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# Het microbioom als sleutel tot reizigersdiarree en Prikkelbare Darmsyndroom?



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# The gut microbiome: a highly diverse ecosystem



**100 trillion inhabitants**



**1,000-2,000 species**



**Unique composition**

# Ecosystems of this time



# Functions of our microbiome

## MAKE

vitamins, including  
B12, K AND FOLATE



## PRODUCE

IMPORTANT MOLECULES  
that travel around the body



## IN/RE-ACTIVATE

XENOBIOTICS AND DRUGS



## TEACH

THE IMMUNE SYSTEM  
to tell friends from foes



## INFLUENCE

the calories you harvest



## DEFEND

against harmful  
MICROORGANISMS



## HELP

PRODUCE SEROTONIN,  
important for optimal  
GUT FUNCTION



# Microbial perturbations

## MAKE

vitamins, including  
B12, K AND FOLATE



## TEACH

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## IN/RE-ACTIVATE

XENOBIOTICS AND DRUGS



# Dysbiosis affects different organs & diseases



Anxiety  
Depression



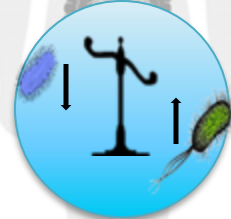
Diabetes (T1 & T2)



Inflammatory  
bowel disease  
& Infections



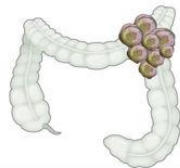
Pulmonary disease  
Atopy & Allergies



Non-alcoholic  
fatty liver disease



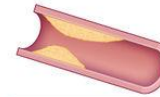
Traveler's diarrhea &  
Irritable Bowel  
Syndrome



Carcinogenesis

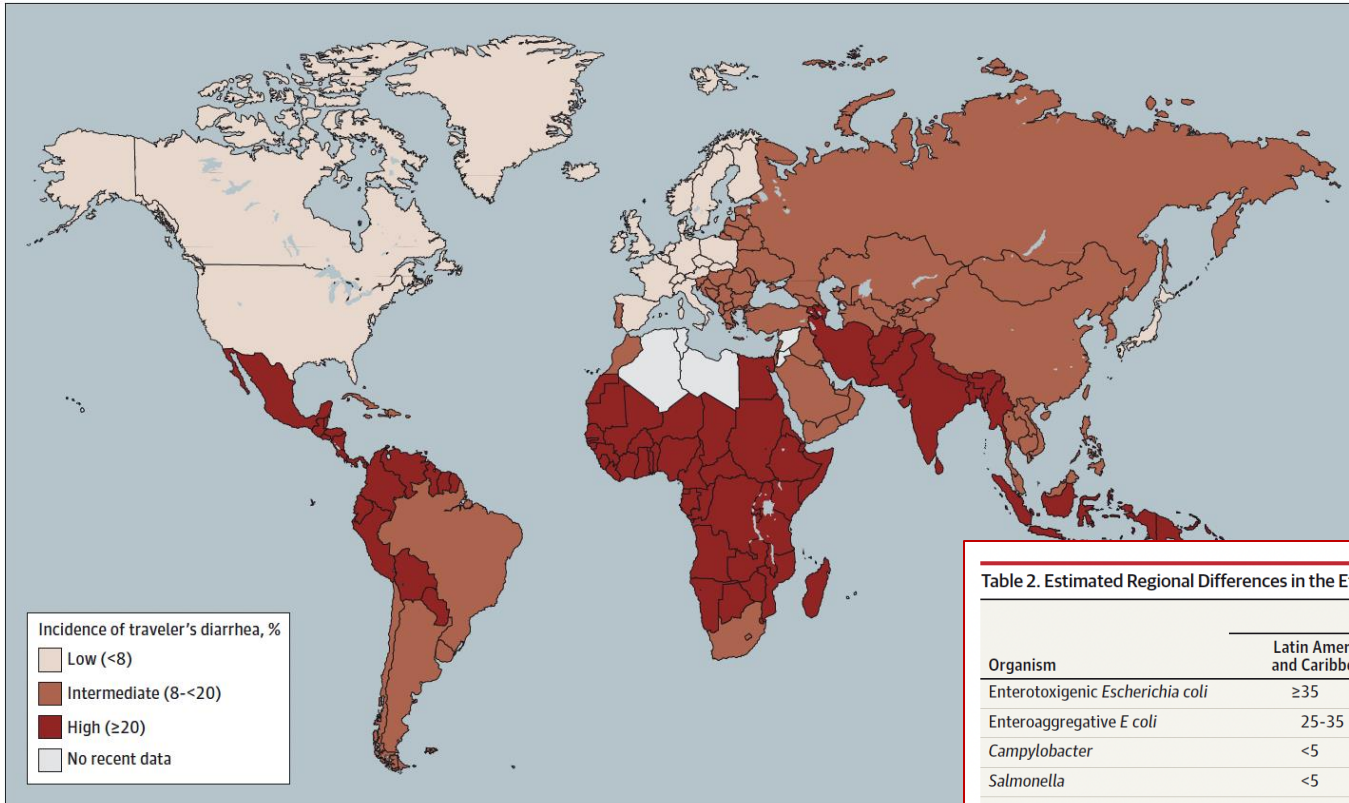


Obesity



Atherosclerosis

# Traveler's diarrhea



Incidence: 10-40%

## Risk factors:

- Adventure travel, VFR
- Younger age
- Lack of caution in food selection
- PPI use
- Certain genetic factors

Table 2. Estimated Regional Differences in the Etiology of Traveler's Diarrhea<sup>a</sup>

Organism	Reported Pathogens, %			
	Latin America and Caribbean	Africa	South Asia	Southeast Asia
Enterotoxigenic <i>Escherichia coli</i>	$\geq 35$	25-35	15-25	5-15
Enteroaggregative <i>E coli</i>	25-35	<5	15-25	No data
<i>Campylobacter</i>	<5	<5	15-25	25-35
<i>Salmonella</i>	<5	5-15	<5	5-15
<i>Shigella</i>	5-15	5-15	5-15	<5
Norovirus	15-25	15-25	5-15	<5
Rotavirus	15-25	5-15	5-15	<5
<i>Giardia</i>	<5	<5	5-15	5-15

# Carriage Of Multidrug Bacteria After Travel (COMBAT) study

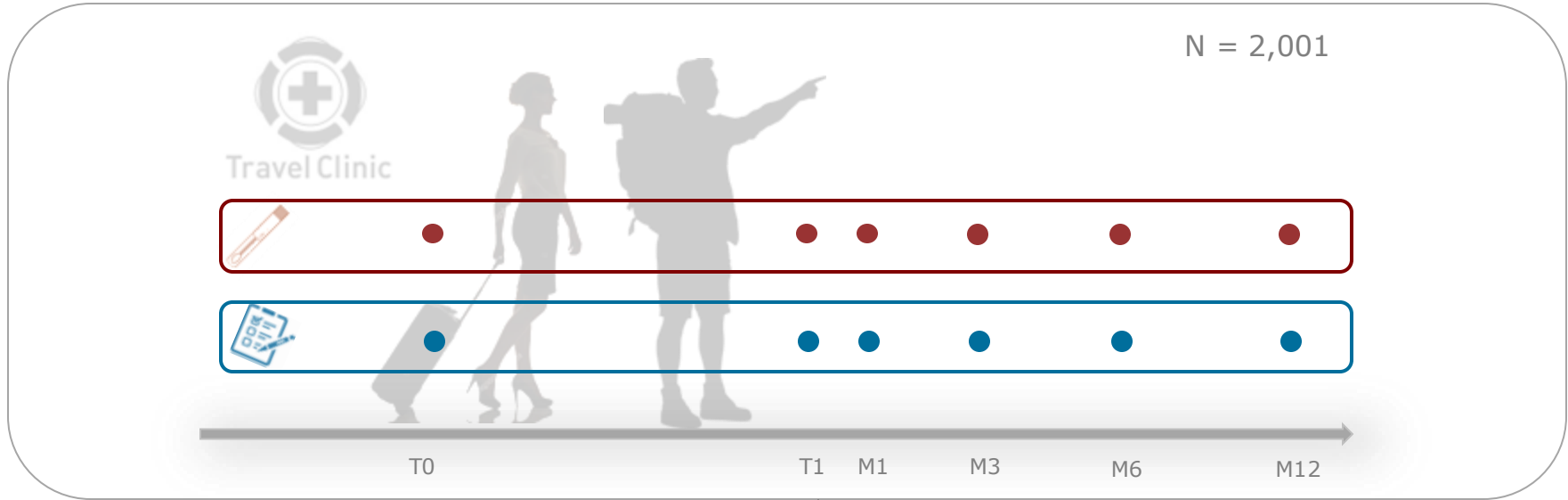


Jarne van Hattem



Maris Arcilla

Aim - acquisition of multidrug resistant Enterobacteriaceae



Incidence TD: ~ 36%

\*ESBL-E: extended-spectrum  $\beta$ -lactamase producing Enterobacteriaceae



# Traveler's diarrhea

Travel Medicine and Infectious Disease 19 (2017) 33–36



Travel-related acquisition of diarrhoeagenic bacteria, enteral viruses and parasites in a prospective cohort of 98 Dutch travellers



Jarne M. van Hattem<sup>a,\*</sup>, Maris S. Arcilla<sup>b</sup>, Martin P. Grobusch<sup>c</sup>, Aldert Bart<sup>a,d</sup>, Martin C. Bootsma<sup>e,f</sup>, Perry J. van Genderen<sup>g</sup>, Tom van Gool<sup>d</sup>, Abraham Goorhuis<sup>c</sup>, Jaap J. van Hellemond<sup>b</sup>, Richard Molenkamp<sup>h</sup>, Nicky Molhoek<sup>h</sup>, Astrid M. Oude Lashof<sup>b</sup>, Ellen E. Stobberingh<sup>b</sup>, Bob de Wever<sup>c</sup>, Henri A. Verbrugh<sup>b</sup>, Damian C. Melles<sup>b</sup>, John Penders<sup>i</sup>, Constance Schultsz<sup>j</sup>, Menno D. de Jong<sup>a</sup>

Pre-travel carriage and acquisition rates of diarrhoeagenic bacteria, parasites and viruses.

Pathogen	Pre travel (n-98)	Acquisition <sup>a</sup>					
		TD <sup>b</sup>	%	No TD <sup>b</sup>	%	Total	
<b>Bacteria</b>							
<i>Campylobacter</i> spp.	0	0%	3/34	9%	1/63	2%	4/98 (4%)
<i>Clostridium difficile</i>	0	0%	1/34	3%	0/63	0%	1/98 (1%)
<i>Plesiomonas shigelloides</i>	1	1%	1/34	3%	6/62	10%	7/97 (7%)
<i>Salmonella</i> spp.	0	0%	2/34	6%	1/63	2%	3/98 (3%)
<i>Shigella</i> spp.	2	2%	4/34	12%	1/61	2%	5/96 (5%)
<i>Yersinia enterocolitica</i>	3	3%	2/33	6%	0/61	0%	2/95 (2%)
<b>Parasites</b>							
<i>Blastocystis</i> spp.	31	32%	3/25	12%	4/41	10%	7/67 (10%)
<i>Cryptosporidium</i> spp.	0	0%	0	0%	0	0	0
<i>Dientamoeba fragilis</i>	19	19%	1/24	4%	4/54	7%	5/79 (6%)
<i>Entamoeba histolytica</i>	0	0%	0	0%	0	0	0
<i>Giardia lamblia</i>	0	0%	0/34	0%	1/63	2%	1/98 (1%)
<b>Viruses</b>							
Adenovirus (40, 41, 52)	0	0%	0	0%	0	0	0
Astrovirus	0	0%	0	0%	0	0	0
Enterovirus	0	0%	1/34	3%	0/63	0%	1/98 (1%)
Hepatitis E virus	2	2%	0	0%	0	0	0
Norovirus	1	1%	2/34	6%	0/62	0%	2/97 (2%)
Parvovirus	0	0%	0/34	0%	2/63	3%	2/98 (2%)
Rotavirus (A, C)	0	0%	0	0%	0	0	0
Sapovirus	0	0%	0	0%	0	0	0

TD: traveller's diarrhoea.

Numbers add up to 97 instead of 98 because of one missing post travel questionnaire.

<sup>a</sup> Acquisition rates were calculated for travellers with a negative pre-travel test: the 'population at risk'.

<sup>b</sup> Denominator is the number travellers with and without TD.

Travel Medicine and Infectious Disease 31 (2019) 101362



Original article

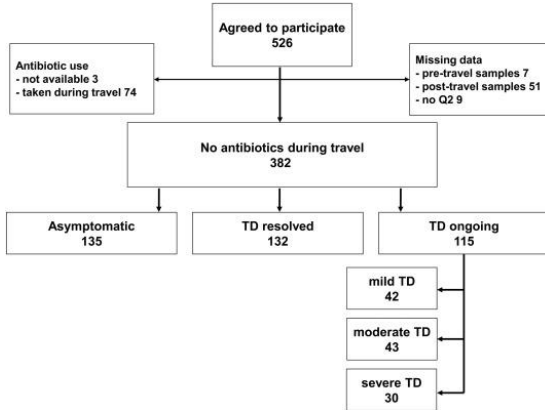
Risk of acquisition of human diarrhoeagenic *Escherichia coli* virulence genes in intercontinental travellers: A prospective, multi-centre study



Jarne M. van Hattem<sup>a,\*</sup>, Adriana Cabal<sup>b,c,1</sup>, Maris S. Arcilla<sup>d</sup>, Julio Alvarez<sup>b,e</sup>, Menno D. de Jong<sup>a</sup>, Damian C. Melles<sup>d</sup>, John Penders<sup>e,g</sup>, COMBAT consortium (Martin C.J. Bootsma<sup>h,i</sup>, Perry J. van Genderen<sup>h</sup>, Abraham Goorhuis<sup>i</sup>, Martin Grobusch<sup>l</sup>, Nicky Molhoek<sup>h</sup>, Astrid M.L. Oude Lashof<sup>m</sup>, Ellen E. Stobberingh<sup>m</sup>, Henri A. Verbrugh<sup>n</sup>), Christian Gortázar Schmidt<sup>c</sup>, Constance Schultsz<sup>h,h</sup>

- ETEC virulence genes most strongly associated with TD
- Diarrhoeagenic microorganisms and virulence genes also frequently identified in asymptomatic travellers

# The importance of an asymptomatic control group



	Ongoing TD at time of sampling		No TD at time of sampling		Univariate statistics					
	Total n (%)	Ongoing TD n (%)	Asymptomatic n (%)	Resolved TD n (%)	Ongoing TD versus asymptomatic OR (95% CI)	p	Ongoing TD versus resolved TD OR (95% CI)	p	Resolved TD versus asymptomatic OR (95% CI)	p
Total:	382	115 (30)	135 (35)	132 (35)						
Any bacterial pathogen	287 (75)	96 (83)	82 (61)	109 (83)	3.3 (1.8–6.0)	<0.001	0.9 (0.5–1.8)	0.851	3.1 (1.7–5.4)	<0.001
Two or more pathogens	152 (40)	61 (53)	34 (25)	57 (43)	2.5 (1.3–4.5)*	0.004	1.6 (0.9–2.8)*	0.105	1.5 (0.9–2.6)*	0.139
Diarrhoeagenic <i>E. coli</i> (DEC)	277 (73)	95 (83)	81 (60)	101 (77)	2.2 (1.3–3.7)	0.004	1.5 (0.8–2.7)	0.239	3.2 (1.8–5.7)	<0.001
EPEC	174 (46)	63 (55)	57 (42)	54 (41)	1.7 (1.0–2.7)	0.048	1.6 (1.1–2.9)	0.030	0.9 (0.6–1.5)	0.828
EAEC	171 (45)	58 (50)	38 (28)	75 (57)	2.6 (1.5–4.4)	<0.001	0.7 (0.5–1.3)	0.316	3.4 (2.0–5.6)	<0.001
ETEC	76 (20)	42 (37)	12 (9)	22 (17)	5.9 (2.9–11.9)	<0.001	2.9 (1.6–5.2)	<0.001	2.1 (1.0–4.3)	0.060
EHEC	34 (9)	7 (6)	10 (7)	17 (13)	0.8 (0.3–2.2)	0.680	0.4 (0.2–1.1)	0.079	1.8 (0.8–4.2)	0.143
Non-DEC	36 (9)	12 (10)	4 (3)	20 (15)	3.8 (1.2–12.2)	0.024	0.7 (0.3–1.4)	0.273	5.8 (1.9–17.6)	0.002
<i>Campylobacter</i>	26 (7)	9 (8)	1 (1)	16 (12)	11.4 (1.4–91.2)	0.022	0.6 (0.3–1.5)	0.268	18.5 (2.4–141.5)	0.005
<i>Salmonella</i>	29 (2)	3 (3)	3 (2)	3 (2)	1.2 (0.2–6.0)	0.842	1.1 (0.2–5.8)	0.864	1.0 (0.2–5.2)	0.978
EIEC/ <i>Shigella</i>	3 (1)	1 (1)	0 (0)	2 (2)	N/A	0.996	0.6 (0.1–6.4)	0.648	N/A	0.996
<i>Vibrio cholerae</i>	0 (0)	0 (0)	0 (0)	0 (0)	N/A	N/A	N/A	N/A	N/A	N/A
<i>Yersinia</i> spp.	0 (0)	0 (0)	0 (0)	0 (0)	N/A	N/A	N/A	N/A	N/A	N/A

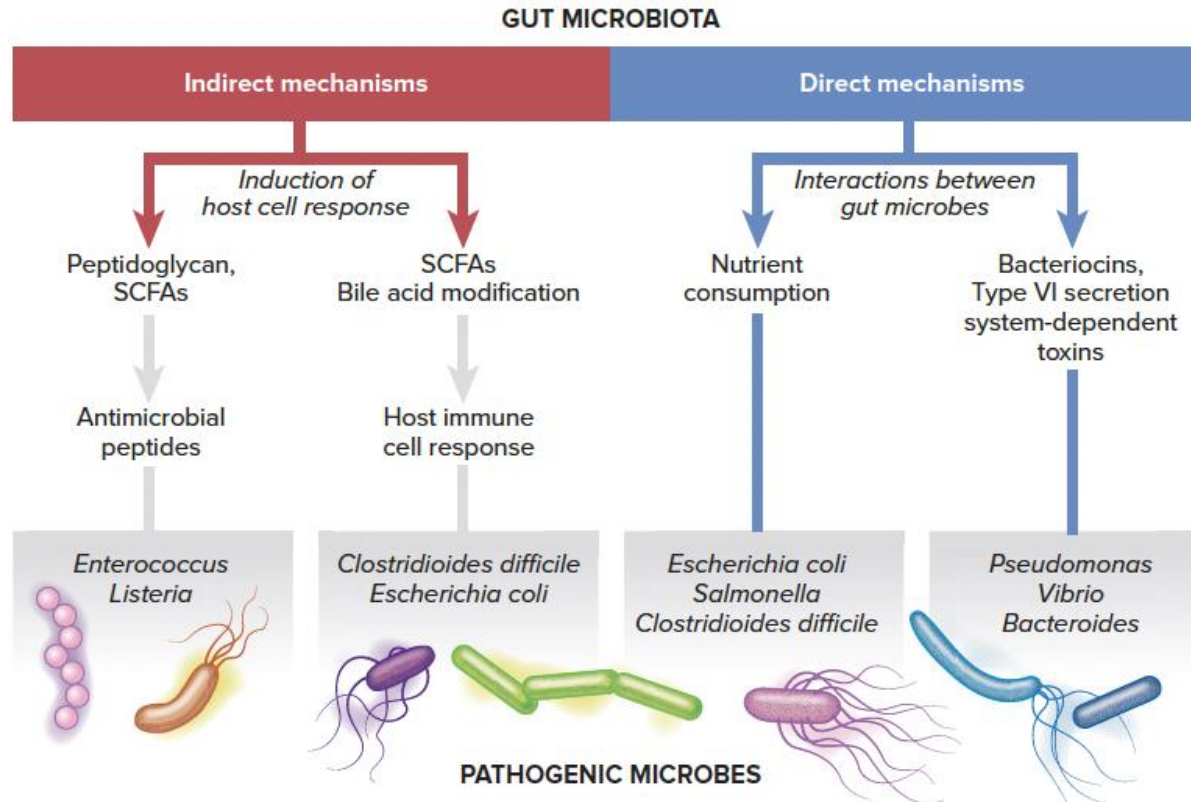
Results are presented in relation to symptoms during travel and at the time of post-travel stool sampling. The following bacterial pathogens were explored: enteropathogenic (EPEC), enteroaggregative (EAEC), enterotoxigenic (ETEC), enterohaemorrhagic (EHEC) and enteroinvasive (EIEC) *Escherichia coli* or *Shigella*, as well as *Salmonella* spp., *Yersinia* spp., *Vibrio cholerae* and *Campylobacter coli*/jejunii. Pathogens were found in all groups: Ongoing TD, Resolved TD and Asymptomatic, frequency decreasing respectively.

\*Compared with one pathogen.

<sup>1</sup>WHO definition: passage of three or more loose or liquid stools per day or, alternatively, more frequently than is normal for the individual [20].

## Colonization resistance of the microbiome?

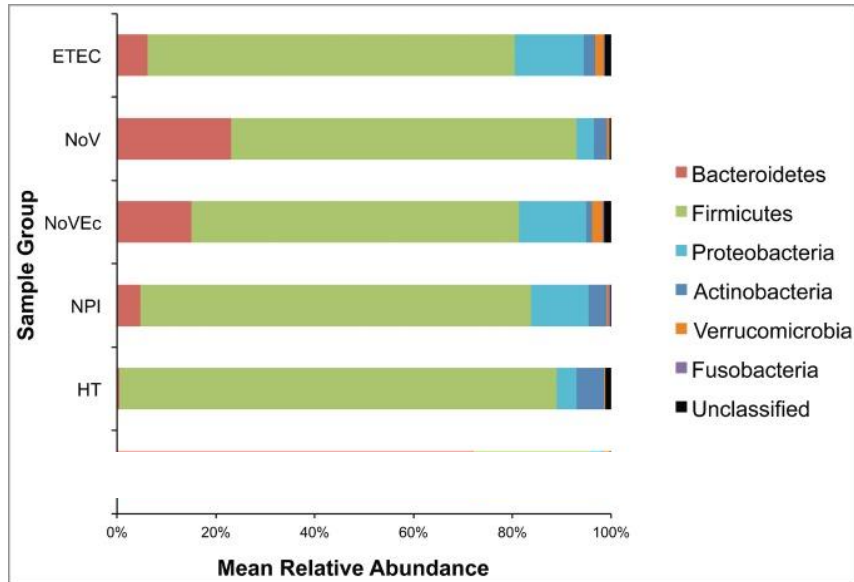
# Colonization resistance – competitive exclusion



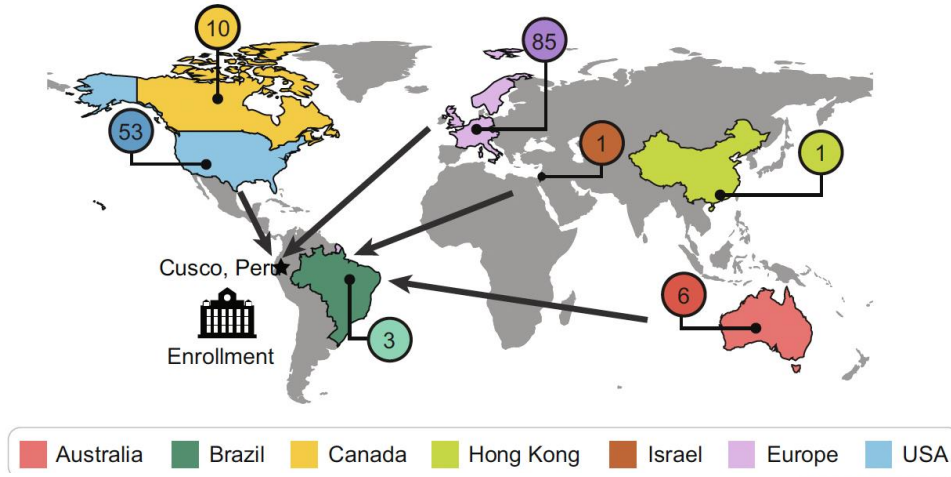
# The role of the microbiome in TD

## *Diarrhea disturbs the microbiome*

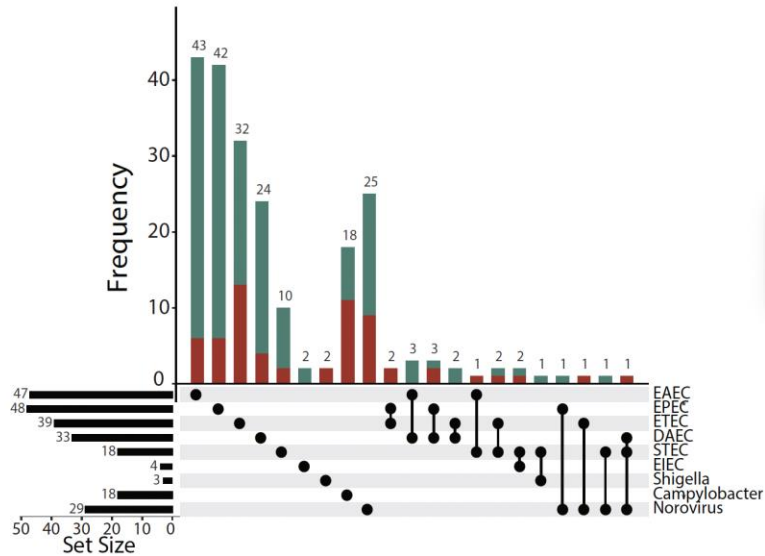
- Retrospective study – 111 returning travellers from Central America/India to US
- Diarrhea samples: ETEC, NoV, NoV + ec, NPI
- Diarrhea-free travelers included as healthy controls



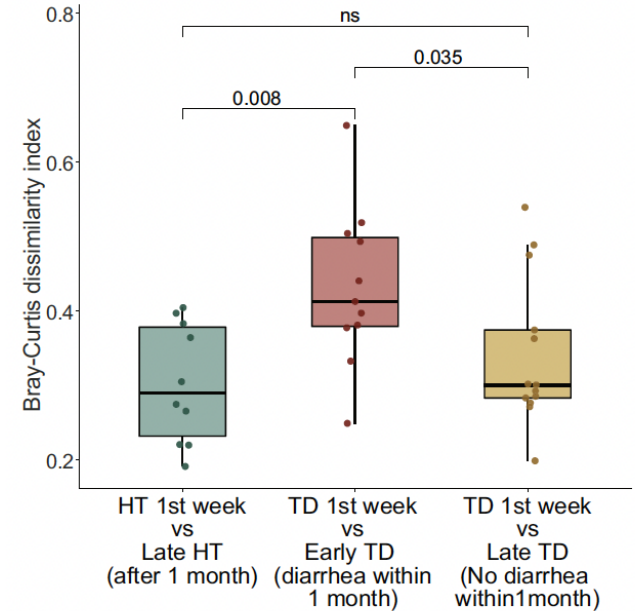
# International travel, diarrhea and the gut microbiome



# International travel, diarrhea and the gut microbiome



Large shifts in microbiome in first month upon arrival associated with diarrhea within 1 months



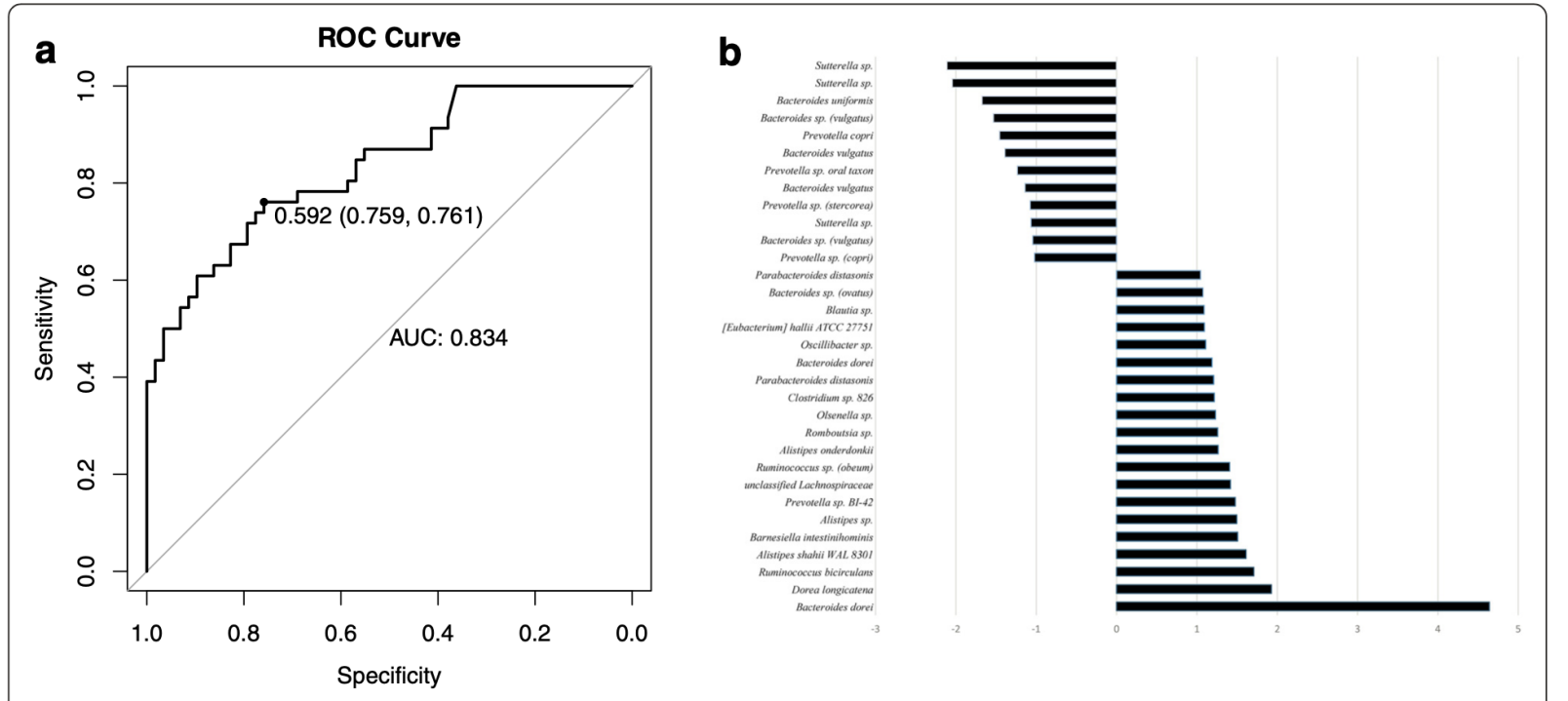
Overall shift in microbiome during travel does not predict who will get diarrhea

# Does the microbiome protect against ETEC-induced diarrhea?

## Study description

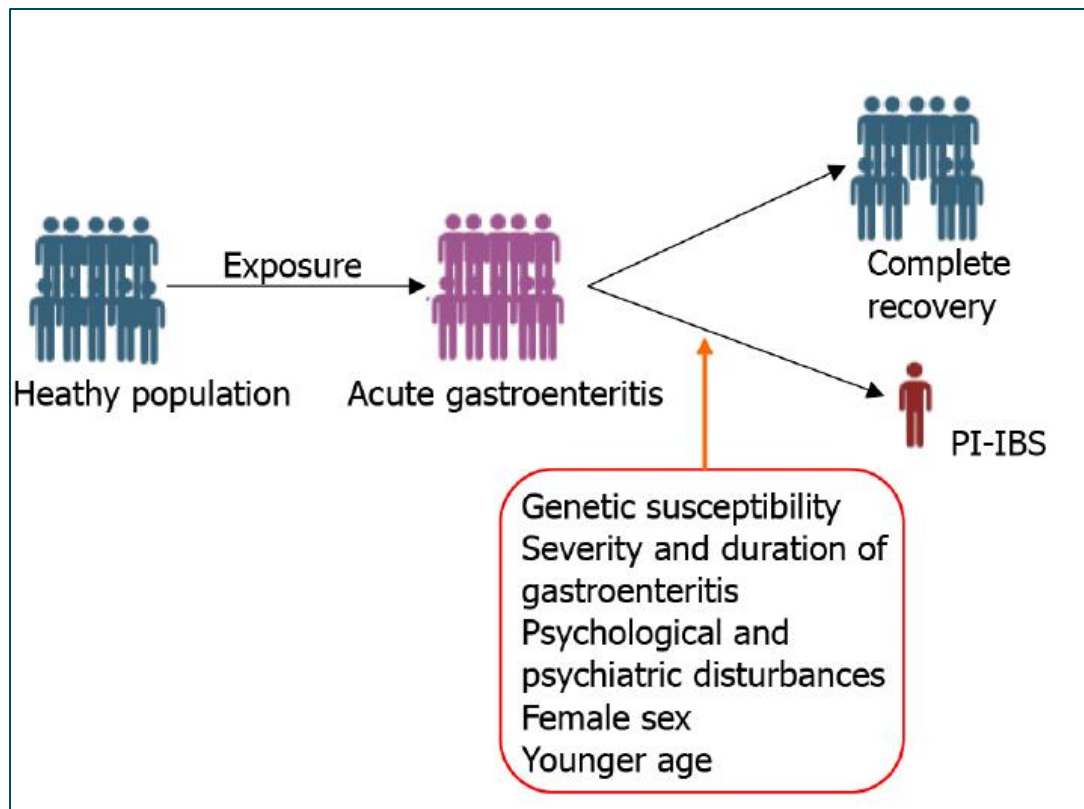
- 30 healthy adults challenged with ETEC ( $10^5$ - $10^6$  CFU)
- Fecal samples collected at prior to challenge (day -1, 0) and repeatedly after infection
- All subjects treated with ciprofloxacin at day 5 (3 days, 500 mg twice daily) or earlier when symptomatic
- Microbiota analysis for 5 subjects developing diarrhea and 7 asymptomatic subjects

# The pre-infection microbiota – a predictor of disease onset?





## Traveler's diarrhea, the microbiome and post-infectious IBS



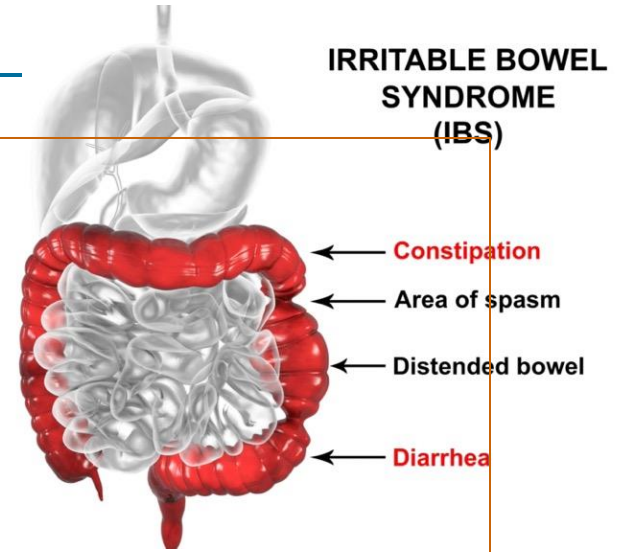
# Irritable Bowel Syndrome (IBS)

## Frequent gastrointestinal disorder

- 5-15% of global population
- More common among women
- Onset mostly before the age of 50

## Symptoms

- **Abdominal pain: cramping, discomfort**
  - **Altered bowel habits: diarrhea, constipation, mixed**
  - **Bloating, gas production**
  - **Tiredness**
- Functional disorder: absence organic disease or biochemical abnormalities



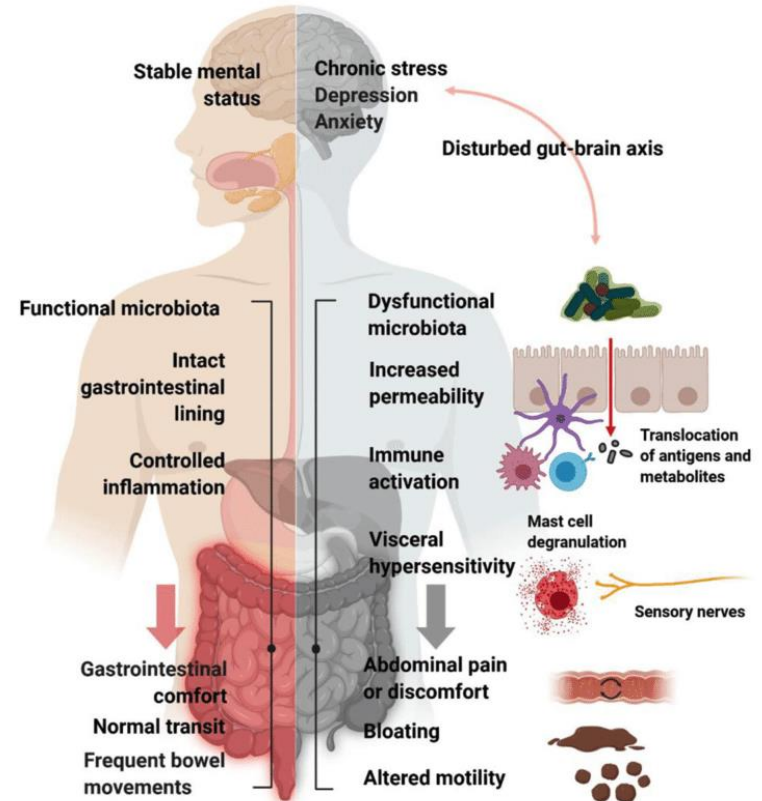
# Pathophysiology of IBS

## Pathophysiology

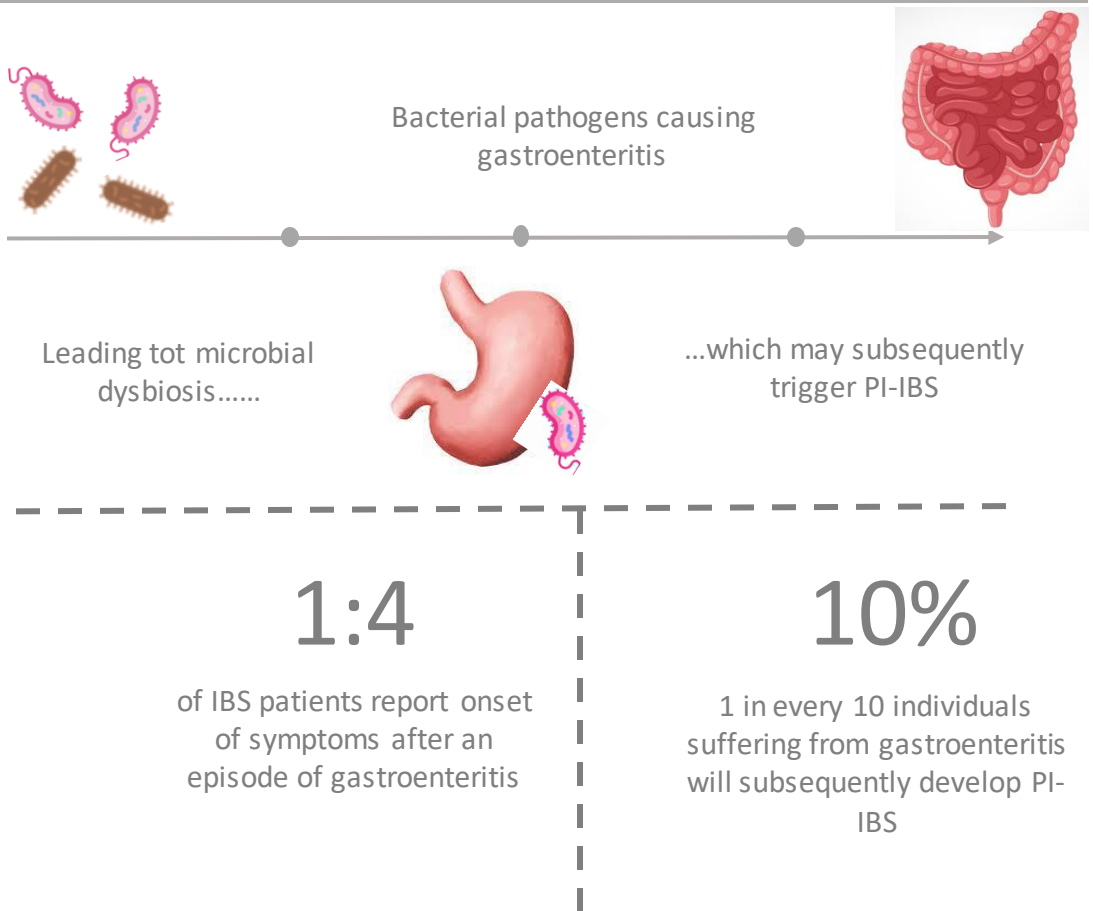
- Gut-Brain axis
- Intestinal motility
- Low-grade inflammation
- **Altered microbiome**
- Increased permeability

## Triggering factors


- Diet
- Stress
- Hormones
- Other diseases: **bacterial infections, gastro-enteritis.**



# Postinfectious IBS



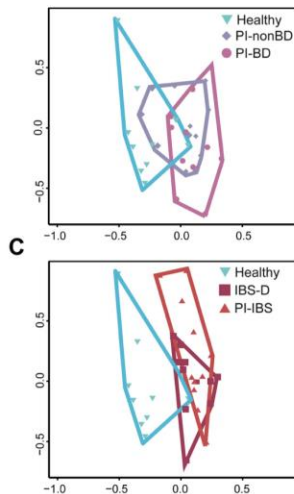
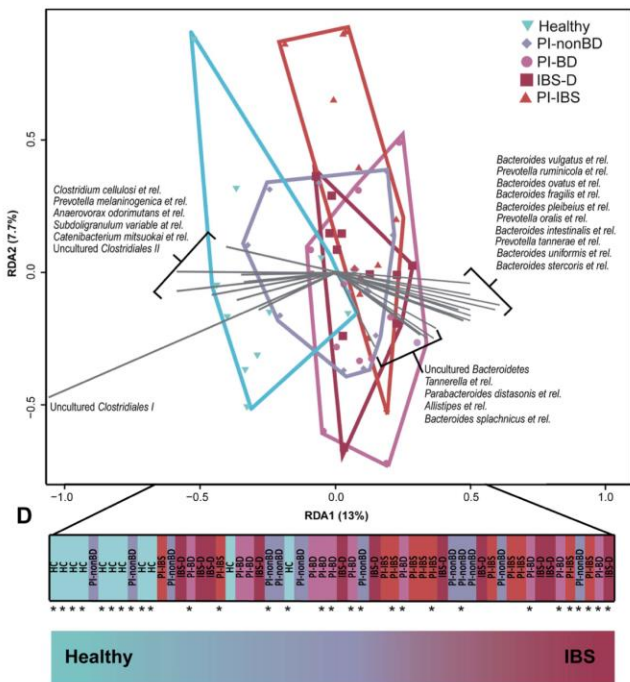
# Post-infectious irritable bowel syndrome after intercontinental travel: a prospective multicentre study

Jiyang Chan , MSc<sup>1</sup>, Niels van Best, PhD<sup>1,2</sup>, Markia Ward, MSc<sup>1</sup>, Maris S. Arcilla, MD, PhD<sup>3</sup>, Jarne M. van Hattem, MD, PhD<sup>4</sup>, Damian C. Melles, MD, PhD<sup>5</sup>, Menno D. de Jong, MD, PhD<sup>4</sup>, Constance Schultsz, MD, PhD<sup>4,6</sup>, Perry J.J. van Genderen, MD, PhD<sup>7</sup>, John Penders, PhD<sup>1,8,\*</sup> and On behalf of the COMBAT-study consortium

Traveler's diarrhea: 35.5%

New onset PI-IBS: 65/539, 12.0% of travelers with TD

# The microbiome and post-infectious IBS



## Microbial profile separated:

- HC from (PI-)IBS patients
- Patients with/without persistent bowel dysfunction (PD) following infection
- gradual change in microbiota from healthy to PI-IBS

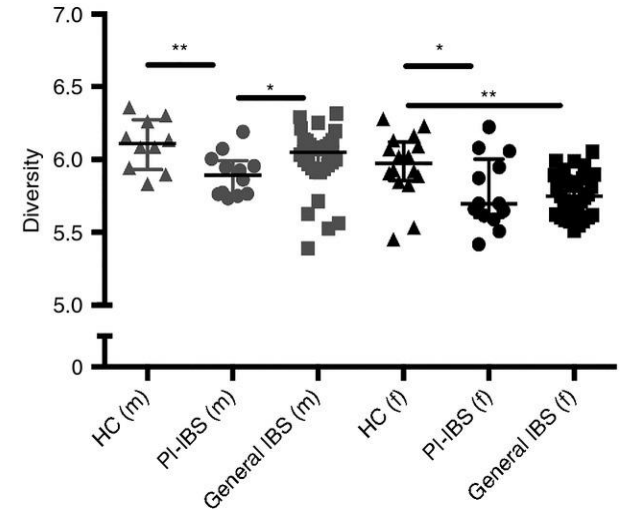
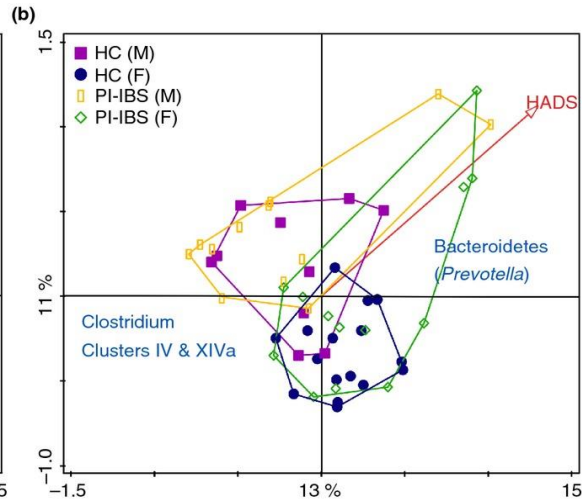
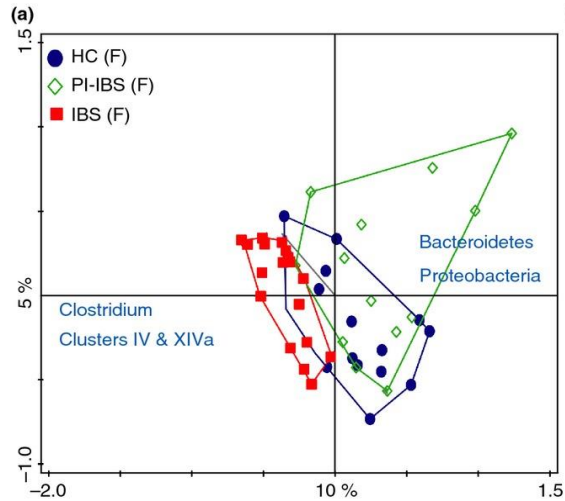
## Microbiota analysis in feces of 57 subjects:

- diagnosed PI-IBS after symptomatic *C. jejuni* infection
- with persisting symptoms after symptomatic *C. jejuni* infection
- without persisting symptoms after symptomatic *C. jejuni* infection
- with IBS-D
- healthy controls

# The microbiome and post-infectious IBS

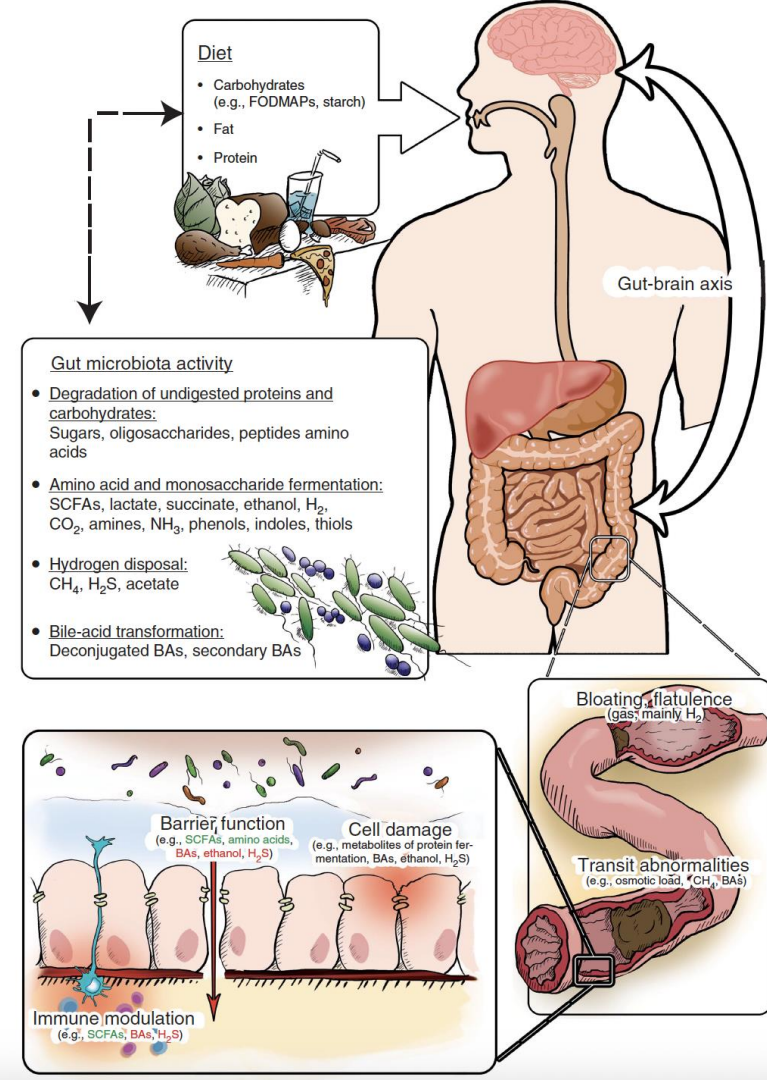
## Microbiota analysis (HIT-Chip) in feces and mucosal biopsies:

- 13 PI-IBS patients (enteropathogens not determined)
- 19 IBS patients
- 16 Healthy controls



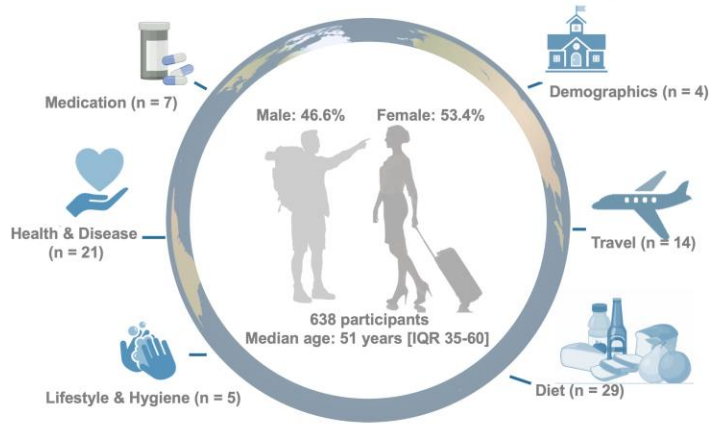
# Intestinal Microbiota And Diet in IBS: Causes, Consequences, or Epiphenomena?

Mirjana Rajilić-Stojanović, PhD<sup>1</sup>, Daisy M. Jonkers, PhD<sup>2</sup>, Anne Salonen, PhD<sup>3</sup>, Kurt Hanevik, MD, PhD<sup>4</sup>, Jeroen Raes, PhD<sup>5</sup>, Jonna Jalanka, PhD<sup>6</sup>, Willem M. de Vos, PhD<sup>3,6,7</sup>, Chaysavanh Manichanh, PhD<sup>8</sup>, Natasa Golic, PhD<sup>9</sup>, Paul Enck, PhD<sup>10</sup>, Elena Philippou, PhD<sup>11</sup>, Fuad A. Iraqi, PhD<sup>12</sup>, Gerard Clarke, PhD<sup>13</sup>, Robin C. Spiller, MD, PhD<sup>14</sup> and John Penders, PhD<sup>15</sup>





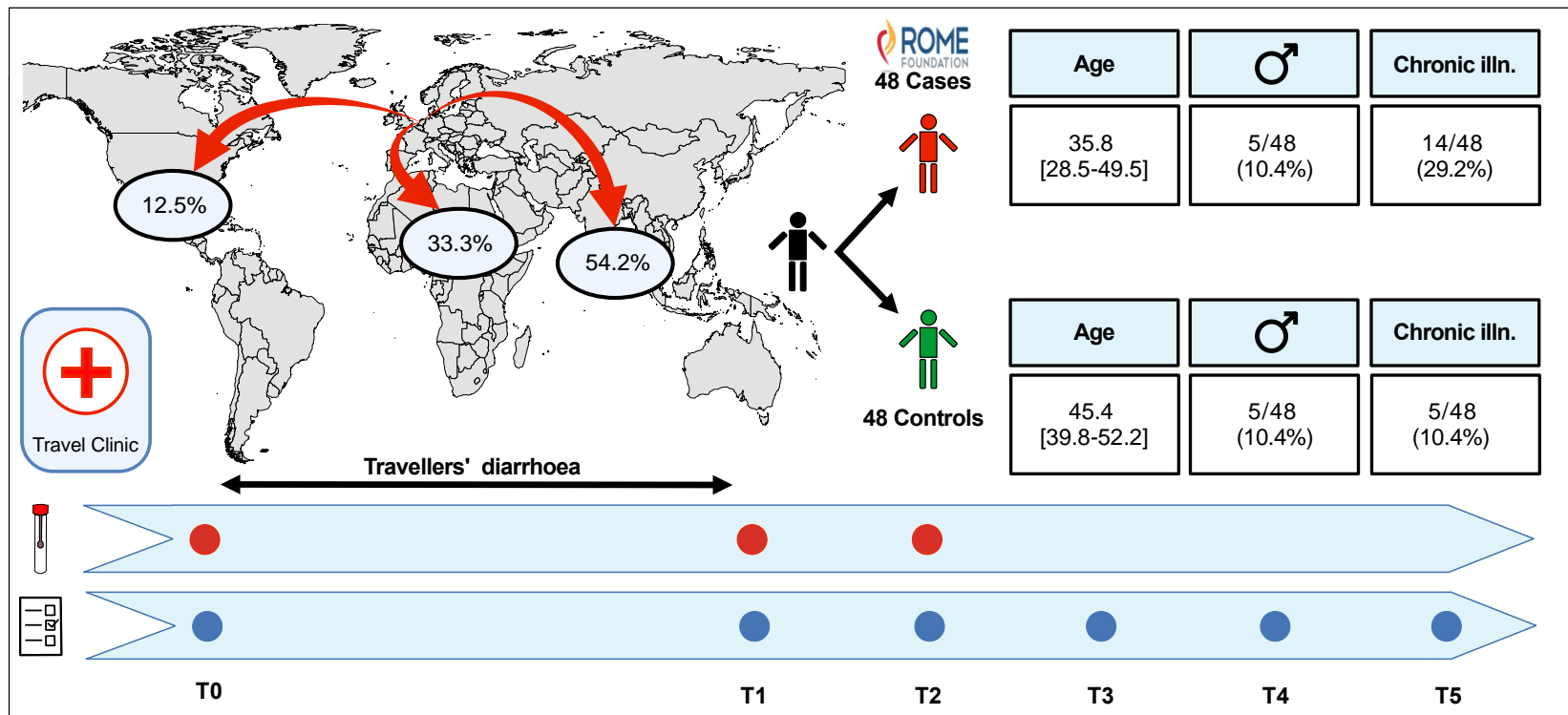
# Travel impacts microbial community structure



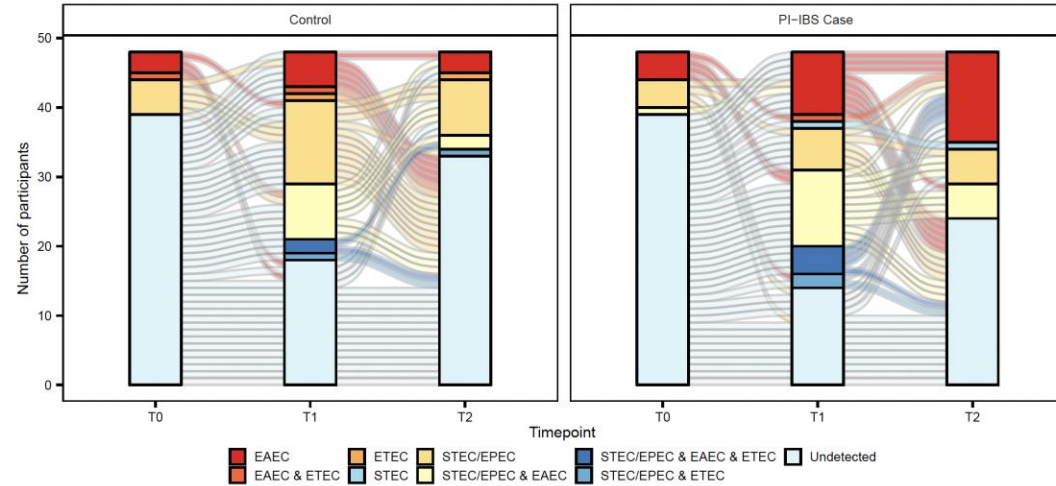


Jiyang Chan

# Nested case-control study on PI-IBS



# Is PI-IBS a result of delayed clearance of enteropathogens?

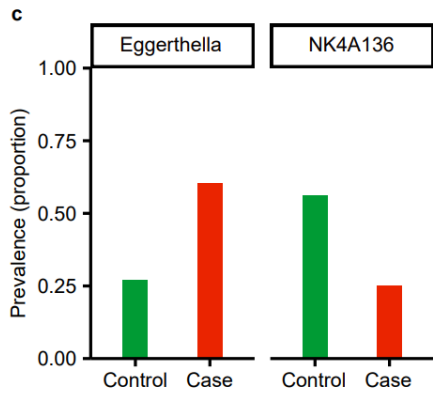
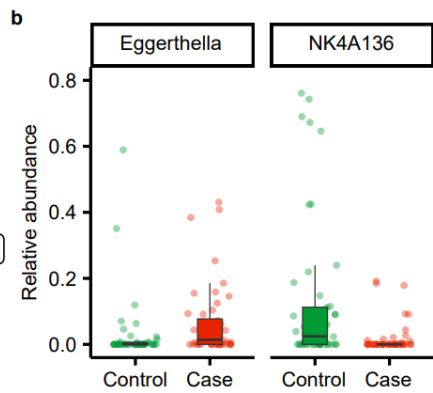
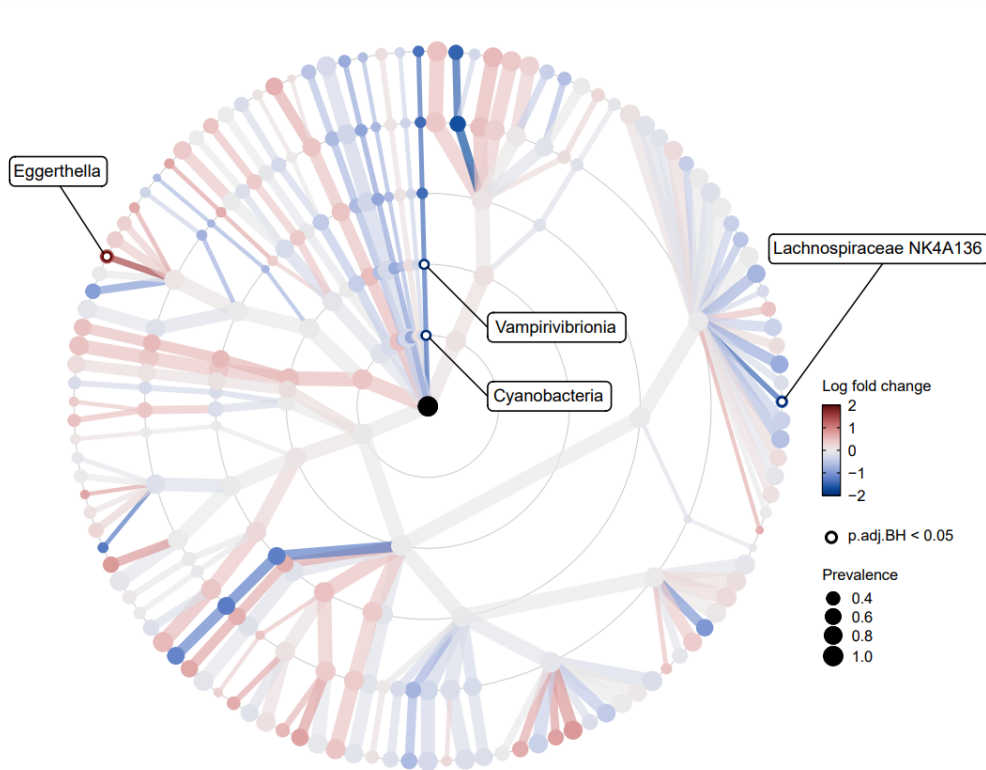


## Reduced microbial diversity prior to PI-IBS onset

# Differential abundant taxa in PI-IBS cases



David Barnett



Can we target the microbiome to boost colonization resistance and prevent or treat traveler's diarrhea?

# Do probiotics help with travelers diarrhea?



Cochrane  
Library

Cochrane Database of Systematic Reviews

## Probiotics for treating acute infectious diarrhoea (Review)

Collinson S, Deans A, Padua-Zamora A, Gregorio GV, Li C, Dans LF, Allen SJ

### Authors' conclusions

Probiotics probably make little or no difference to the number of people who have diarrhoea lasting 48 hours or longer, and we are uncertain whether probiotics reduce the duration of diarrhoea. This analysis is based on large trials with low risk of bias.

Figure 4. Funnel plot of comparison: 1 Primary diarrhoea outcomes, outcome: 1.1 Diarrhoea lasting  $\geq 48$  hours.

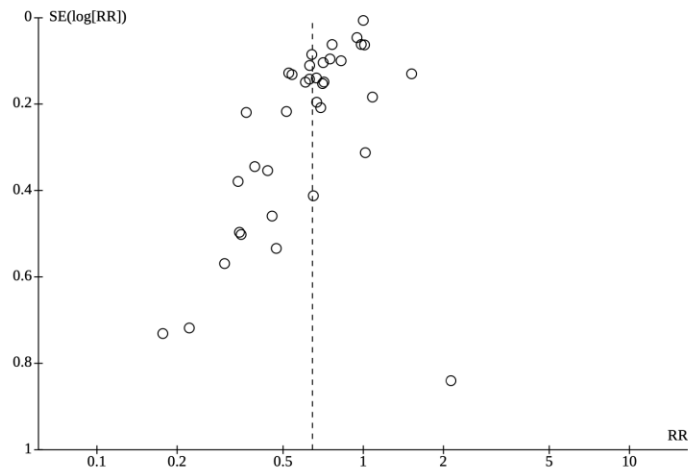
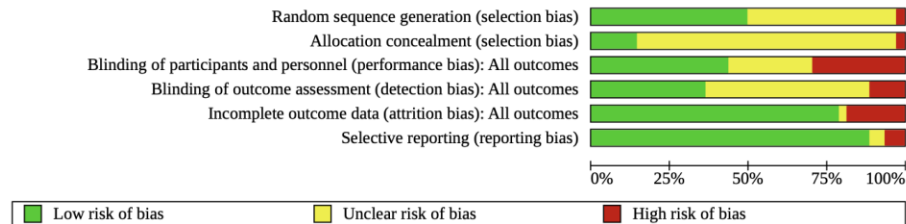


Figure 3. Risk of bias graph: review authors' assessments about each risk of bias item presented as percentages across all included studies.



# Do probiotics help with travelers diarrhea?

Systematic Review and Meta-Analysis

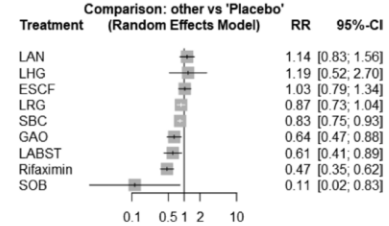
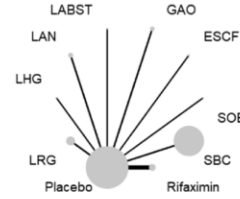
Medicine

OPEN

## Probiotics and rifaximin for the prevention of travelers' diarrhea

### A systematic review and network meta-analysis

Hao Fan, PhD<sup>1,2</sup>, Lei Gao, MD<sup>2</sup>, Zidan Yin, PhD<sup>3</sup>, Sheng Ye, PhD<sup>4</sup>, Hua Zhao, MD<sup>5</sup>, Qi Peng, MD<sup>1\*</sup>



**Figure 3.** The comparative effectiveness of differential probiotics and rifaximin. CI = confidence interval, ESCF = *Enterococcus faecium* SF68 + *S. cerevisiae* CNCM I-4444 + fructo-oligosaccharide, GAO = galacto-oligosaccharide, LABST = *L. acidophilus* + *L. bulgaricus* + *Bifido.bifidum* + *Strept. Thermophilus*, LAN = *L. acidophilus* nr, LHG = *L. helveticus* ATCC33409 + *L. gasseri* ATCC4962, LRG = *L. rhamnosus* GG, RR = relative ratio, SBC = *S. boulardii* CNCM I-745, SOB = sodium butyrate.

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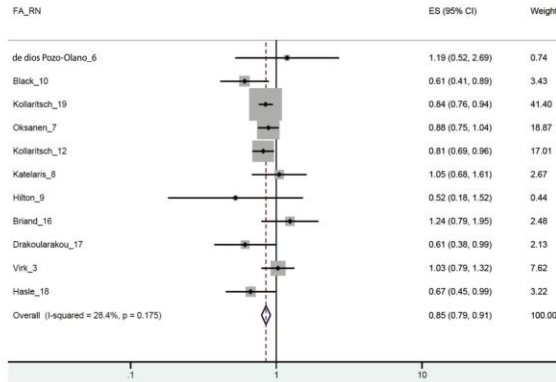
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BRIEF COMMUNICATION

## Prophylactic efficacy of probiotics on travelers' diarrhea: an adaptive meta-analysis of randomized controlled trials

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Available at www.sciencedirect.com

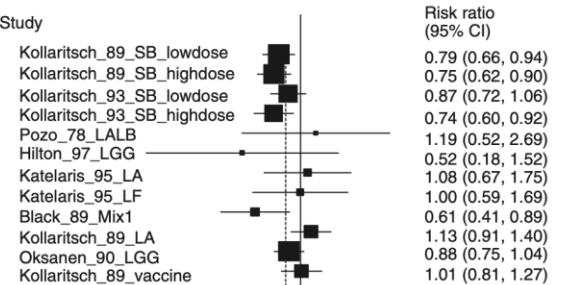
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## Meta-analysis of probiotics for the prevention of traveler's diarrhea<sup>1,2</sup>

Lynne V. McFarland<sup>1,2,\*</sup>

Study



Overall

Favors Probiotic Favors Placebo



## Take home messages

- TD – **significantly disturbs the microbiota** composition
- **Mechanistic studies strongly support role of microbiome** in GI infections, but fewer data for diarrheagenic *E. coli* as compared other bacterial pathogens (e.g., *C. difficile*, *Salmonella*)
- Limited and weak quality human studies on role microbiome in protecting against TD
- **Probiotic** trials on TD prevention are heterogenous and **effects at most limited**
- **Microbiome perturbations in PI-IBS** – yet no consistent microbial signature
- Studies on **microbiome in PI-IBS** mostly contain **heterogenous patients groups** (e.g., various pathogens) and are **cross-sectional**
- First evidence for **role microbiome in new-onset PI-IBS**

*Time for next-generation probiotics and personalized interventions?*

# The EXPLORE-Rs study

Aim – understand microbiome dynamics during travel





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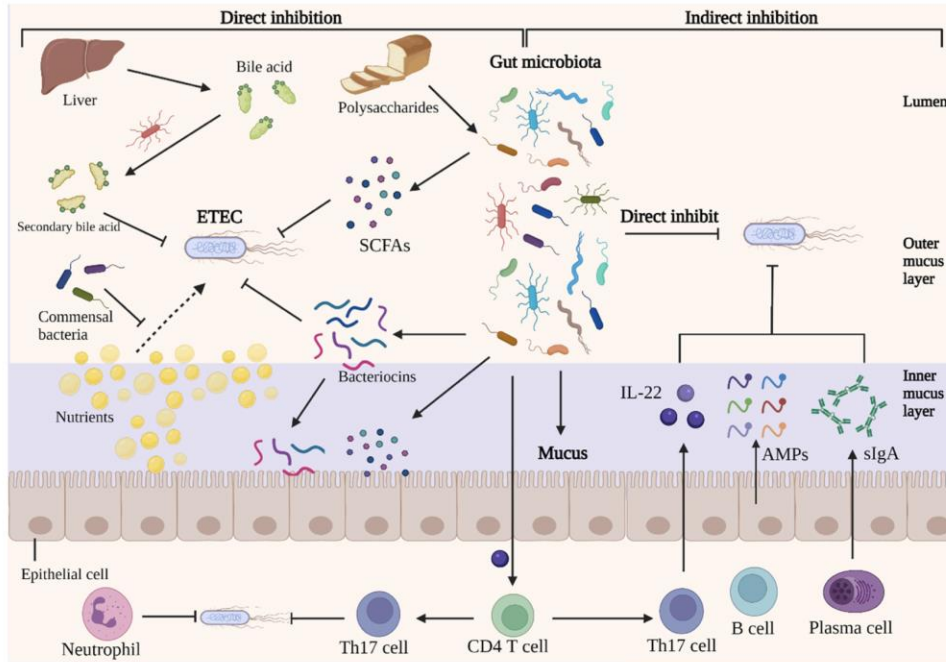
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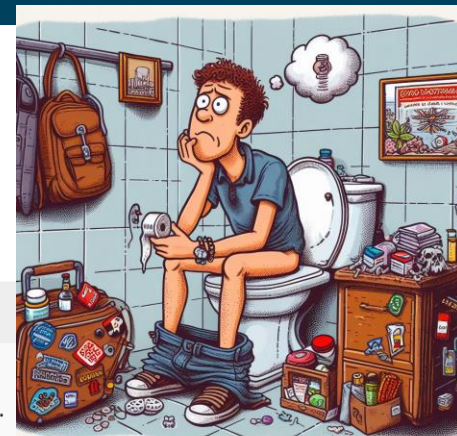
## Other evidence for the role of the microbiome in ETEC infection?

- Mouse models for ETEC infection require pre-treatment with antibiotics to disrupt the microbiome to facilitate infection



# Traveler's diarrhea

Type	Symptoms	Management
Mild	Few unformed stools. Generally tolerable. Does not interfere with daily activities.	Increase fluid intake and use oral rehydration solution. Anti-motility agents or bismuth subsalicylate may be used. Antibiotic is not recommended.
Moderate	Loose or liquid stools; cramps or nausea may also occur. Becomes distressing and interferes with daily activities. No blood in the stool.	Increase fluid intake and use oral rehydration solution. Anti-motility agent or bismuth subsalicylate may be used. An antibiotic is not encouraged but may be used.
Severe	Loose or liquid stools that become debilitating. Cramps, nausea, chills, severe thirst, or inability to keep liquids down. Prevents all planned activities. Blood in the stool.	Increase fluid intake. Use an antibiotic. Seek medical attention if symptoms do not improve or if blood in stool is present.



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