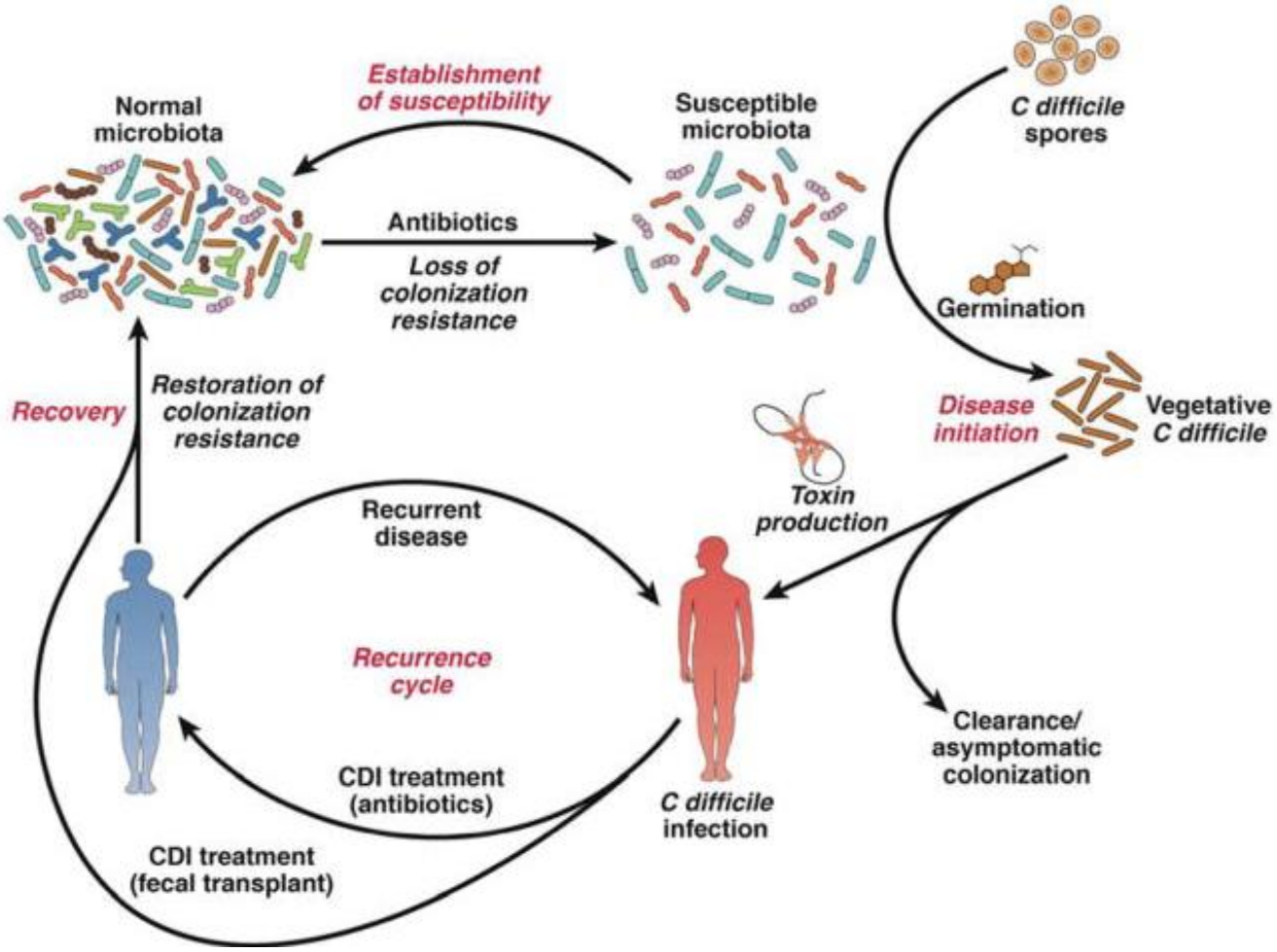
Bohnhoff et al.,1954; Van der Waaij et al.,1971; Barza et al.,1987; Lawley & Walker, 2013; Newton et al., 2013; Gahan and Hill, 2014; Pham and Lawley, 2014; Malys et al., 2015; Perez-Cobas et al., 2015; Sassone-Corsi and Raffatellu, 2015; Sassone-Corsi and Raffatellu, 2015; Stecher, 2015; Brugiroux et al., 2016; Zipperer et al., 2016; Isaac et al., 2017

Colonization Resistance and *Clostridium difficile*

Major goals:

- build a **shared understanding** of microbial benefits and risks with stakeholders (**21st century science**);

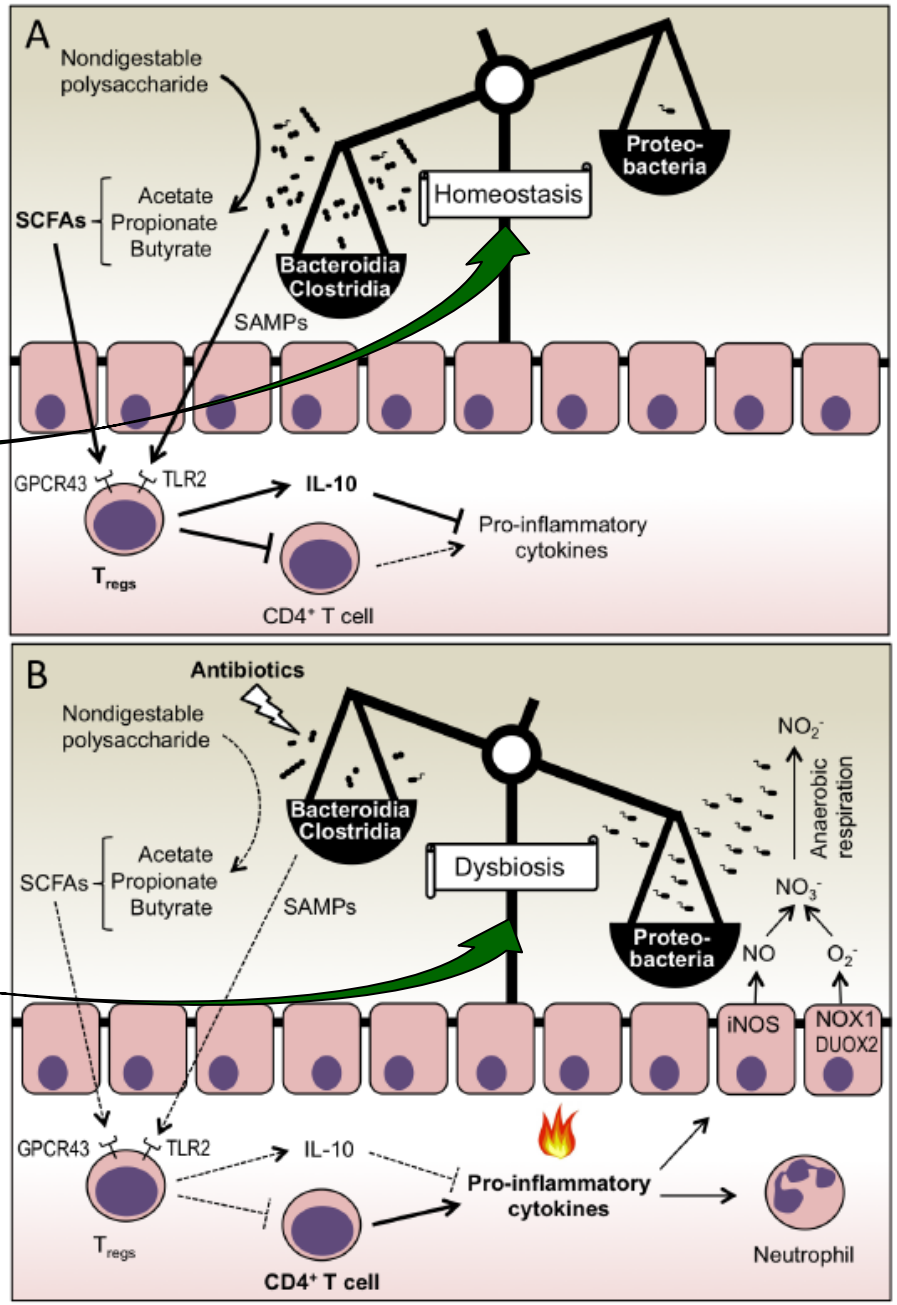
- facilitate a **paradigm shift** for an expanded framework for microbial benefit-risk assessment that incorporates the '**superorganism**', food microbiota, and their roles in contributing to health and disease.



Britton and Young Gastroenterology, 2014; Dietert, 2017, 2018

Colonization Resistance in Homeostatis,

Disrupted in Dysbiosis

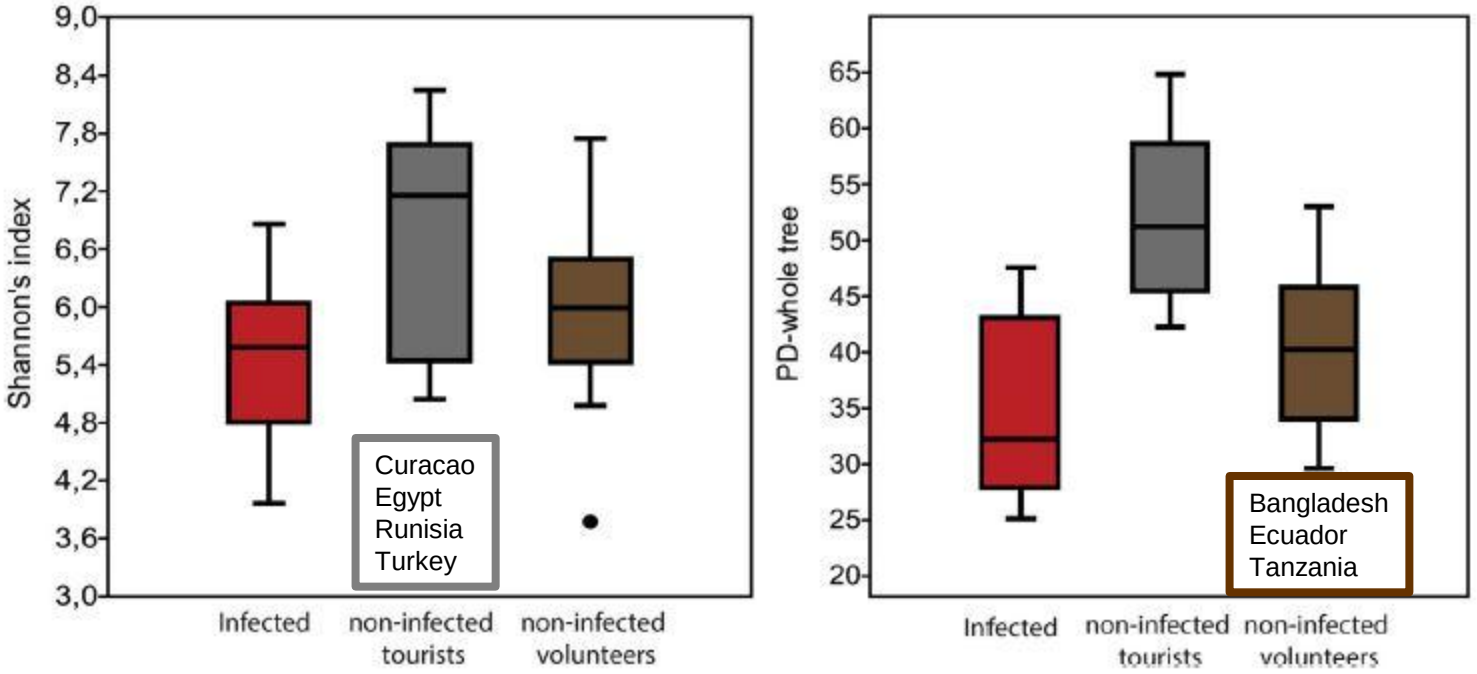


Additional Mechanistic Examples of Colonization Resistance:

- Lawley and Walker, 2012
- Masanta et al., 2013
- Ostaff et al., 2013
- Pham and Lawley, 2014

(Spees et al., 2013)

Colonization Resistance to *Campylobacter* with Increased Microbiota Diversity



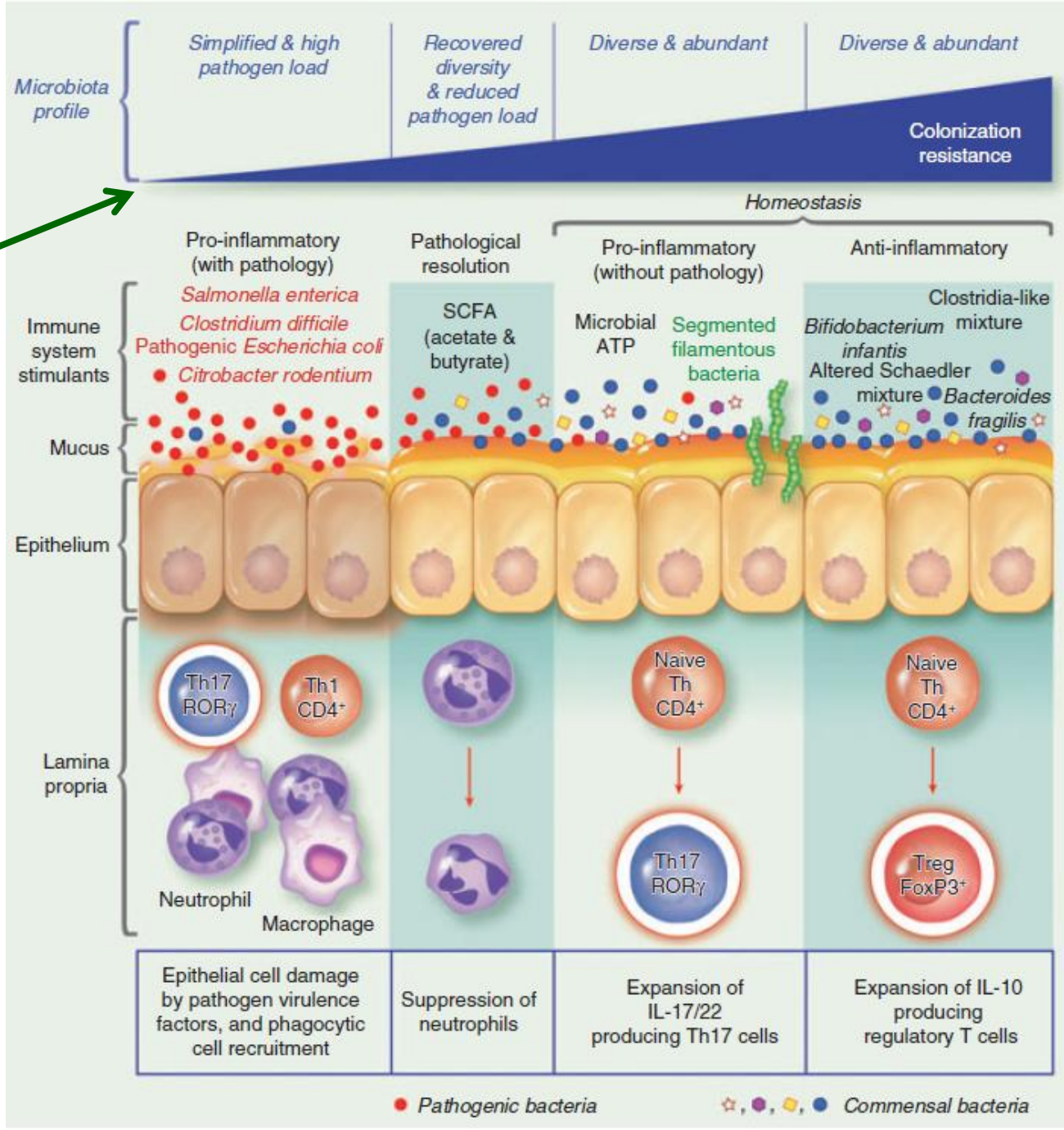
Healthy tourists 18-64 years of age who traveled in groups from Sweden and acquired travelers' diarrhea

Composition of human faecal microbiota in resistance to *Campylobacter* infection

C. Kampmann[1,2,3], J. Dicksved[2,4], L. Engstrand[5,6] and H. Rautelin[2,7]

2016

Microbiology and Immunology of Colonization Resistance

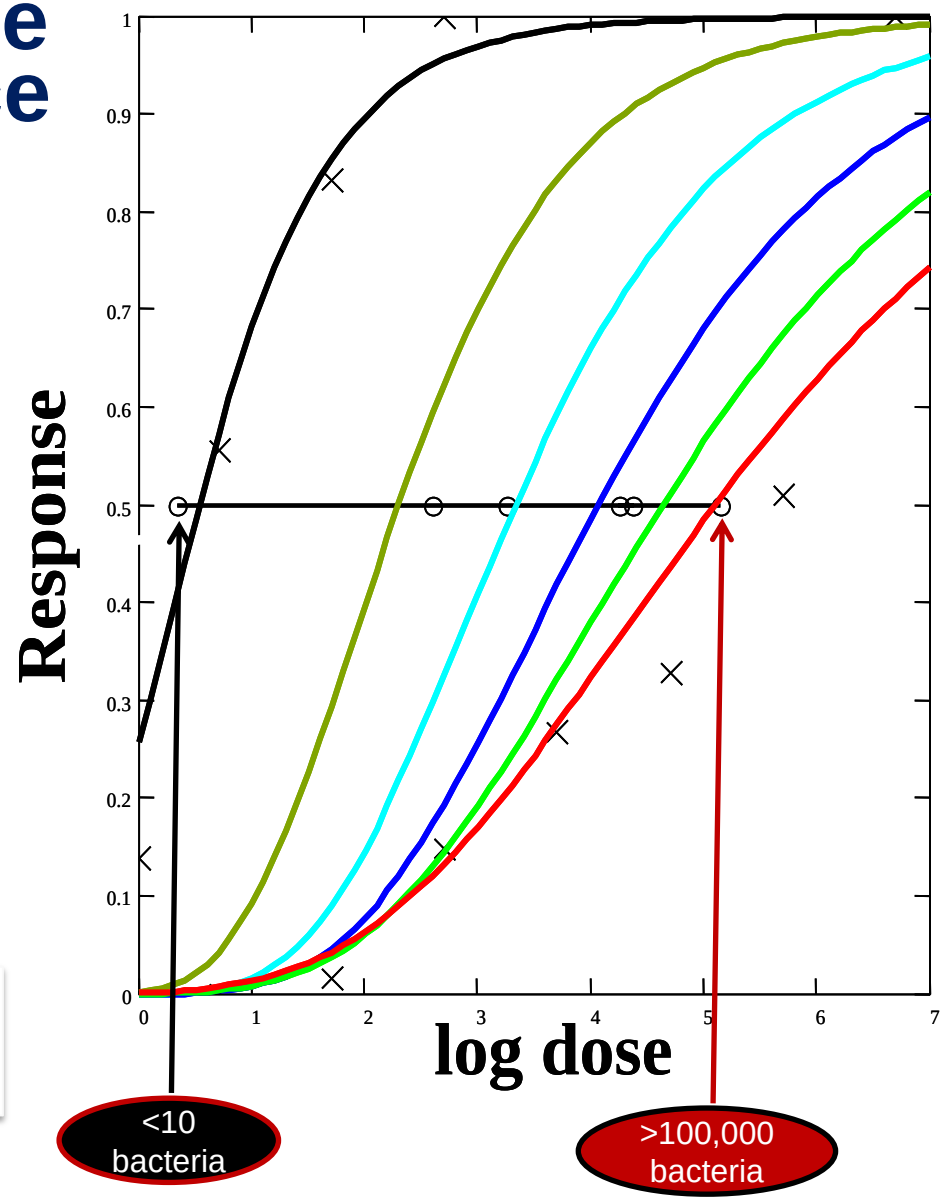


Lawley and Walker, 2012

Colonization Resistance to Salmonellosis in Mice

- **Normal** animal **challenges** with increasing doses of *Salmonella enteritidis* (**red line**)
- **Antibiotic** 1 day before challenge disrupts colonization resistance and increases susceptibility (**black line**)
- **Microbiota recovers** within 5 days (**bright green line**) to normal magnitude of colonization resistance

Host susceptibility increases five orders of magnitude!

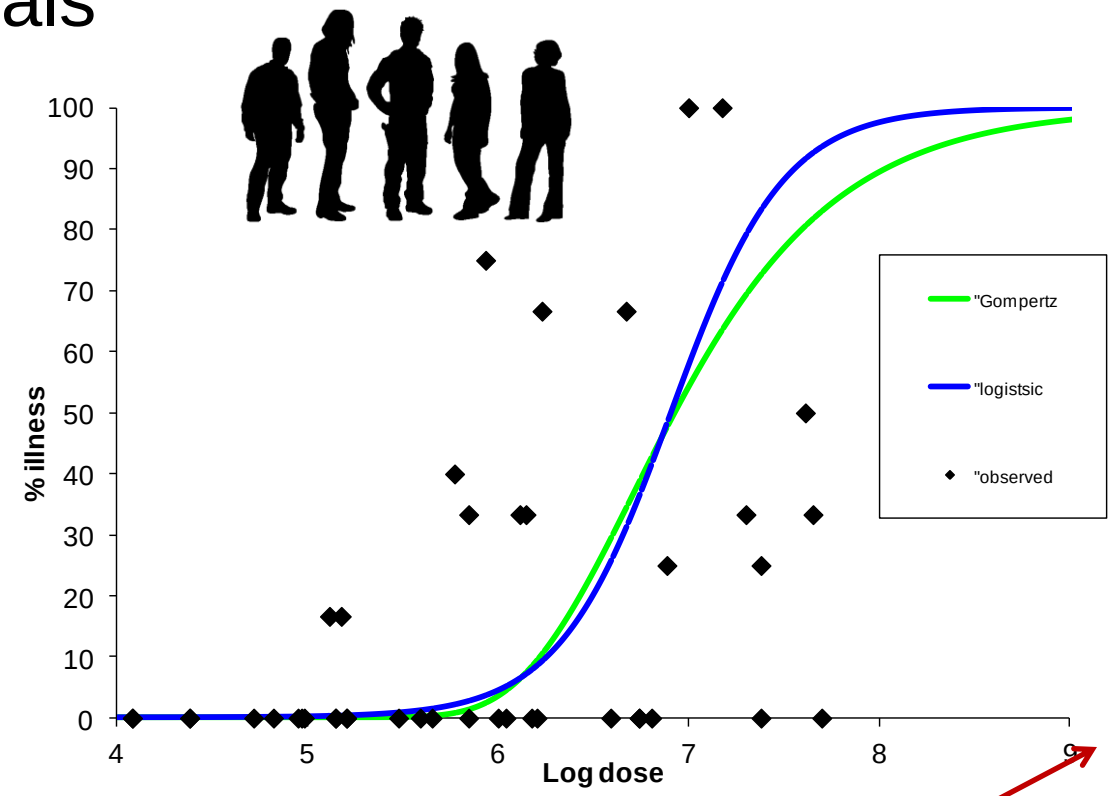


(Coleman and Marks,1999, 2000; Coleman et al., 2017)

Salmonella Strains Administered to Humans

Human clinical trials

- anatum
- bareilly
- derby
- meleagridis
- newport

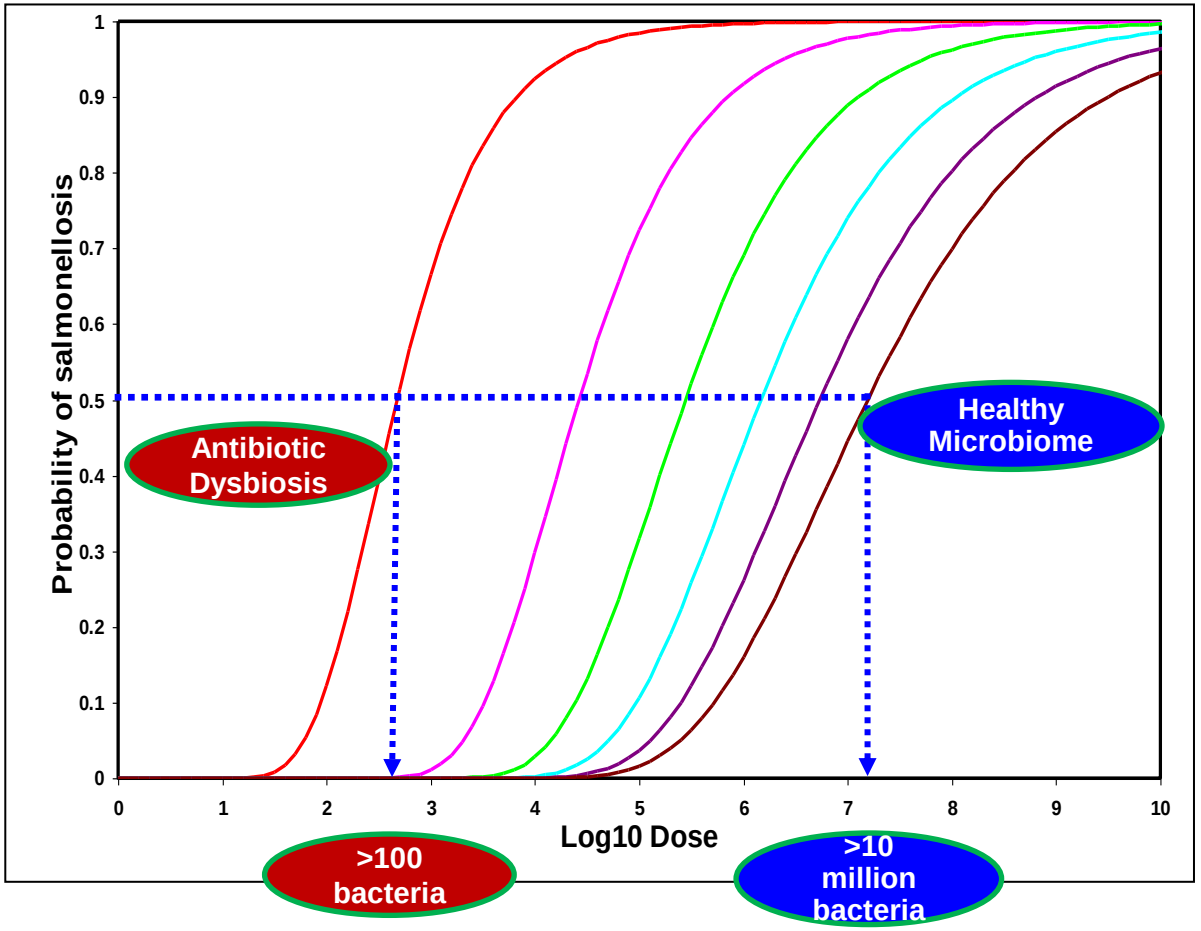


- **pullorum** (statistically significant threshold at 10^9 bacteria)

(Coleman and Marks, 1998; Coleman et al., 2017)

Increased Susceptibility for Antibiotic-Induced Loss of Colonization Resistance

- Half of healthy volunteers ill after dosing with ~10^7 (ten million) *Salmonella* bacteria (**brown line**)
- Half of volunteers with antibiotic dysbiosis likely ill after dosing with **~10^2** (>100) (**red line**)
- Microbiota recovers over time (2 days, pink line; 3 days, green, 4 days, aqua line, 5 days navy line)
- Indirect evidence of **10^5** magnitude of **colonization resistance** (mouse and human data)



(Coleman and Marks, 1999; Coleman et al., 2017)

Opportunistic Pathogens

Can cause nosocomial (hospital-acquired) infections, serious infections in neonates and immunocompromised people, and those on ventilators and other medical devices, with wounds, and with antibiotic-disrupted microbiomes.

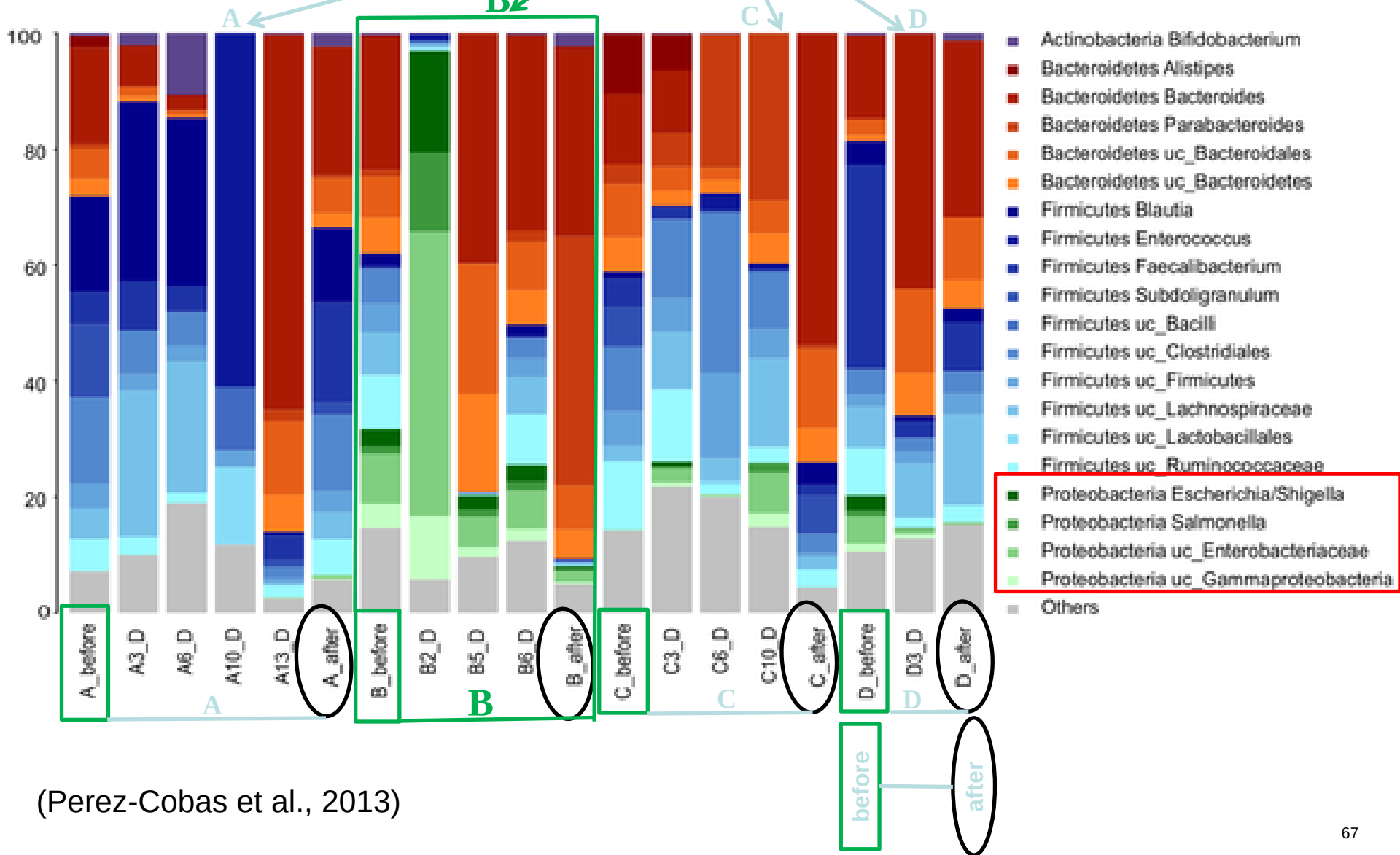
Examples in the news:

- ***Clostridium difficile***
- *Ebola, Zika viruses*
- *Enterococcus*
- *Escherichia coli*
- *Mycobacterium tuberculosis*
- *Pseudomonas*
- *Staphylococcus aureus*
- *Streptococcus*

Antibiotics Shift Gut Microbiota in Four Volunteers

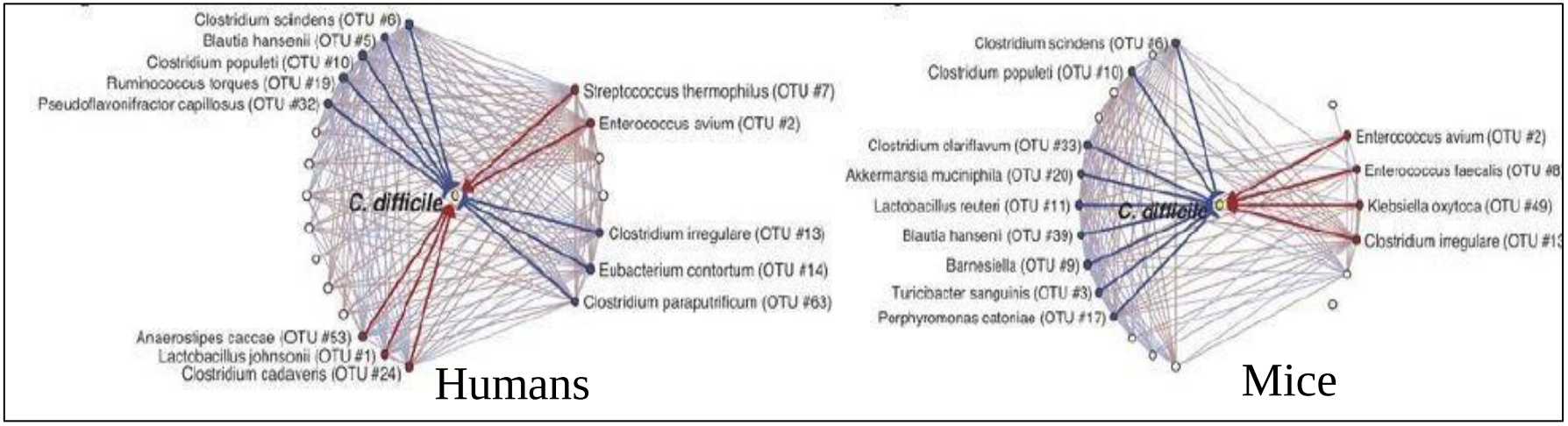
DYSBIOSIS

disruption of the indigenous gut microbiota; factors include antibiotic administration, infection, malnutrition, stress, changes in diet, and travel



(Perez-Cobas et al., 2013)

Resistance and Susceptibility to *C. difficile*



Similar patterns from inference modelling of subnetworks of metageomic data.

- **Blue lines** mark **resident** microbiota predicted to inhibit *C. difficile* growth blooms in **healthy hosts.**

- **Red lines** mark **dysbiotic microbiota** predicted to promote *C. difficile* growth blooms in **immunocompromised hosts**.

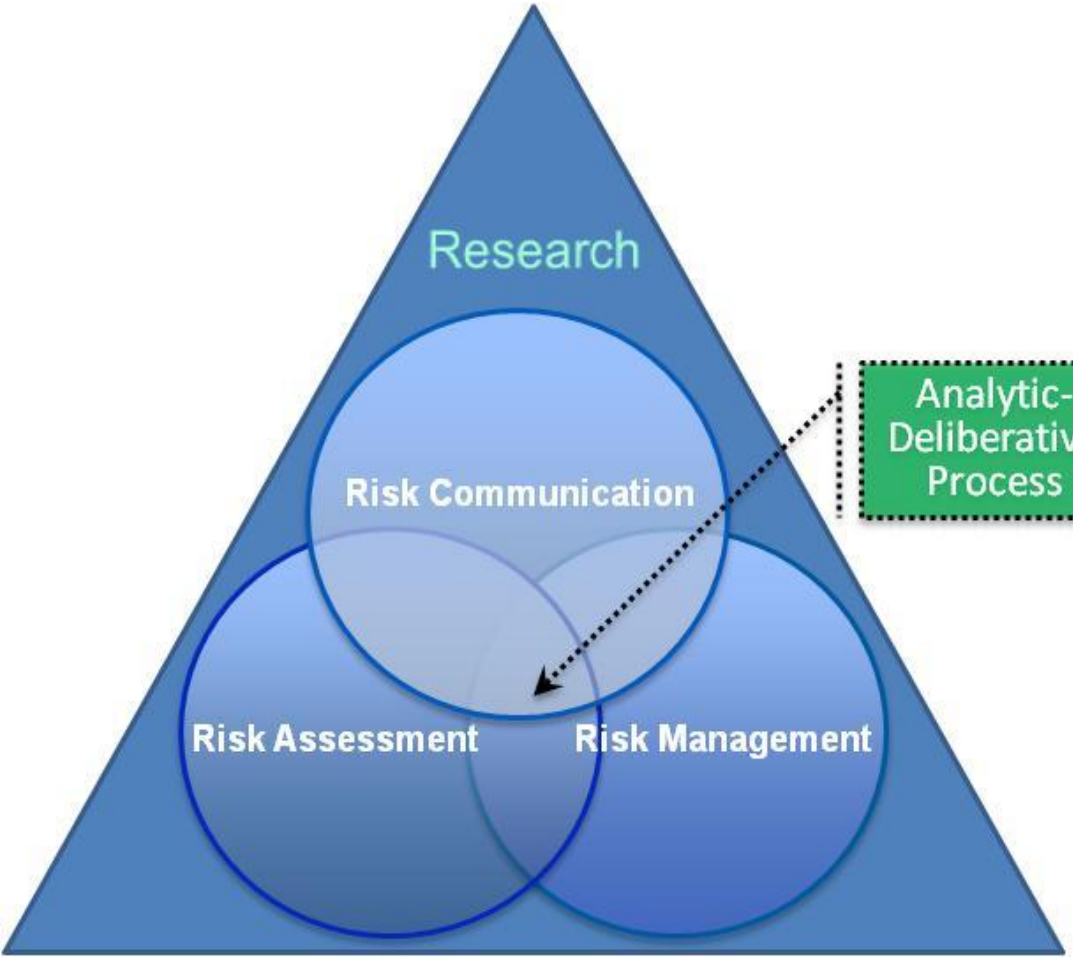
Buffie et al., 2015

Section 3 Summary

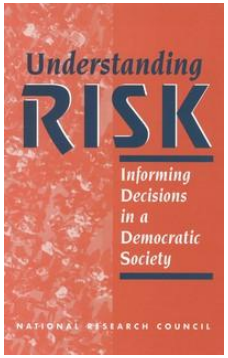
1. The gut microbiota of healthy superorganisms trains and maintains balanced immune systems and provides colonization resistance, innate protection against pathogens under normal conditions.
2. Antibiotic administration disrupts the healthy microbiome, causing dysbiosis that increases susceptibility to many pathogens and left-shifts dose-response curves.
3. Microbiome studies building on traditional microbial ecology (Lotka Volterra equations) reveal groups of microbes in humans and mice associated with resistance and susceptibility to *Clostridium difficile* and other pathogens.

SECTION 4: FUTURE FOR MICROBIAL RISK ANALYSIS

21st Century Science Reveals Risks & Benefits to Microbes, including Pathogens



Analytic-Deliberative Process



Builds in cycles of **research, analysis**, **deliberation**, and **interpretation** with stakeholders on

- **what goes in** (data, assumptions)

and

- **what comes out** of risk models (**estimates of risk, uncertainty**).

When perceptions of risk don't match up, need analytic-deliberative process.

Context for Analytic-Deliberative Process

Problem: conflicting risk perceptions lack transparency about the supporting scientific evidence, impeding respectful collaborative public discourse and misleading consumers.

Desired Outcome: develop reasoned, coherent, science-based regulations, policies, and communications about benefits and risks

Barriers:

- **Outdated QMRA paradigms** exclude significant 21st century advances in scientific knowledge about the human 'superorganism' and food microbiota that influence health and disease
- **Debates fueled rather than resolved conflict** because debaters reasoned from selected studies and not the full body of scientific evidence
- **Fear of microbes as germs that will kill us (germophobia)**

Download this and other risk books FREE from National Academies Press (https://nap.edu/)!

Joint SRA Project
(2017-2019)

http://www.sra.org/upstateny/

Upstate NY SRA is leading a joint project on the microbiota of milks designed to overcome global and local barriers for consumers

- engage **diverse stakeholders** (researchers, risk assessors, risk communicators, risk managers, breast milk advocates, consumers seeking whole foods, small farmers);

- describe the **full body of evidence**, including 21st century data on microbiomes, partnering with Professor Rod Dietert from Cornell University;

- conduct **balanced objective analyses** incorporating the human superorganism into analysis of benefits and risks to consumers; and

- deliberate findings and knowledge gaps for assessing magnitude of **benefits and risks**.

Section 4 Summary

1. Including 21st century science from microbiome studies in Next Generation Quantitative Microbial Risk Assessment methodology could improve the balance for risks and benefits associated with microbes
2. Risk practitioners (Society for Risk Analysis, National Academies of Science) acknowledge the need for investment in analytic-deliberative processes with stakeholders when perceptions and estimates of risks and benefits don't add up for controversial global problems
3. Students, faculty, and others seeking the stimulation of interdisciplinary collaborations on risks and benefits can join SRA and its Regional Organizations, including Upstate NY SRA

Upstate New York SRA

SRA

Society for Risk Analysis

http://www.sra.org/upstateny/

Questions?

Connect on social media:

COLEMAN SCIENTIFIC CONSULTING

Science and Raw Milk

Email: peg@colemanscientific.org

Backup Slides

Risk Management Solution: Remove HIGH CONCENTRATIONS of Environmental Microbes

Source: *Vibrio cholerae*-contaminated drinking water near cesspits in industrialized 19th century London

Simple Solution:

John Snow advised **removing the handle** on London water pump **near cesspit** that caused clusters of fatal cholera cases

- Removed **highly contaminated water** from drinking supply
- Scientific knowledge of **importance of sanitation** informed control of outbreaks

Sherman, 2007; http://www.csiss.org/classics/content/8

Risk Management Solution: Remove HIGH CONCENTRATIONS of Environmental Microbes

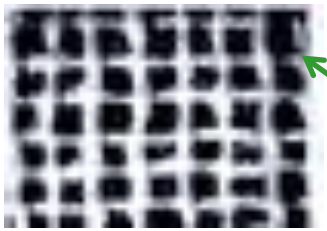
Source: *Vibrio cholerae*-contaminated surface waters in 21st century developing countries

Simple Solution:

Rita Colwell & Anwar Huq trained villagers to filter river water with common **cloth** (sari cloth)

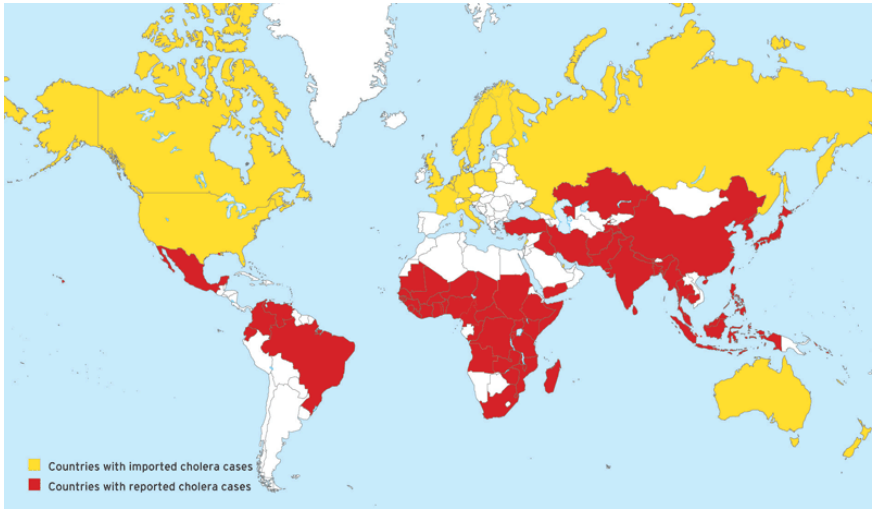
- Removed copepods that **concentrate** bacteria to **high doses**
- Scientific knowledge of **ecological link** of **copepods** and **cholera outbreaks** informed solution

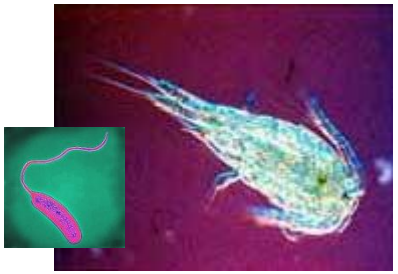
Colwell et al., 2003; Huq et al., 2005



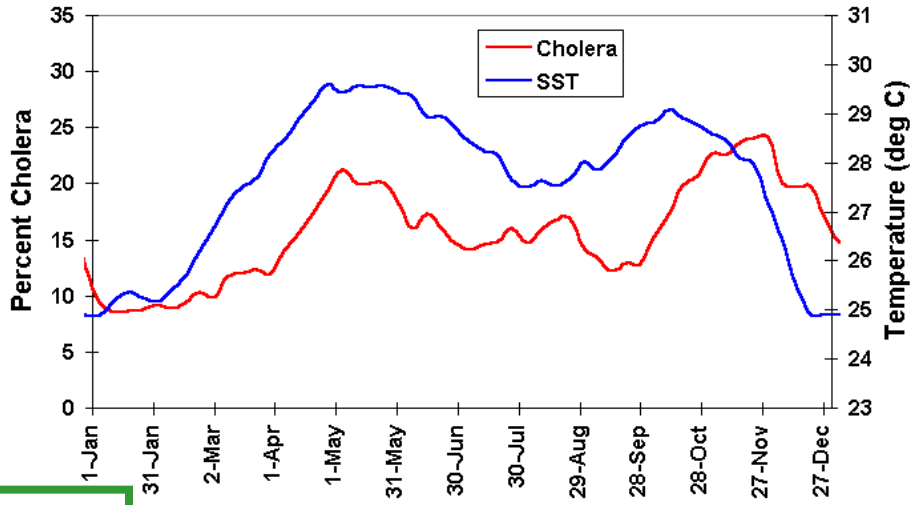
cloth filters **copopods,** NOT *Vibrio* bacteria

Remote Sensing as Predictive Tool for Bacteria in Surface Waters???





- Surveillance at microscopic level **unnecessary** for cholera
- Remote sensing of **plankton blooms** and **water temperature** sufficient due to concentration of *V. cholerae* in copepods



Knowledge of ecosystem interactions enables testing at resolution appropriate to protect health

Microbiomes of Natural and Built Environments: Subways

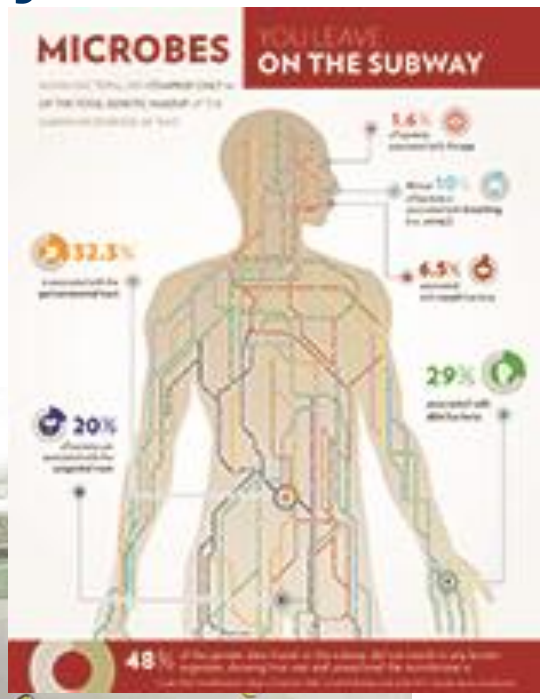
Science News *from research organizations*

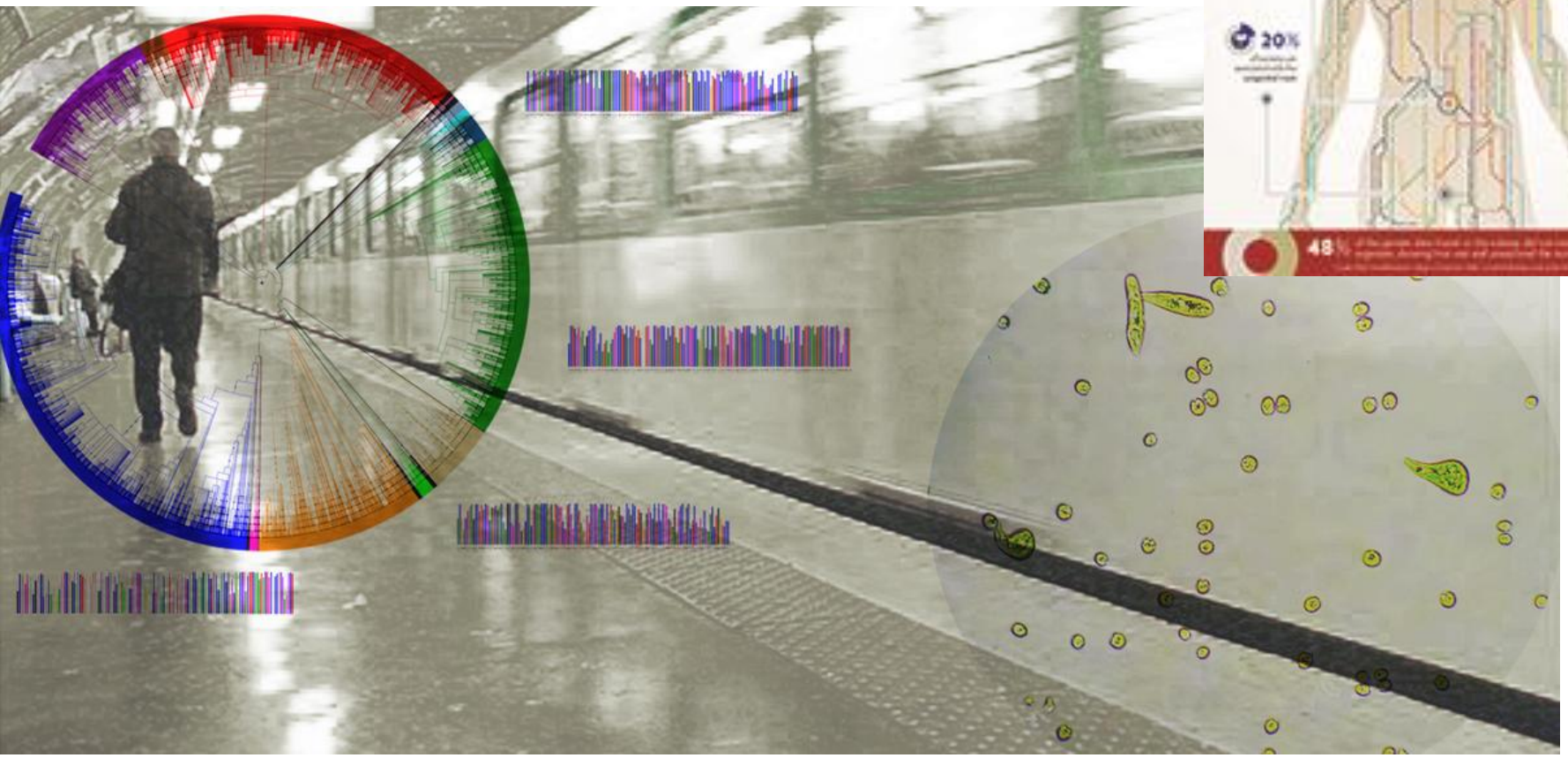
Mapping the subway's microbiome

Date: June 21, 2016

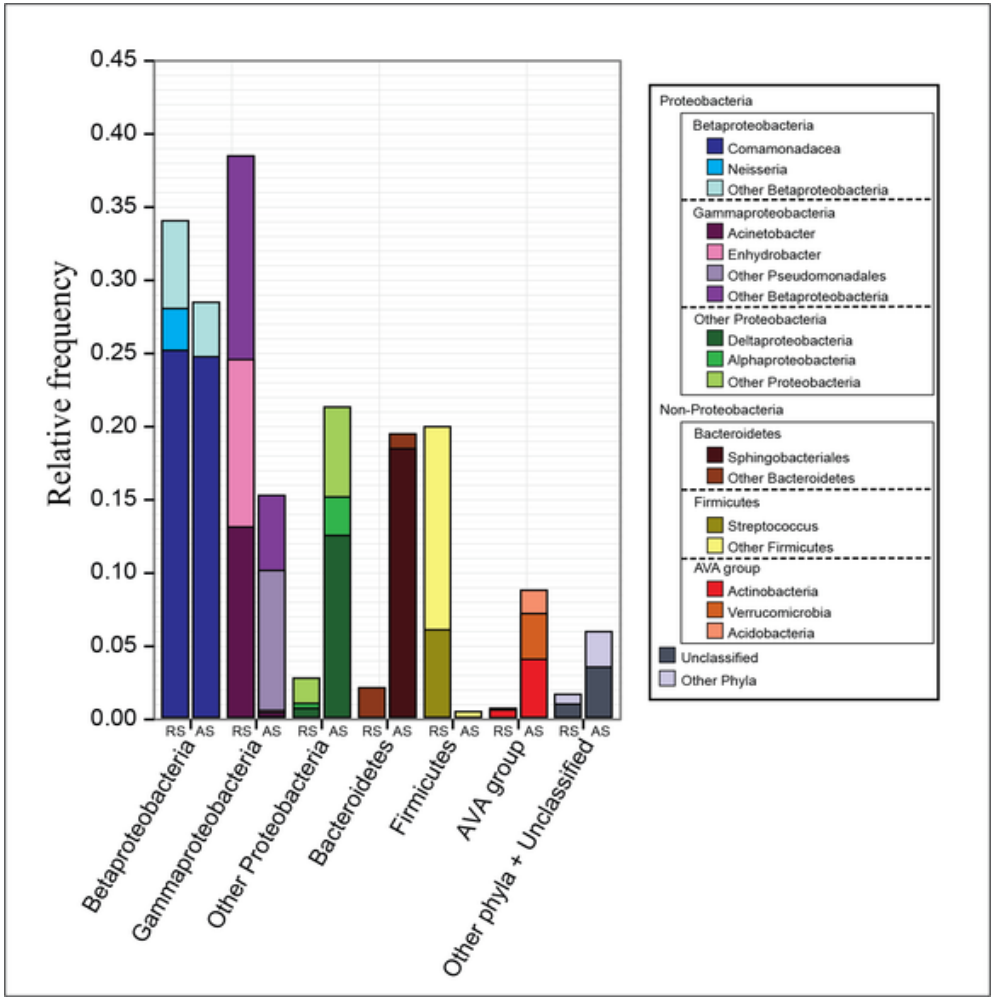
Source: Centre for Genomic Regulation

Summary: Researchers aims to map the microbiome of public transit systems in 54 cities worldwide, including New York, Hong Kong, Paris or Sydney.





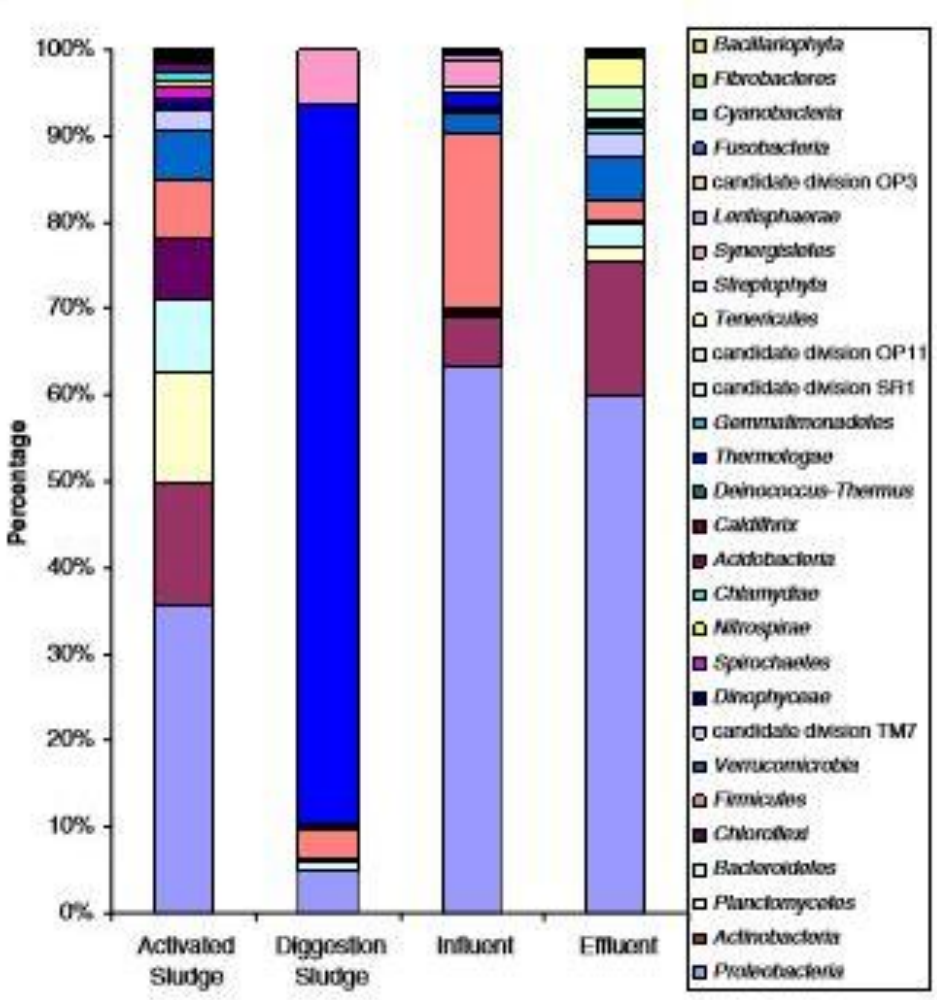
Microbiomes of Natural and Built Environments: Sewage Treatment



Paiva MC, Ávila MP, Reis MP, Costa PS, Nardi RMD, et al. (2015) The Microbiota and Abundance of the Class 1 Integron-Integrase Gene in Tropical Sewage Treatment Plant Influent and Activated Sludge. PLOS ONE 10(6): e0131532. doi:10.1371/journal.pone.0131532
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0131532

PLOS ONE | TENTH ANNIVERSARY

Microbiomes of Wastewaters



Bacterial Communities in Different Sections of a Municipal Wastewater Treatment Plant Revealed by 16S rDNA 454 Pyrosequencing. (Ye and Zhang, 2013)

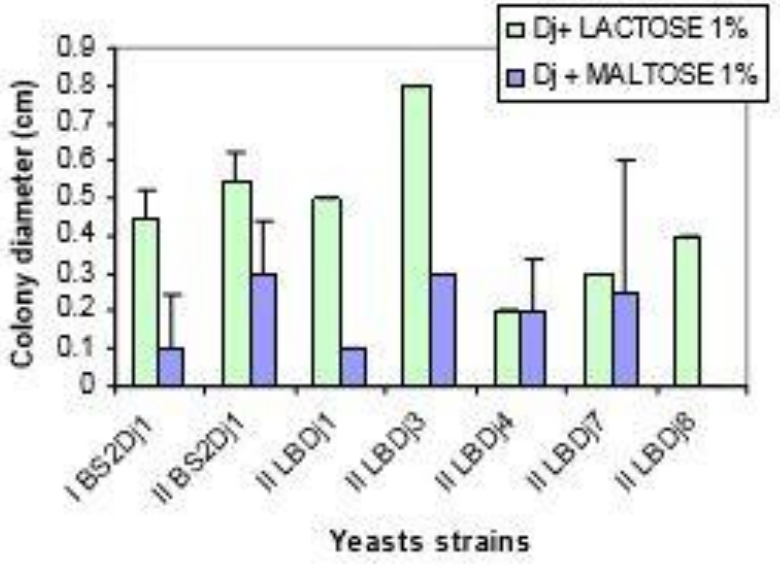
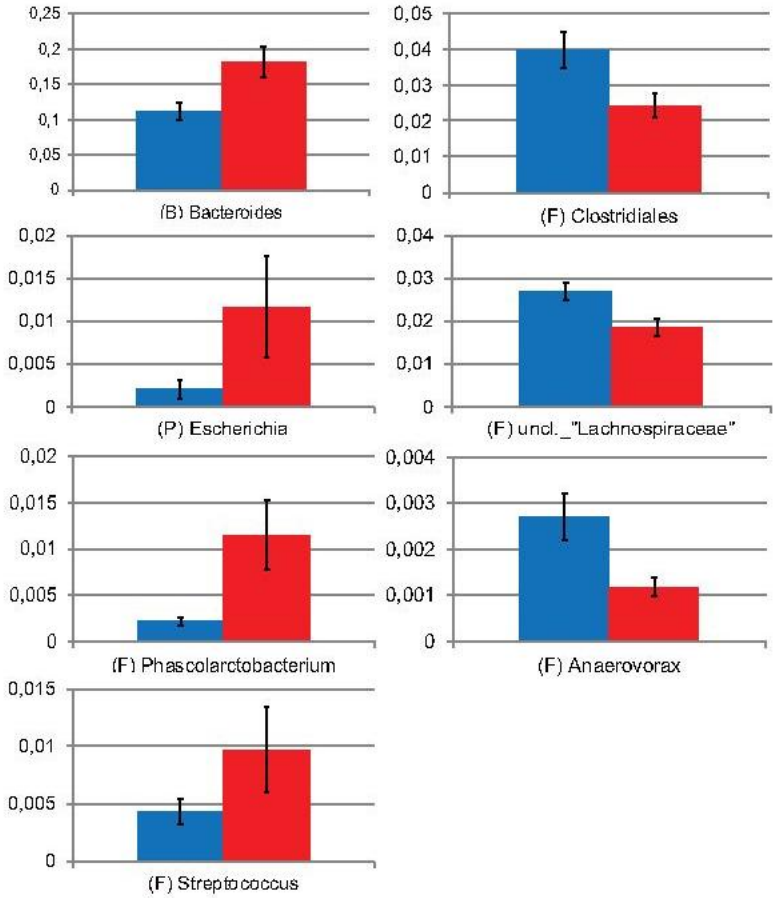


Figure 7. Food industry wastewater microbiota capacity to metabolise the simple glucides

Microbiological and Biochemical Characterisation of Dairy and Brewery Wastewater Microbiota (Palela, Ifrim and Bahrim, 2015)

Microbiomes of Workers in Built Environments: Poultry Abattoirs

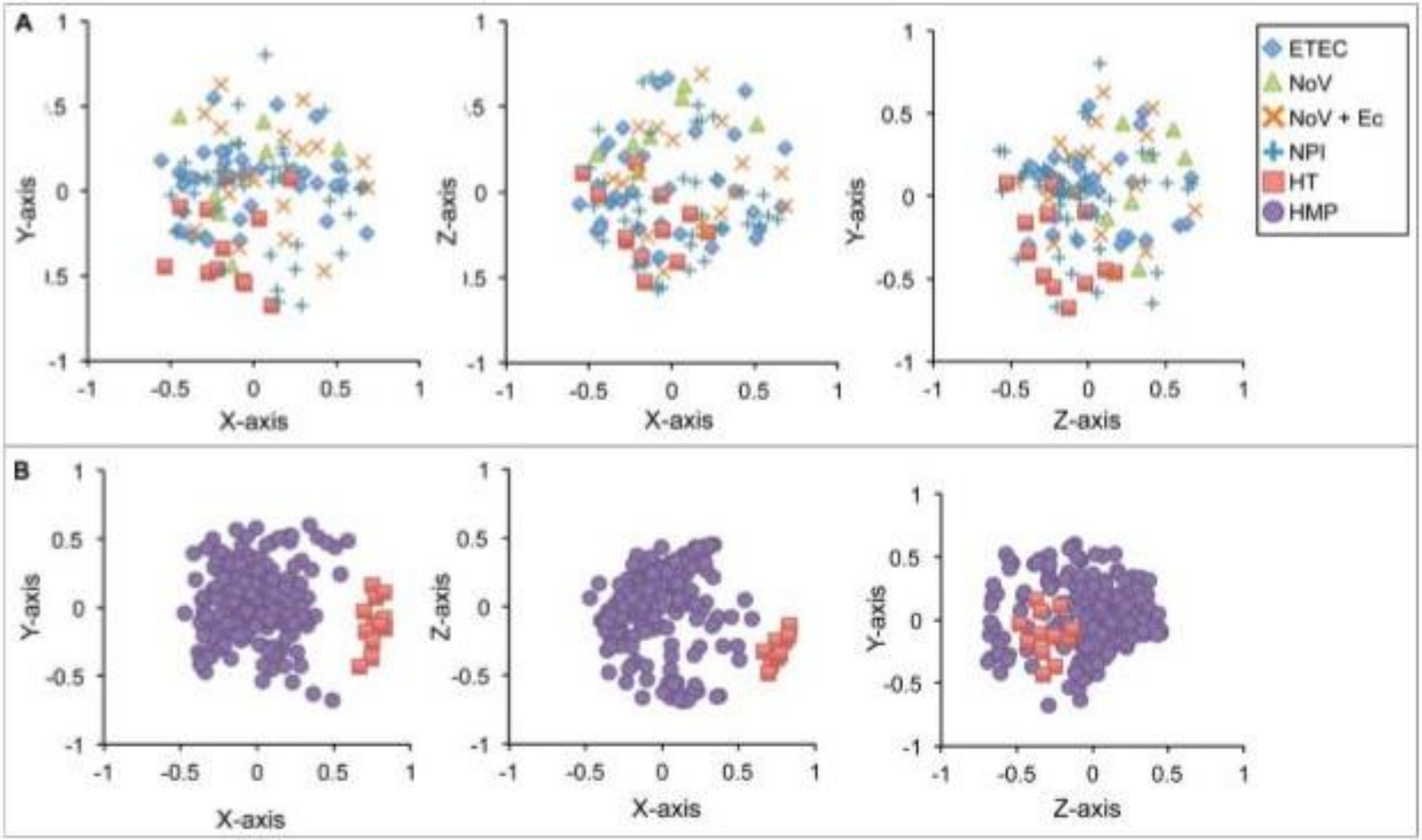
- Gut microbiota (N=24 poultry abattoir workers) monitored during peak *Campylobacter* exposures (all likely exposed)
- Culture-positive for pathogen (**red**)
  6 of 7 asymptomatic, significant long-term changes in gut microbiome
- Culture-negative for pathogen (**blue**)
  - significantly lower abundance of *Bacteriodes, Escherichia, Phascolarctobacterium,* and *Streptococcus*
  - significantly higher abundance *Clostridiales, Lachnospiraceae,* and *Anaerovorax*
- Frequent exposures can provide **colonization resistance** and protection from illness



Susceptibility to *Campylobacter* Infection Is Associated with the Species Composition of the Human Fecal Microbiota

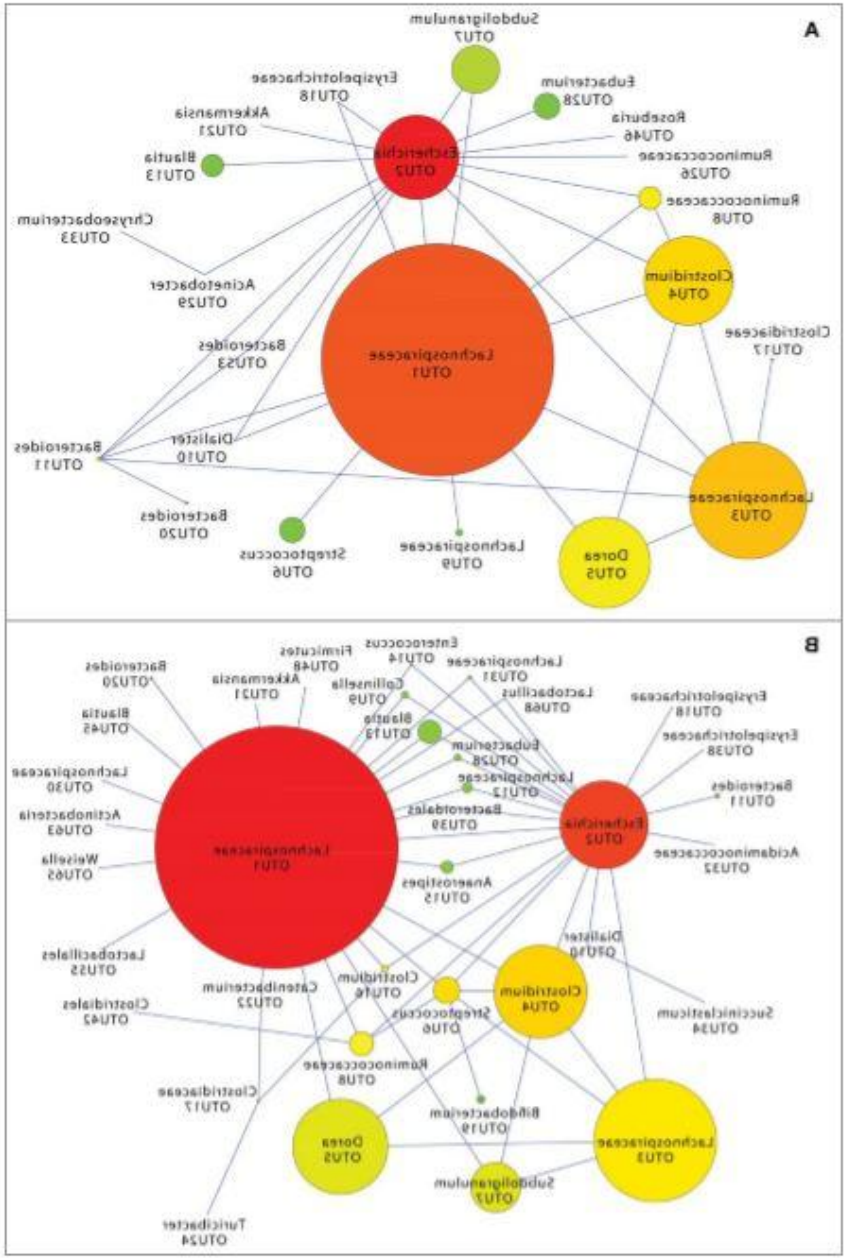
Johan Dicksved,[a,b] Patrik Ellström,[a] Lars Engstrand,[c] Hilpi Rautelin[a,d] 2014

Community Structures: Healthy and Diseased



Youmans et al., 2015. Characterization of the human gut microbiome during travelers' diarrhea

Community Structures: Healthy and Diseased



Characterization of the human gut microbiome during travelers' diarrhea

Bonnie P Youmans[1,#], Nadim J Ajami[1,2], Zhi-Dong Jiang[3], Frederick Campbell[4], W Duncan Wadsworth[4], Joseph F Petrosino[1,2], Herbert L DuPont[3,5,6,7], and Sarah K Highlander[8,*] 2015

Childhood and Travelers' Diarrhea in Developing Countries

Bacterial Pathogens:

- *Campylobacter*
- Entero-Toxigenic *E. coli* (ETEC)
- Entero-Pathogenic *E. coli* (EPEC)
- *Salmonella*
- *Shigella*
- *Vibrio*

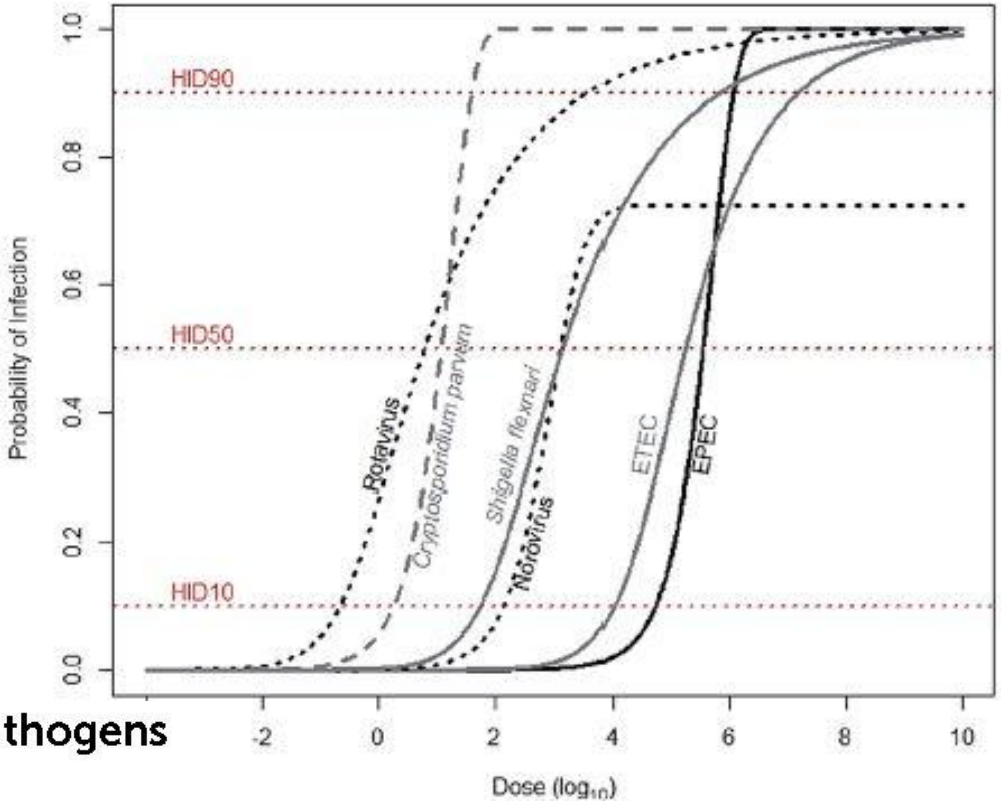
Viral Pathogens:

- Norovirus, Rotavirus

Adults exposed to endemic pathogens develop resistance, but children and travelers susceptible.

Childhood Diarrheal Morbidity (Mortality)

	Effective Dose$_{50}$
1. ETEC	10^5 to 10^8 bacteria
2. EPEC	10^5 to 10^7 bacteria
3. Shigella	10^3 bacteria



Environmental transmission of diarrheal pathogens in low and middle income countries

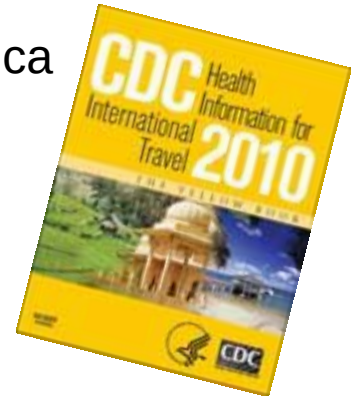
Timothy R. Julian 2017

Clinical trial with probiotic *E. coli* Nissle 1917 yielded significant reduction in childhood diarrhea duration (Henker et al., 2008)

Predicting and Preventing Travelers' Diarrhea

International travelers to high and intermediate risk countries can be exposed to less controlled sanitation (more frequent and higher levels of contamination) in food and water that have caused 40-60% to contract travelers' diarrhea.

- **High risk**: Asia, Middle East, Africa, Mexico, Central/South America
- **Intermediate risk**: E Europe, S Africa, Caribbean islands
- **Low risk**: US, Canada, Australia, New Zealand, N/W EU



Resistant superorganisms?

The Traveling Microbiome

Mark S. Riddle[1] · Bradley A. Connor 2016

Volunteers Administered *Campylobacter*: Study Host Risk Factors for Travelers' Diarrhea

Innate immunity for some volunteers after one high dose (10^9 or 1 billion bacteria)

Immunity from previous exposures

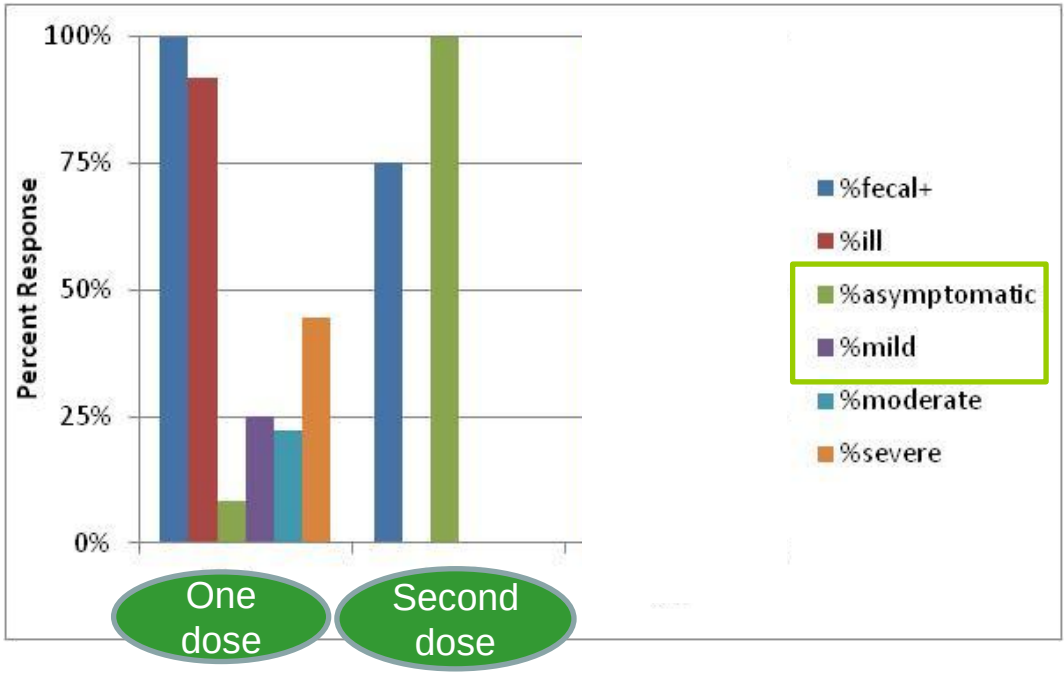
Fatigue and physical stress

Psychological stress

Boredom with ready-to-eat meals

Failure of **public health advice** to prevent travelers' diarrhea in soldiers deployed outside the US

Tribble et al., 2010



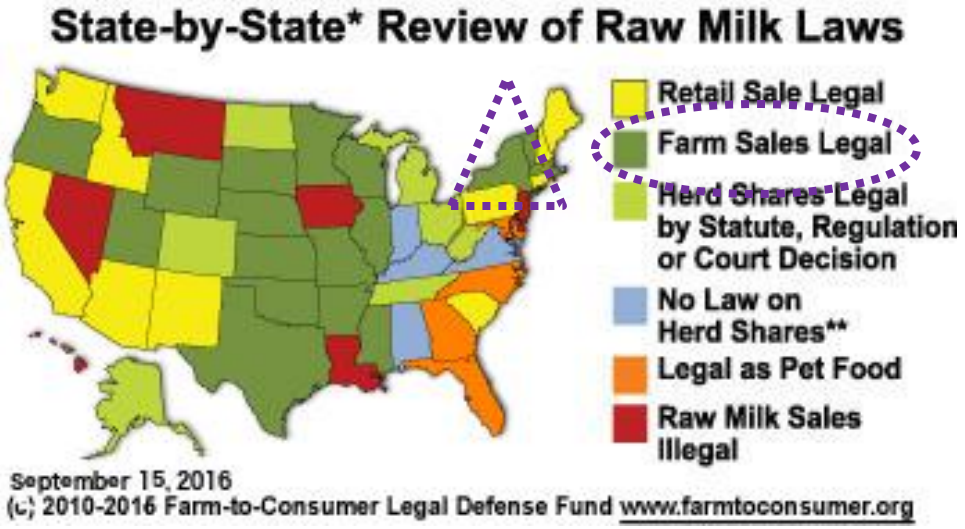
Avoid street vendor foods/beverages, raw and undercooked meat/seafood, raw fruits/vegetables, tap water, ice, unpasteurized dairy products

Range of Perceptions of Raw Milk Risks

- **UK** determined **raw (drinking) milk from licensed farms is SAFE** and **acceptably low risk for healthy adults** (Food Safety Authority, 2015).

- **US states** can **license** dairies for sale of fresh unprocessed (raw) milk at retail, at licensed farm stores, or as 'cow share' operations or **prohibit** sale.

- **New Zealand** permits sale of raw milk from licensed farms via home delivery or from licensed farm stores.



- **Australia** and **Canada** currently prohibit sale of raw milk as **INNATELY HAZARDOUS**.

Benefits AND Risks for Fresh Unprocessed (Raw) Milks?

Some Requirements for Farms Licensed by NY State to Sell Raw Milk

- Brucellosis ring test (No longer required for validated brucellosis-free states; 9 CFR 78.43)
- Tuberculosis test for each animal (No longer required for validated TB-free states)
- Quality Milk Production Services (QMPS) program
  - Each animal tested for *E. coli* and pathogens including *Staphylococcus aureus*
- Monthly milk sample tested for coliforms and pathogens including *Salmonella, Listeria, E. coli O157:H7, Campylobacter, Staphlococci*
- Satisfactory farm water test
- Farm inspections at least twice a year
  - Sanitary conditions
  - Health of cows
  - Health of individuals working on farm