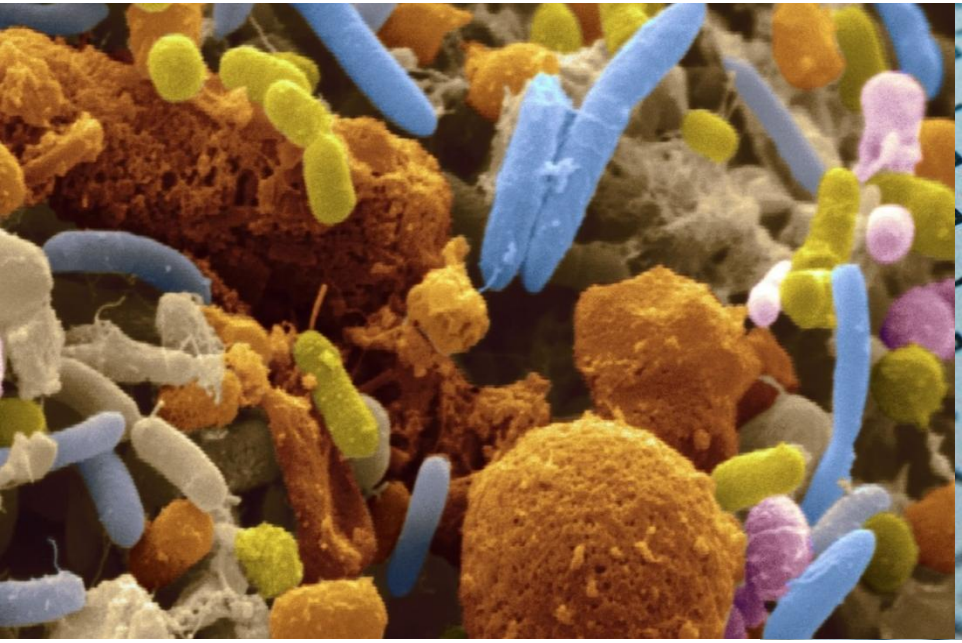
The intestinal microbiota and Intensive care setting.



Etienne Ruppé
AP-HP Hôpital Bichat-Claude Bernard and INSERM/CNRS/Université Paris 7 – Paris 13
Journée OUTCOME REA, May 17, 2018

Disclosures

Consultant for DaVolterra

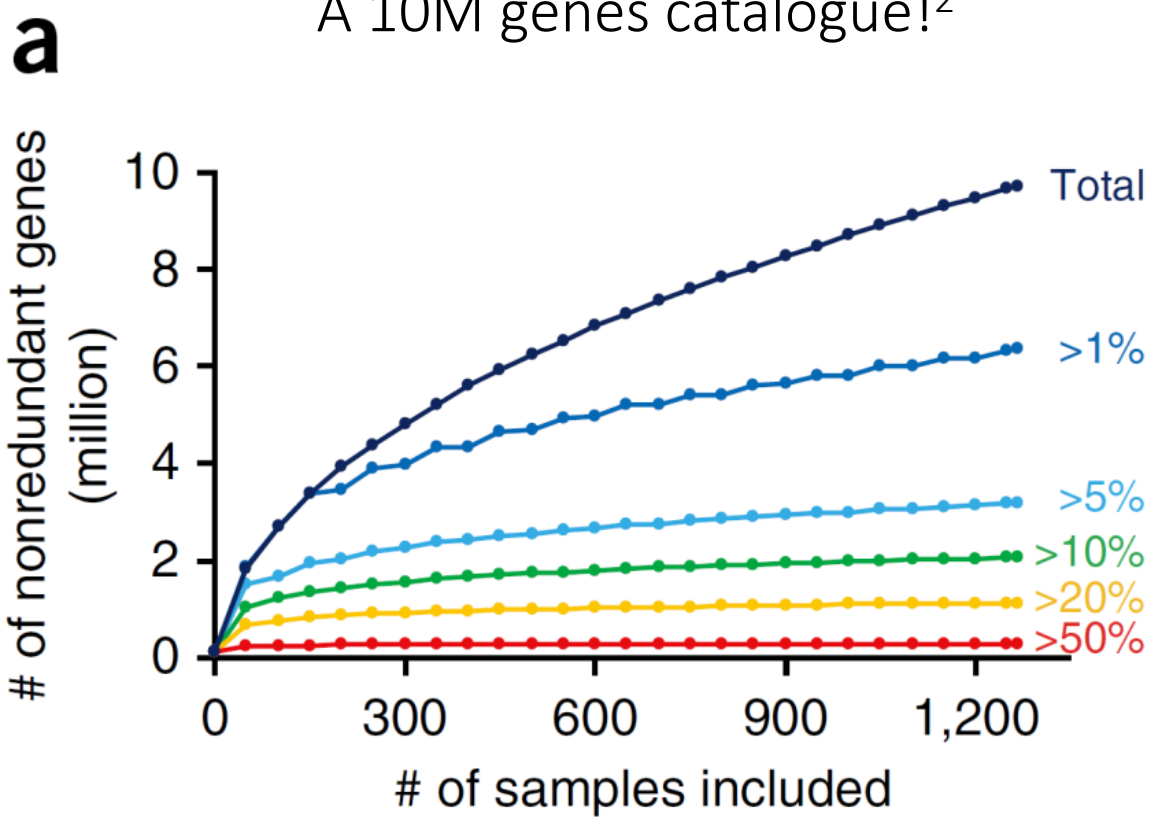
Scientific advisory Board member of Pathoquest and MaaT Pharma

What have metagenomics brought to the understanding of the intestinal microbiota?

16S profiling and metagenomics allowed a more in-depth vision of our microbiota

1. Huge number of bacterial cells ($3.8x10^{13}$)[1], pathogens are subdominant
2. Great diversity (estimated hundreds of species)
3. Mostly un(hardly)culturable bacteria

A 10M genes catalogue![2]



[1]**Sender, R.** *PLoS Biol.* **14,** e1002533 (2016);[2]**Li, J.** *Nat. Biotechnol.* **32,** 834–841 (2014).

Metagenomics allowed a more in-depth vision of our microbiota

Metagenomics applied to the intestinal microbiota can yield:

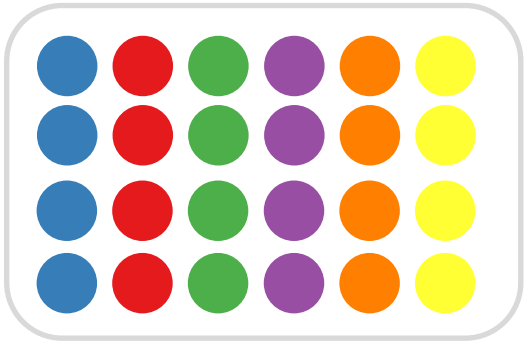
- The **diversity** of bacterial (and other) species
- The **richness** of genes and bacteria
- **Metagenomic species, and subspecies (clonal populations)**
- The content of antibiotic resistance genes (the **resistome**)

Metagenomics applied to the intestinal microbiota cannot (or barely can):

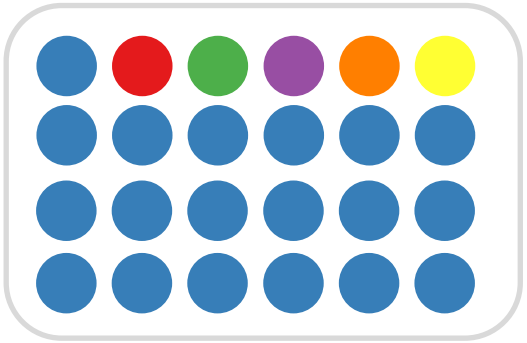
- Identify the conventional drug-resistant Enterobacteriaceae and enterococci and their resistance genes

[1]**Sender, R.** *PLoS Biol.* **14,** e1002533 (2016);[2]**Li, J.** *Nat. Biotechnol.* **32,** 834–841 (2014).

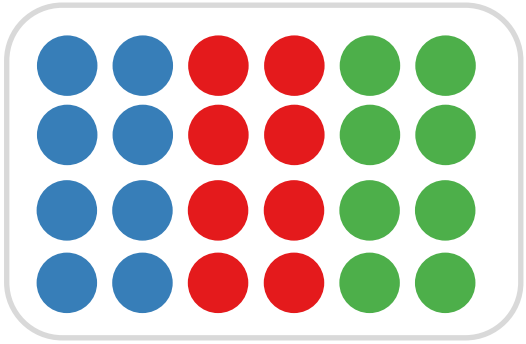
Richness and diversity



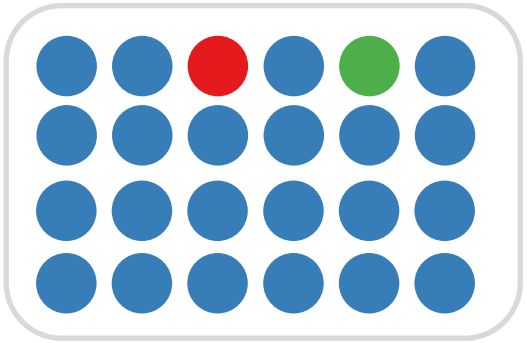
Rich
Diverse



Rich
Not diverse

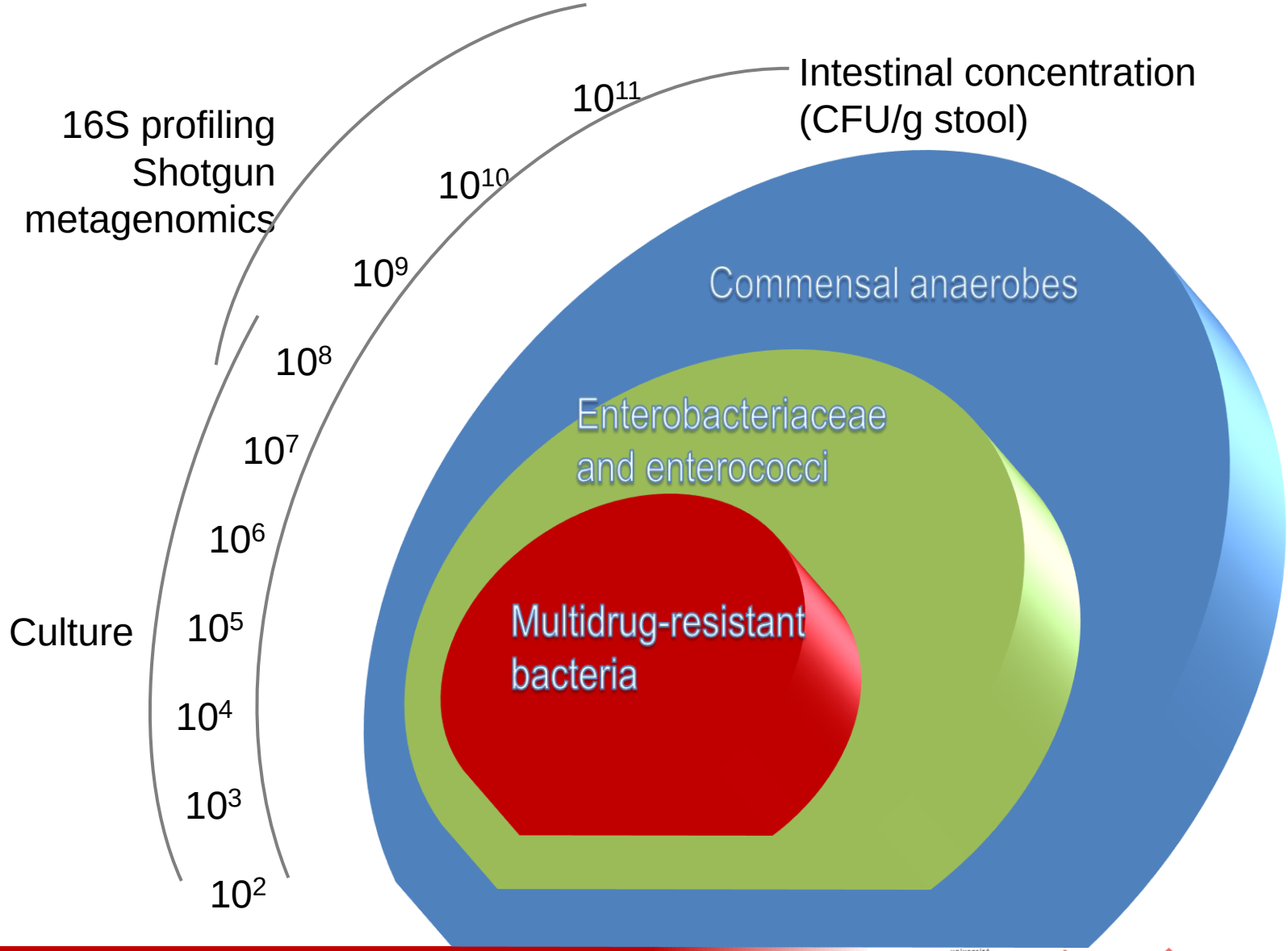


Not rich
Diverse



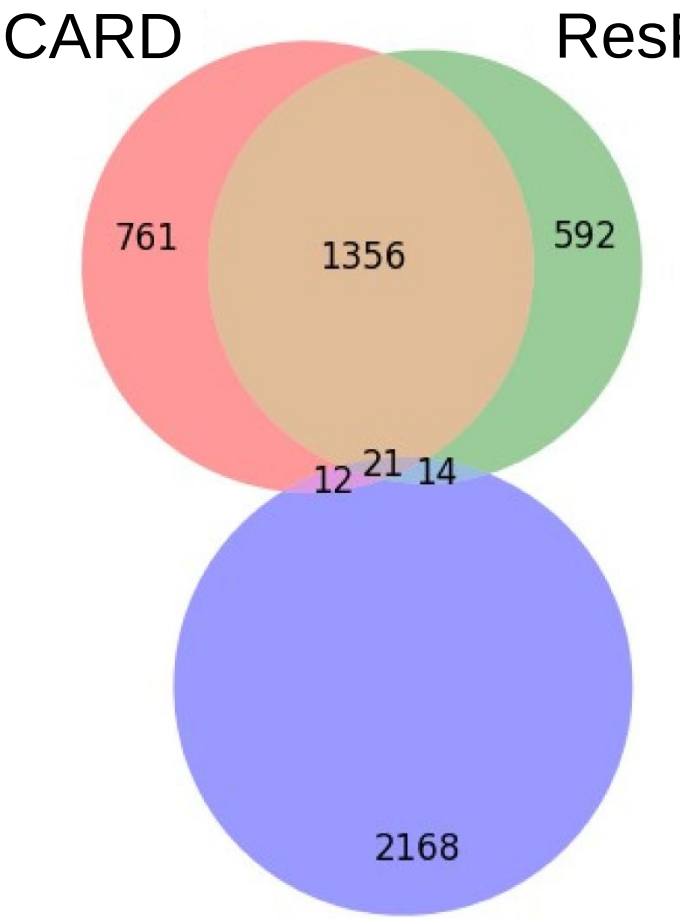
Not rich
Not diverse

Metagenomics lack sensitivity (despite the millions of reads)!



The resistome of pathogens/culturable bacteria is distinct from that of intestinal commensals/environmental bacteria

ResfinderFG includes 2,528 ARDs from functional selections performed on various types of samples: intestinal microbiota, sewage, latrine and soils[1-5].



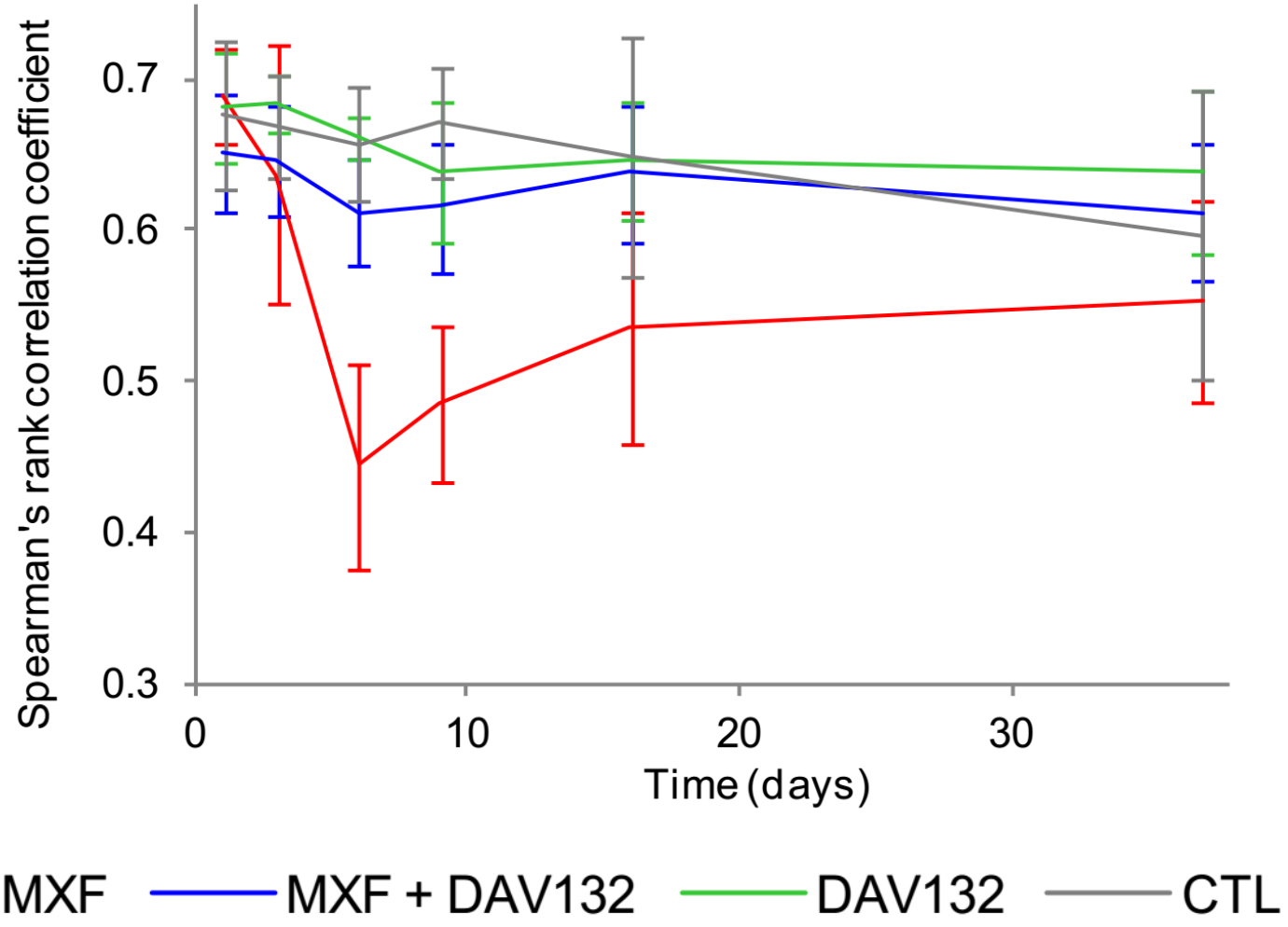
ResFinderFG (from functional metagenomics)

1. **Forsberg, K. J**. et al. Nature 509, 612–616 (2014).; 2. **Gibson, M. K.** et al. Nature Microbiology 16024 (2016); **Moore, A. M**. et al. PLoS ONE 8, e78822 (2013).; 4.; **Pehrsson, E. C**. et al. Nature 533, 212–216 (2016)5. **Sommer, M. O**. et al. Science 325, 1128–1131 (2009)

Impact of antibiotics on the intestinal microbiota

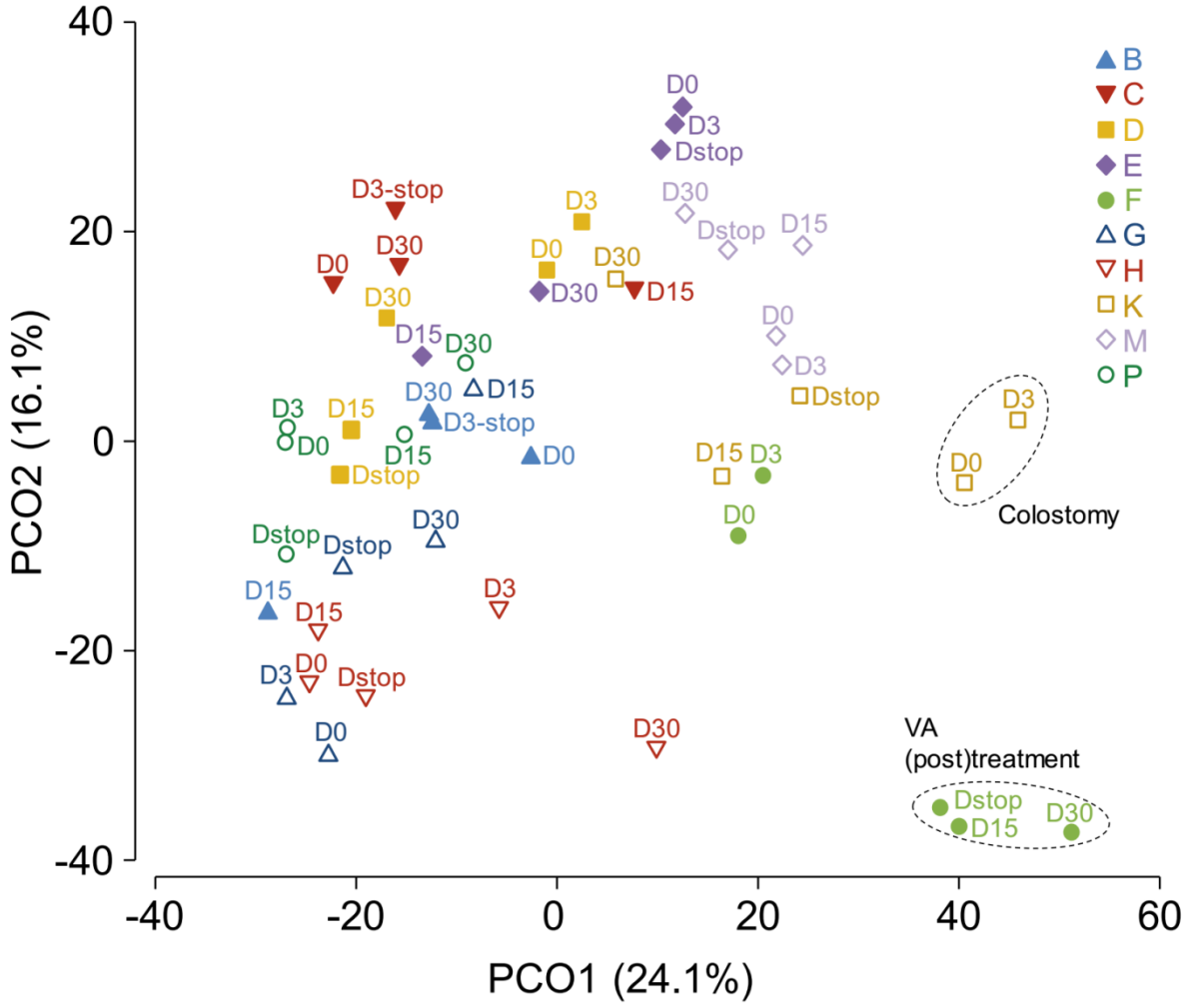
Effect of moxifloxacin on healthy volunteers

The microbiota composition 30 days after the cessation of moxifloxacin differs from that at baseline.



Absence of effect of imipenem on patients?

PCA analysis (Bray-Curtis): samples cluster by patient and not by time point.



3. How about in ICU?

Peculiarities of ICU with respect to microbiome studies.

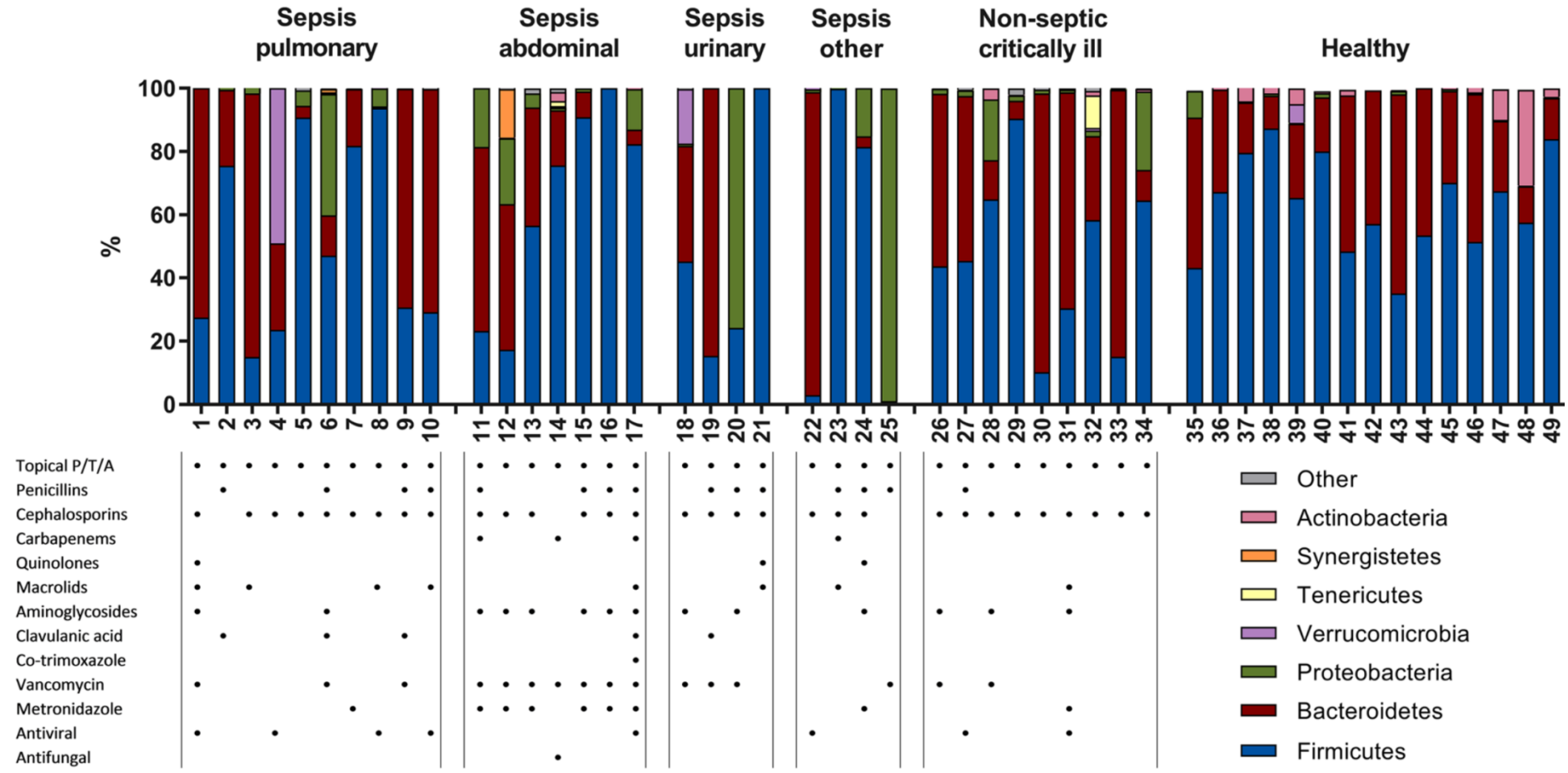
Iterative drug administration:

- Antibiotics (including erythromycin)
- Other drugs that may affect the intestinal microbiota
- Various, changing regimen.

Difficulty to get **faecal** samples.

Variability of the intestinal microbiota of ICU patients

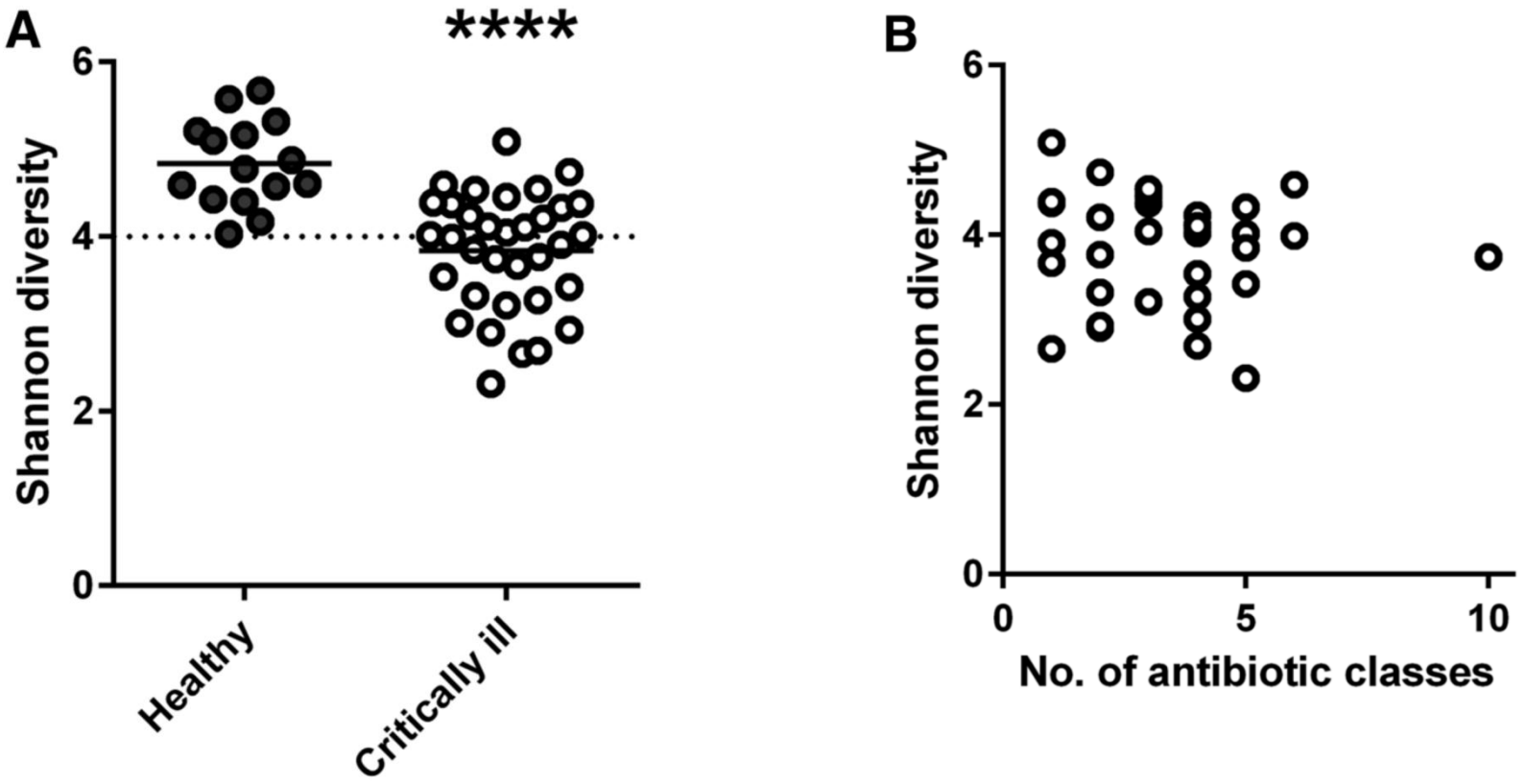
Intestinal microbiota (16S sequencing) of 34 with sepsis and 15 control patients



Lankelma, J.M. et al. *Intensive Care Med* **43**, 59–68 (2017).

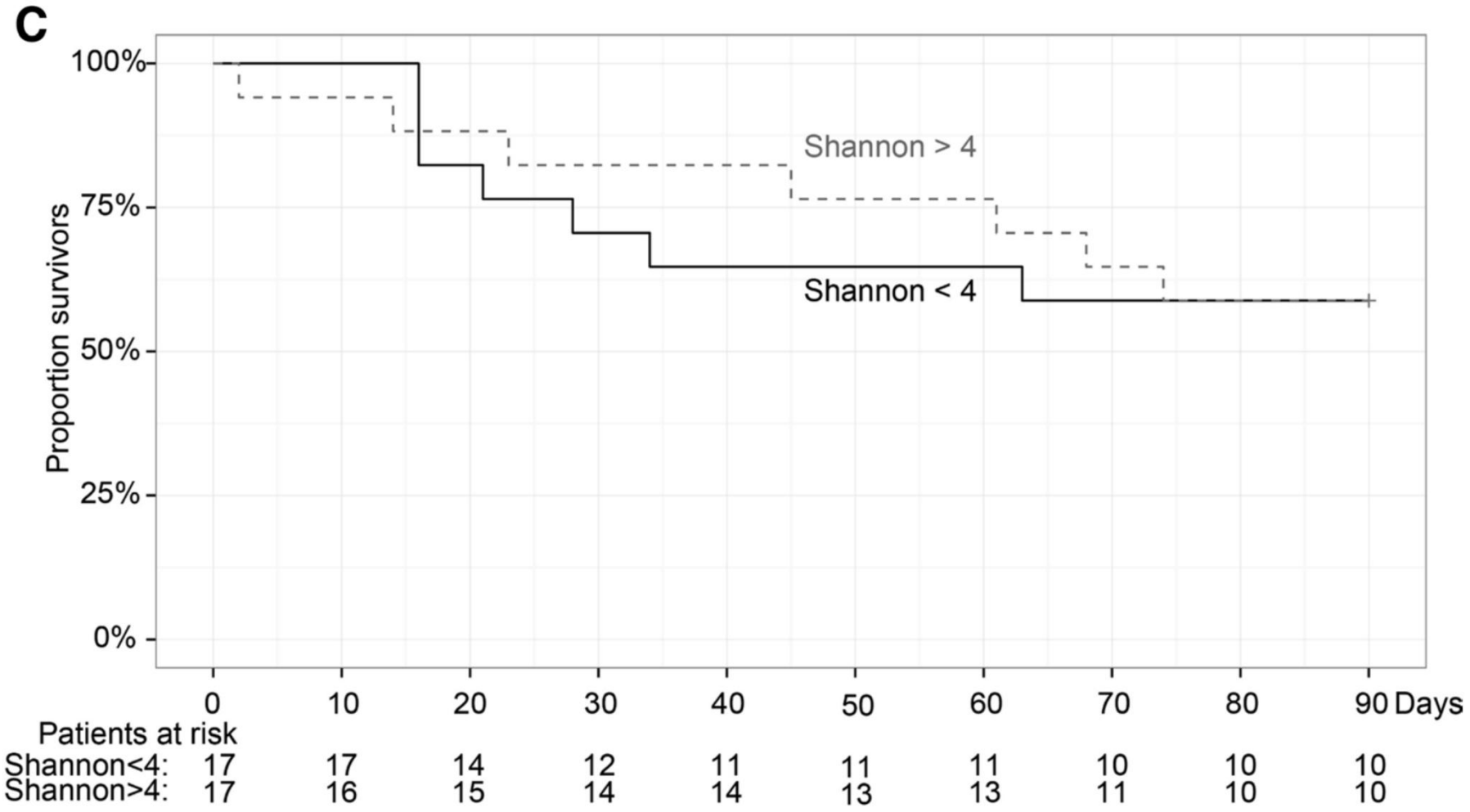
The intestinal microbiota of ICU patients is less diverse

Intestinal microbiota (16S sequencing) of 34 with sepsis and 15 control patients



Link between intestinal diversity and outcome

Intestinal microbiota (16S sequencing) of 34 patients with sepsis.



Lankelma, J.M. et al. *Intensive Care Med* **43**, 59–68 (2017).

Selective digestive decontamination (SDD) affects the composition of the intestinal microbiota

21 patients in the SC regimen, 19 patients in the SOD regimen and 17 patients in the SDD regimen.
Analysis of the microbiota by bacterial group specific PCR.

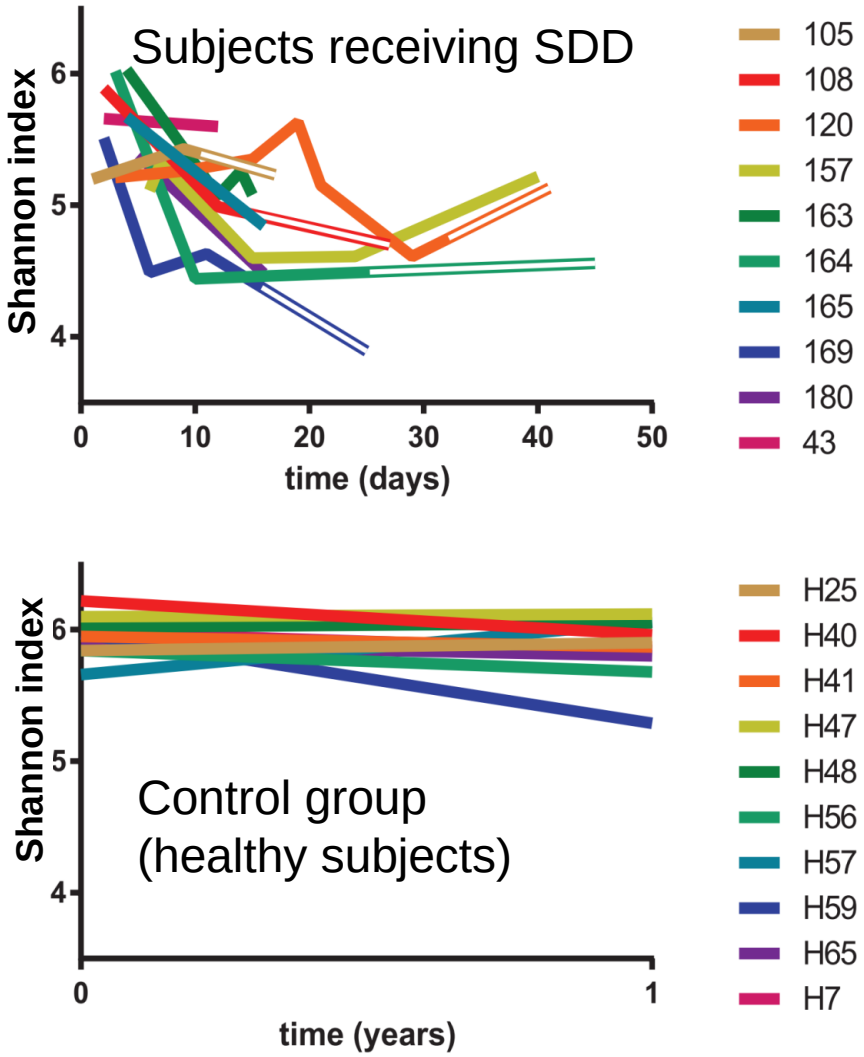
Table 3 Numbers and statistical analysis of the main intestinal microbiota groups

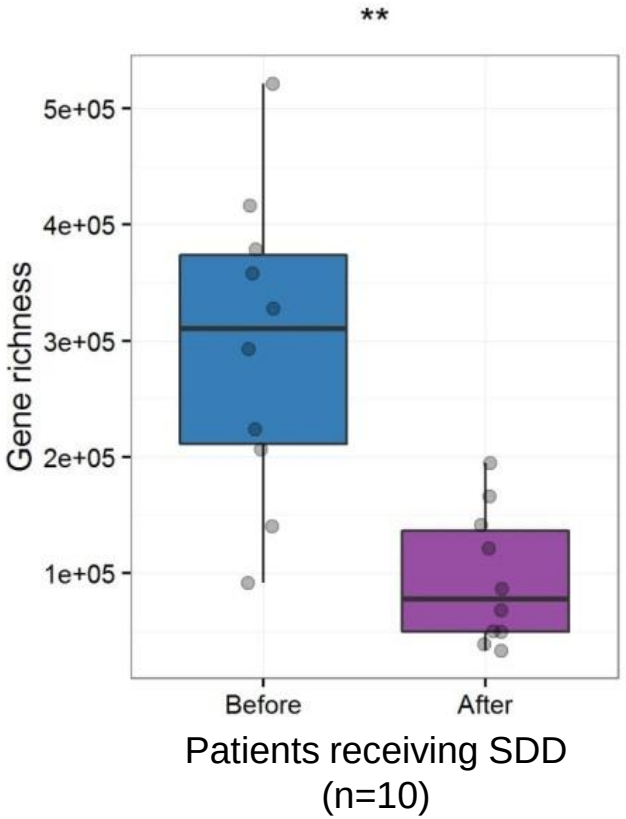
Variable	Regimen:					
	SC (21[a])		SOD (19[a])		SDD (17[a])	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Probe						
Total bacteria	3.7×10^9	2.2×10^9–6.2×10^9	1.6×10^9	7.8×10^8–3.4×10^9	1.9×10^9	8.7×10^8–4.3×10^9
Bacteroides	6.5×10^8	3.5×10^8–1.2×10^9	3.6×10^8	1.4×10^8–9.5×10^8	4.2×10^8	2.1×10^8–8.1×10^8
E. rectale[b]	5.1×10^8	3.0×10^8–8.5×10^8	1.4×10^8	5.4×10^7–3.4×10^8	6.2×10^7	2.6×10^7–1.4×10^8
R. intestinalis[b]	6.8×10^7	3.7×10^7–1.3×10^8	1.8×10^7	7.0×10^6–4.8×10^7	1.1×10^7	4.9×10^6–2.7×10^7
F. prausnitzii[c]	5.5×10^7	2.3×10^7–1.3×10^8	4.0×10^7	1.6×10^7–9.9×10^7	2.9×10^6	1.4×10^6–6.0×10^6
Atopobium	1.3×10^8	6.6×10^7–2.3×10^8	3.5×10^7	1.3×10^7–9.2×10^7	4.2×10^7	1.4×10^7–1.2×10^8
Bifidobacteria	4.4×10^7	1.6×10^7–1.2×10^8	1.6×10^7	5.4×10^6–4.6×10^7	5.8×10^7	1.8×10^7–1.8×10^8
Ruminococci	2.0×10^8	1.3×10^8–3.3×10^8	8.6×10^7	3.8×10^7–2.0×10^8	7.8×10^7	3.1×10^7–1.7×10^8
Enterobacteriaceae[c]	7.2×10^7	3.6×10^7–1.4×10^8	4.8×10^7	1.7×10^7–1.4×10^8	4.1×10^6	2.0×10^6–8.3×10^6

Table 4 Numbers and statistical analysis of enterococci per gram faeces

	SC ($n = 21$)	SOD ($n = 19$)	SDD ($n = 17$)	SC vs. SOD[a]	SC vs. SDD[a]	SOD vs. SDD[a]
E. faecalis	2.6×10^6	7.6×10^6	6.9×10^7	0.002	0.000	0.000
E. faecium	6.3×10^6	9.8×10^6	5.4×10^7	0.142	0.000	0.000

Selective digestive decontamination (SDD) affects the composition of the intestinal microbiota

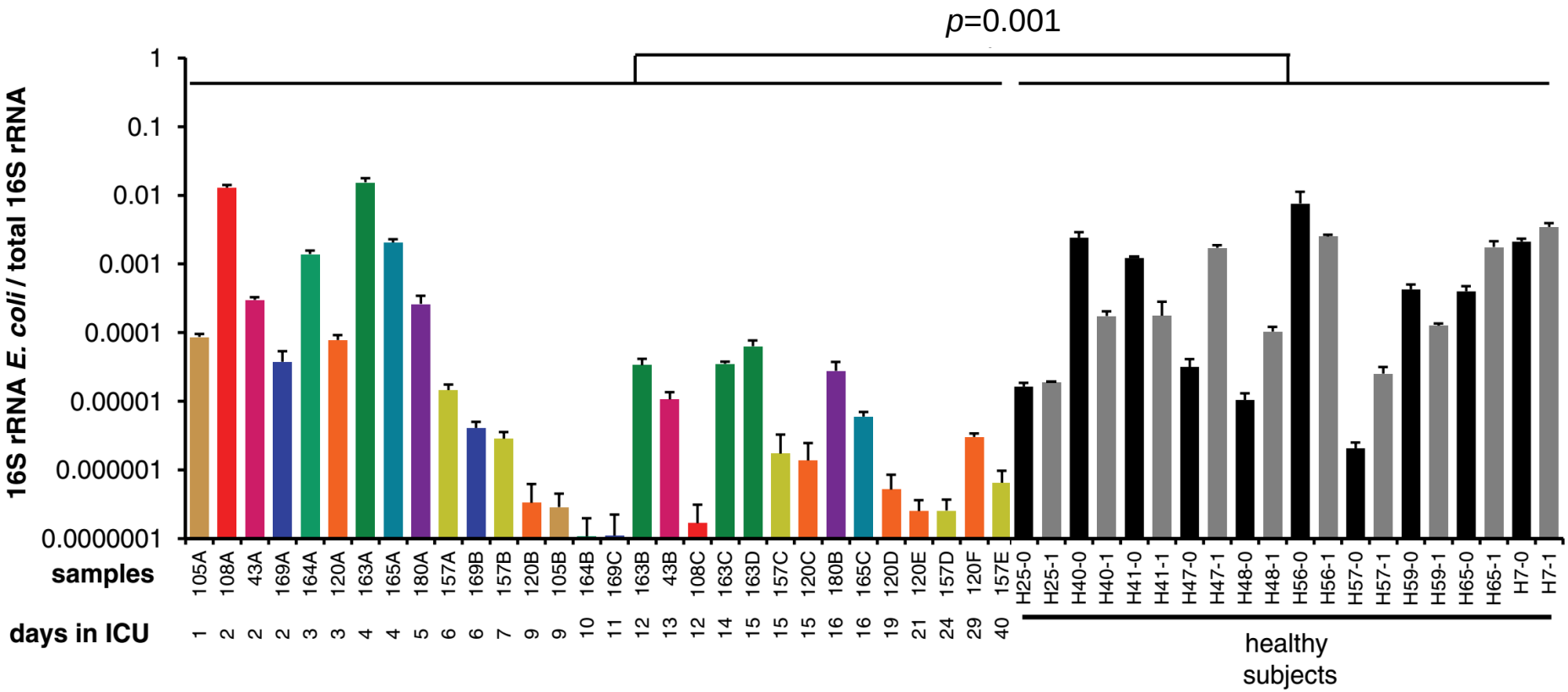




Buelow, E. et al. *Microbiome* **5**, 88 (2017), **Ruppé, E.** et al. *bioRxiv* 196014 (2017).

Selective digestive decontamination (SDD) affects the composition of the intestinal microbiota

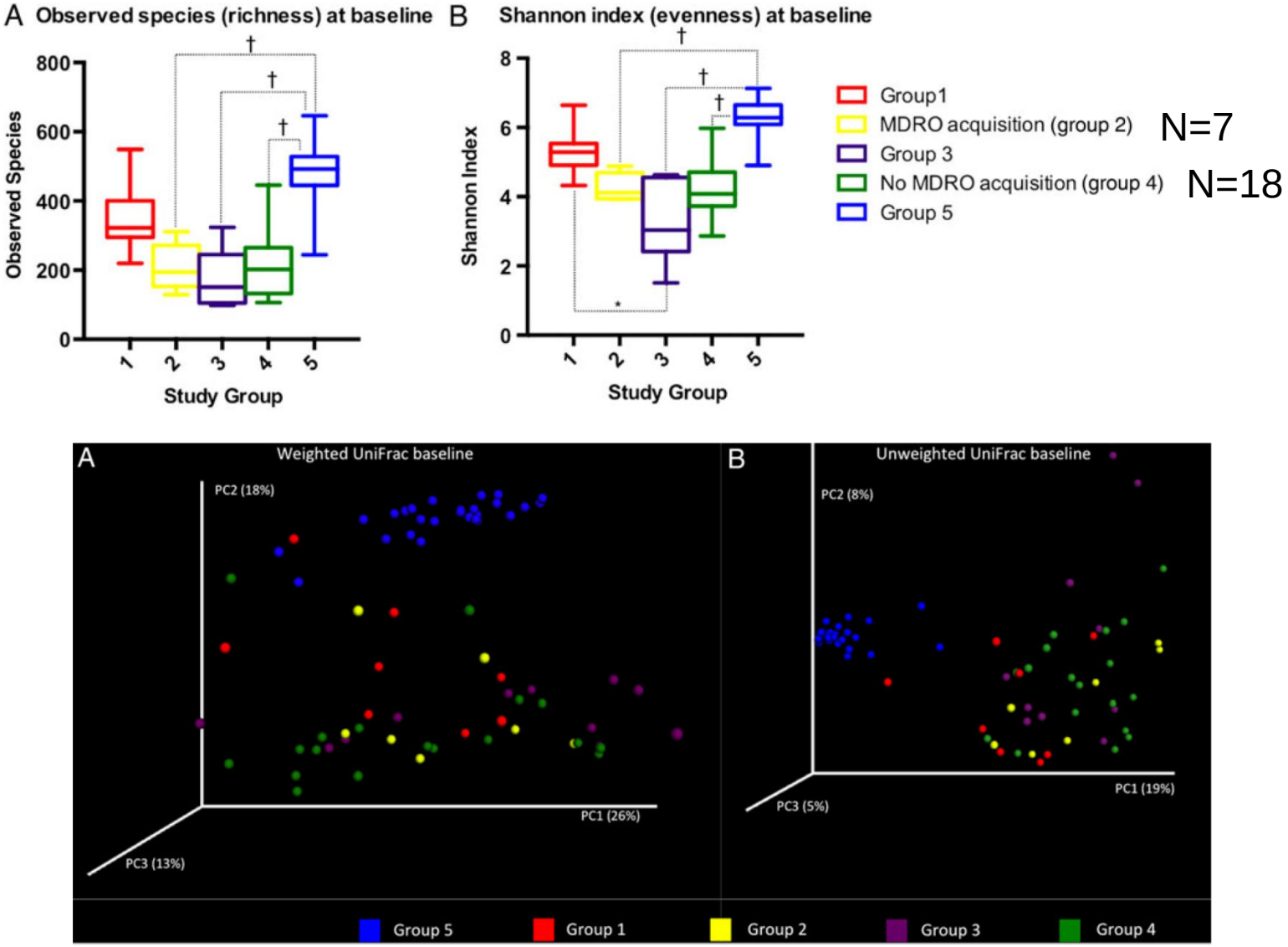
Variability of the relative abundance of *E. coli* in SDD-receiving patients and healthy controls.



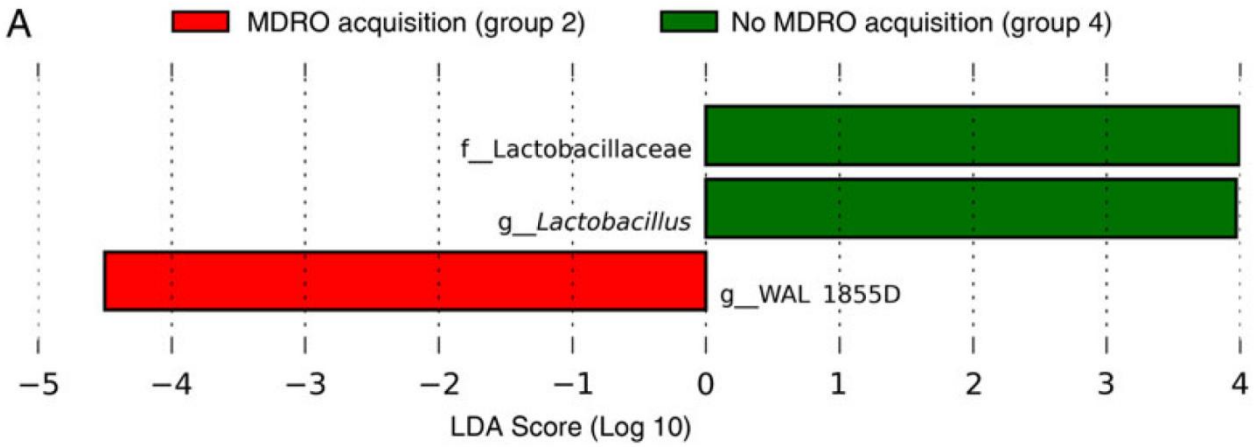
Buelow, E. et al. *Microbiome* **5**, 88 (2017),

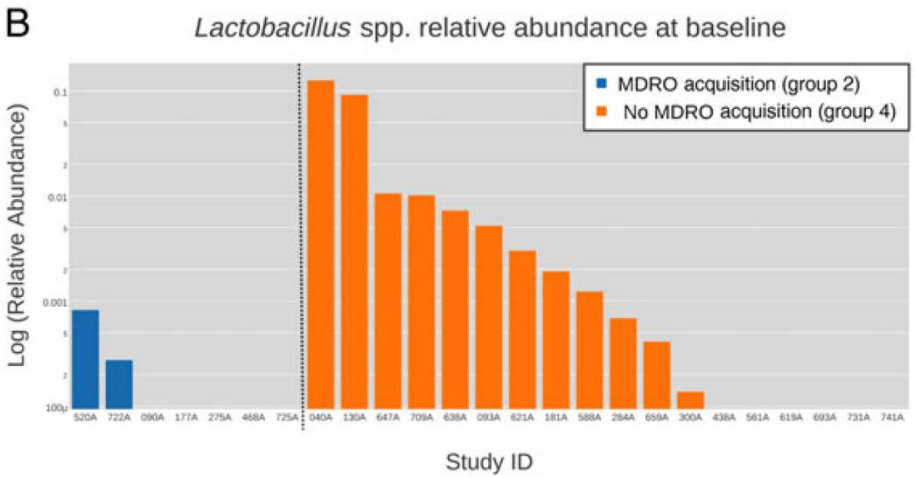
4. Case control studies

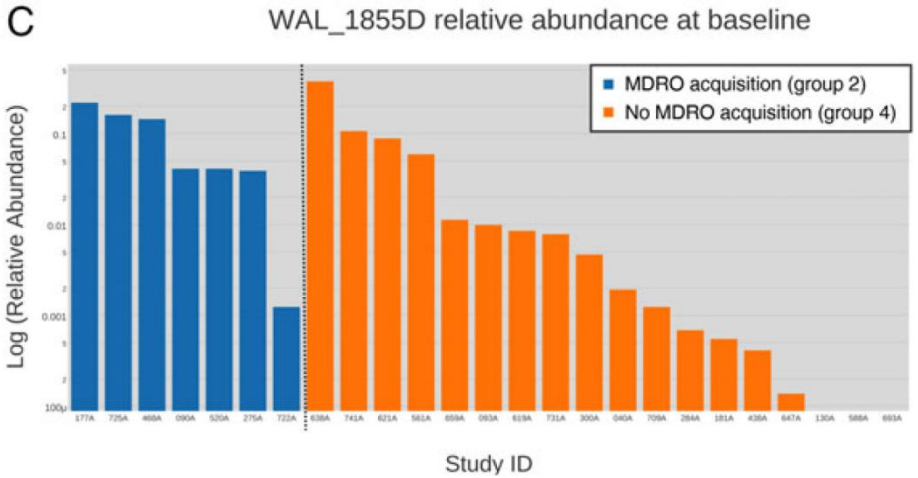
Does the intestinal microbiota oppose to the acquisition/persistence of antibiotic-resistant bacteria?



Does the intestinal microbiota oppose to the acquisition/persistence of antibiotic-resistant bacteria?

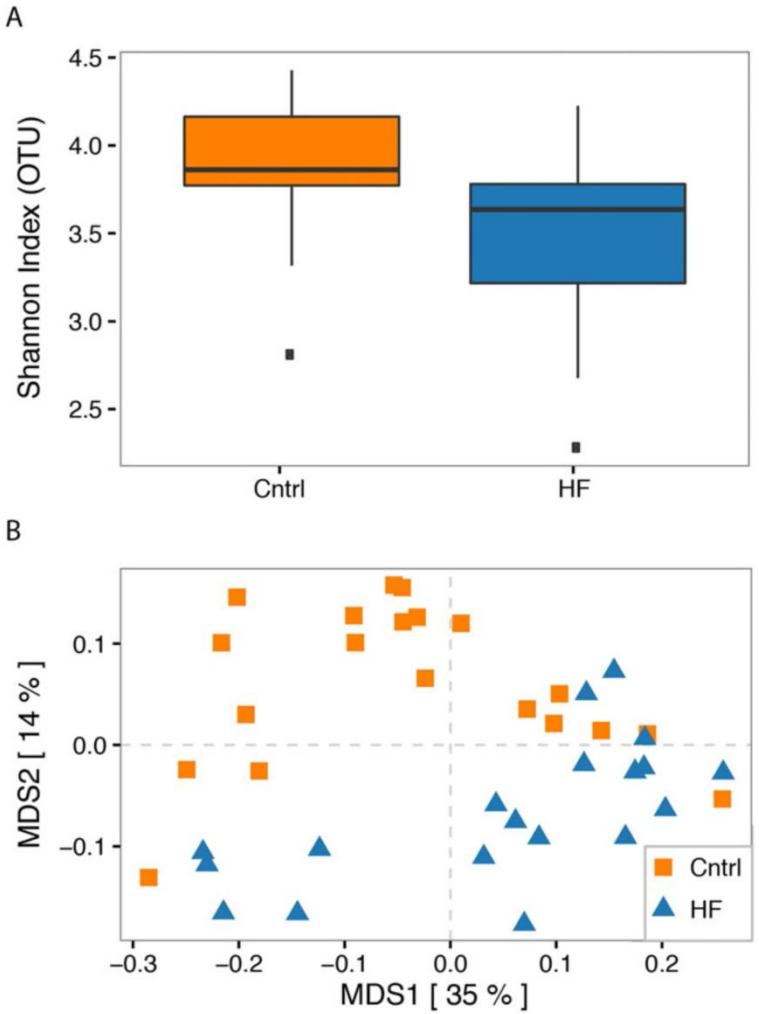






Let's be careful!

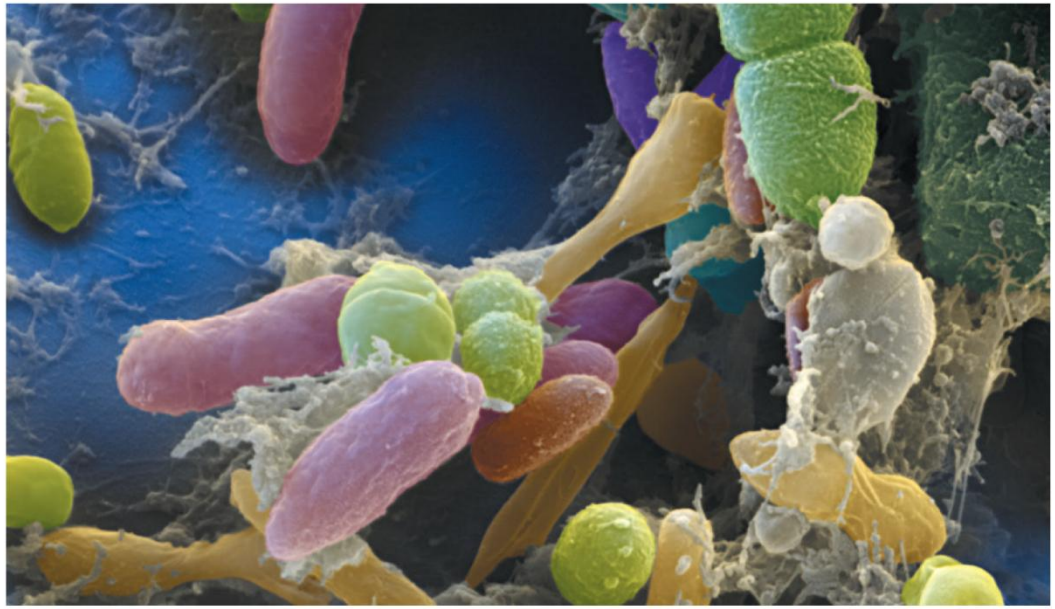
Intestinal microbiota and heart failure?



	HF Patients (n=20)	Controls (n=20)	*P*-value
Age, y	65 ± 3,17	65 ± 3,07	0,941
Men/women	11/9	11/9	1,000
BMI, kg/m^2	29,7 ± 1,44	29,1 ± 1,33	0,768
Smoking habits			0,733
never	11	11	
in the past	7	7	
current	2	2	
HF aetiology	ICMP 55% DCM 45%		
LVEF, %	22,3 ± 2,85	NM	
CRP, mg/L	11,1 ± 2,06	4,22 ± 1,12 (n=15)	0,006
NT-proBNP, ng/L	6564,5 ± 1187,23	109,2 ± 45,91 (n=17)	<0,001
NYHA class			
I	1		
II	4		
III	6		
IV	9		
Medication			
Beta-blockers	80%	15%	<0,001
Diuretics	70%	35%	0,027
ACE-inh/ARBs	70%	35%	0,027
Ald-ags	40%	15%	0,081
Comorbidities			
DM type II	35%	15%	0,152
HTN	70%	40%	0,059

The intestinal microbiota “hype”

EYE OF SCIENCE/SPL



A scanning electron micrograph of bacteria in human faeces, in which 50% of species originate from the gut.

Microbiome science needs a healthy dose of scepticism

1. Can experiments detect differences that matter?
2. Does the study show causation or just correlation?
3. What is the mechanism?
4. How much do experiments reflect reality?
5. Could anything else explain the results?

Hanage, W. P. *Nature News* **512,** 247 (2014).

5. How to move on?

#MicrobiomeinICU

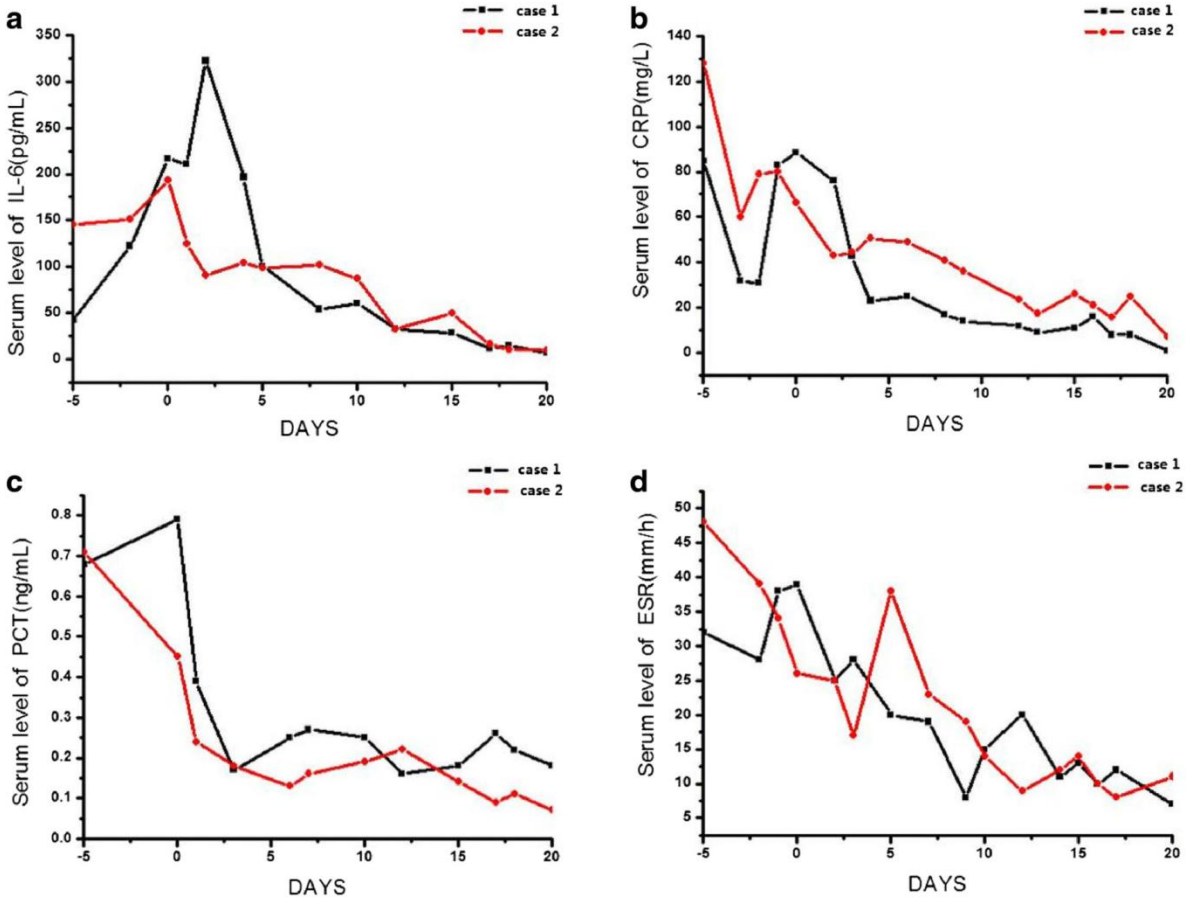
#Protectmymicrobiome #ICU

#Leavemymicrobiotaalone #ICU

#STOPantibiotics

#FMT

Two male patients, a 65-year-old and an 84-year-old, were initially diagnosed with cerebellar hemorrhage and cerebral infarction, respectively, after admission.
The two patients each received a single nasogastric infusion of sterile-filtered, pathogen-free feces from a healthy donor.



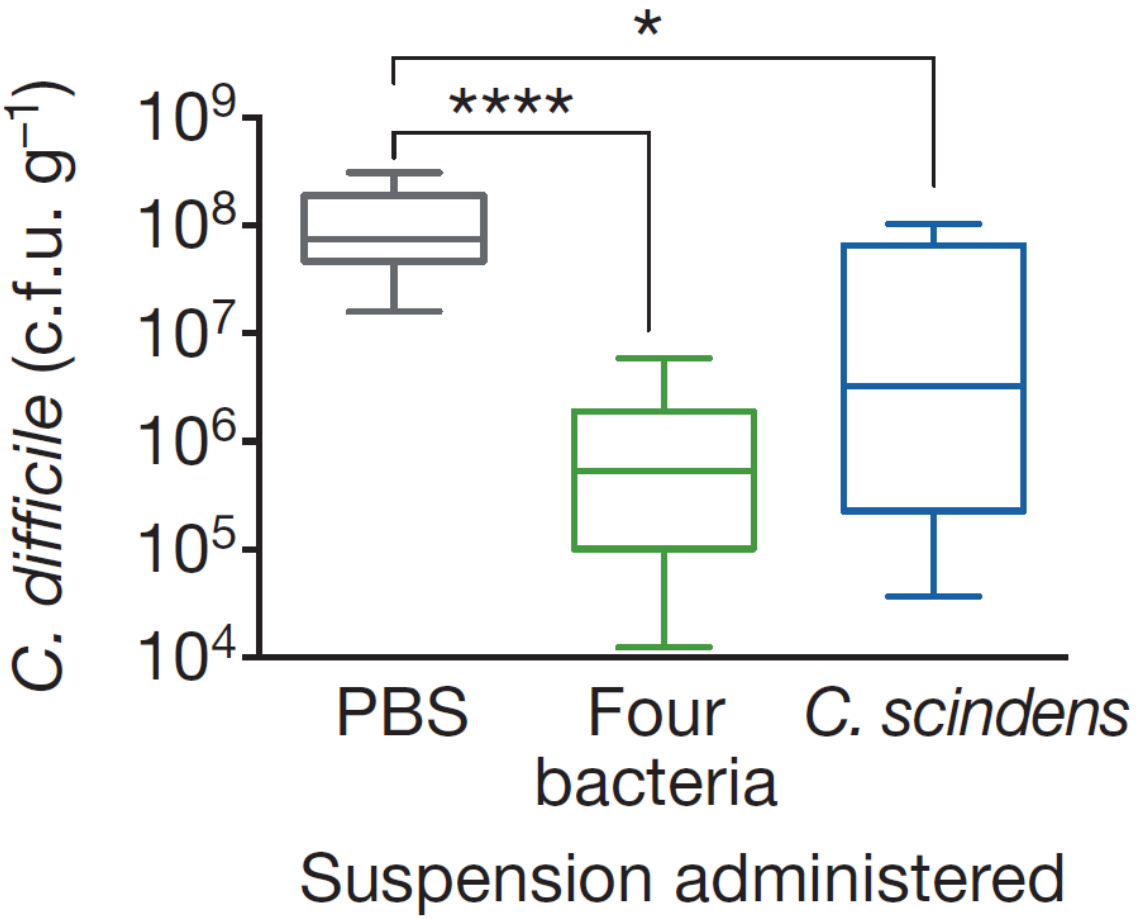
Should FMT be used in ICU?

Table 1. Clinical use of fecal microbial transplantation in critical illness where *Clostridium difficile* has been excluded

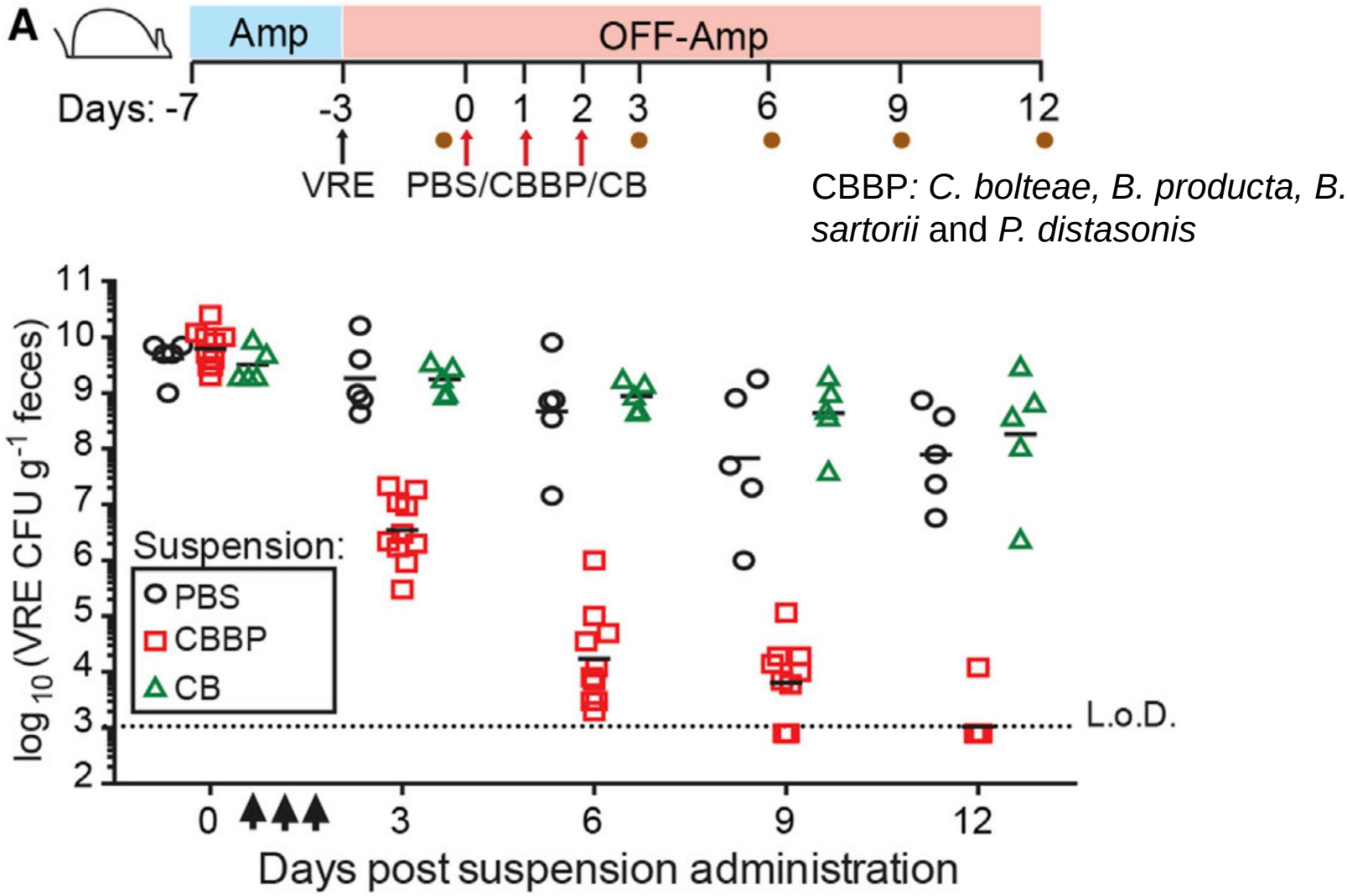
Patient	Presentation	Concomitant Rx	FMT	Recovery
16-year-old girl [7▪▪]	Trauma, TBI, intractable diarrhea, AAA enterocolitis	Dexamethasone, antibiotics Probiotics,	Day 72, donor feces (mother), cecal infusion	2 Days ↓ fever, ↓ diarrhea
29-year-old woman [9]	SIRS, intractable diarrhea, septic shock (H/O UC, colectomy)	antibiotics Probiotics	Day 20, donor feces per NE tube	1 Day ↓ fever, ↓ diarrhea
44-year-old woman [5▪▪]	Septic shock, intractable diarrhea, s/p partial gastrectomy/vagotomy	Antibiotics, probiotics ECMO, CRRT	Day 30, donor feces (brother) per ND tube	2 Days ↓ sepsis, 7 days ↓ diarrhea
65-year-old man [8▪▪]	Cerebral hemorrhage, MODS, septic shock, intractable diarrhea	Antibiotics	Day 20, donor feces (graduate student), sterile-filtered pathogen-free feces per NG tube	1 Day ↓ fever, 7 days ↓ diarrhea
84-year-old man [8▪▪]	Cerebral infarct, MODS, septic shock, intractable diarrhea	Antibiotics, probiotics	Day 7, donor feces (graduate student), sterile-filtered pathogen-free feces per NG tube	1 Day ↓ fever 7 days ↓ diarrhea

AAA, abdominal aortic aneurysm; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; FMT, fecal microbial transplantation; H/O UC, history of ulcerative colitis; MODS, multiorgan dysfunction syndrome; NG, nasogastric; SIRS, systemic inflammatory response syndrome; TBI, traumatic brain injury,

Would specific bacterial formulations work as well?



A consortium of 4 anaerobic bacteria collaborate to eradicate VRE from the gut in mice



Take-home messages

Metagenomics allows to assess the precise composition of the dominant intestinal microbiota

But it is not suitable to monitor conventional MDRB and known resistance genes.

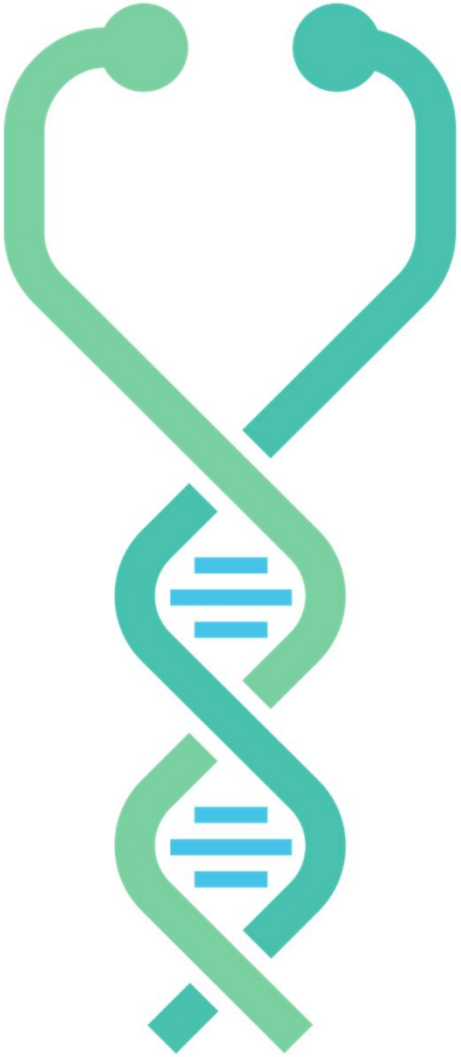
Antibiotics but also other drugs affect the composition of the intestinal microbiota.

Few studies in the ICU setting, compromised by the use of antibiotics.

Perspectives: protect and restore the intestinal microbiota.

Thank you for your attention!

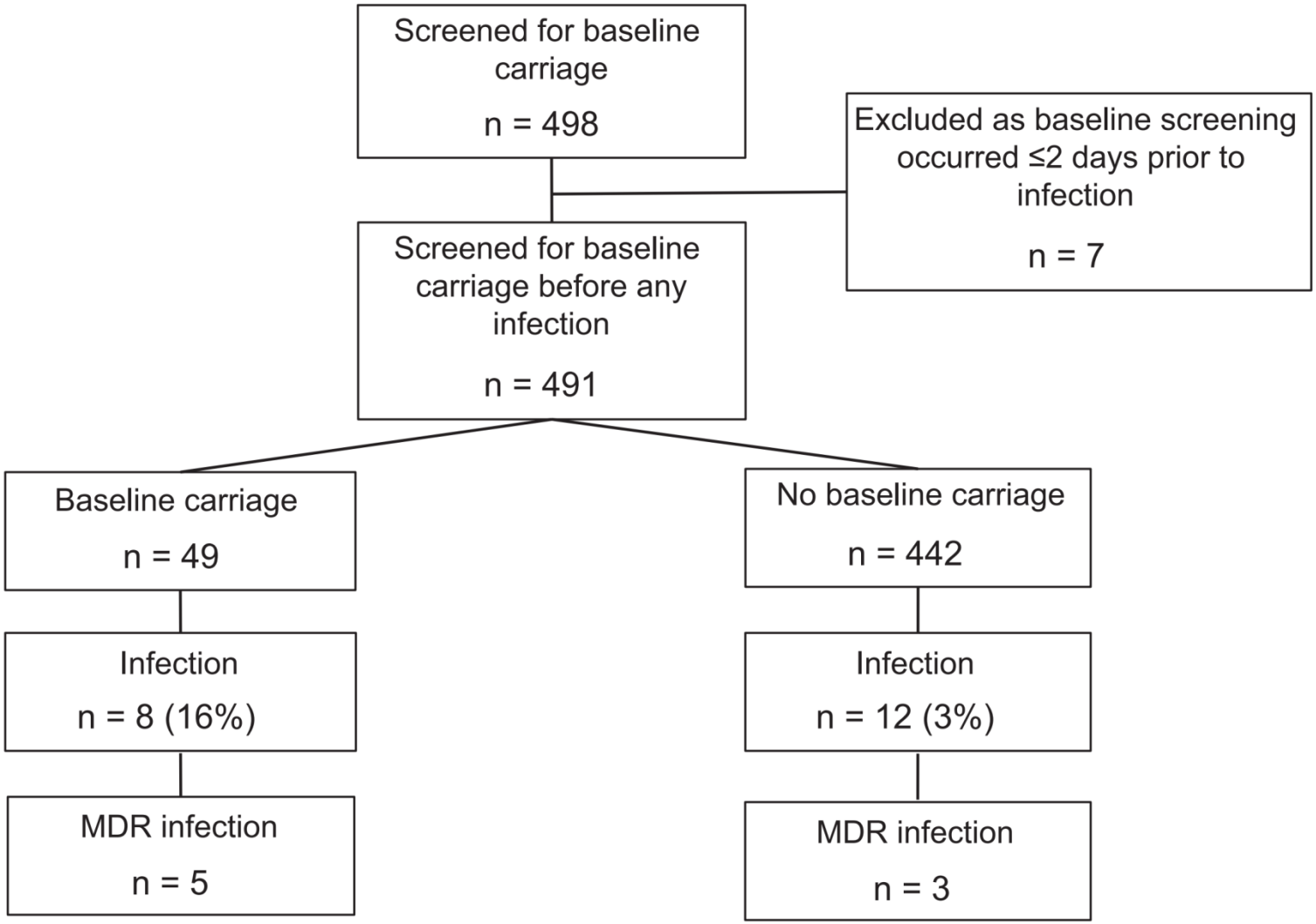
See you in Geneva for more metagenomics!



Third International Conference on Clinical Metagenomics

Campus Biotech, Geneva, 18-19 October 2018

The intestinal microbiota as a reservoir of bacterial pathogens



Gorrie, C.L. et al. *Clin. Infect. Dis.* **65**, 208–215 (2017).