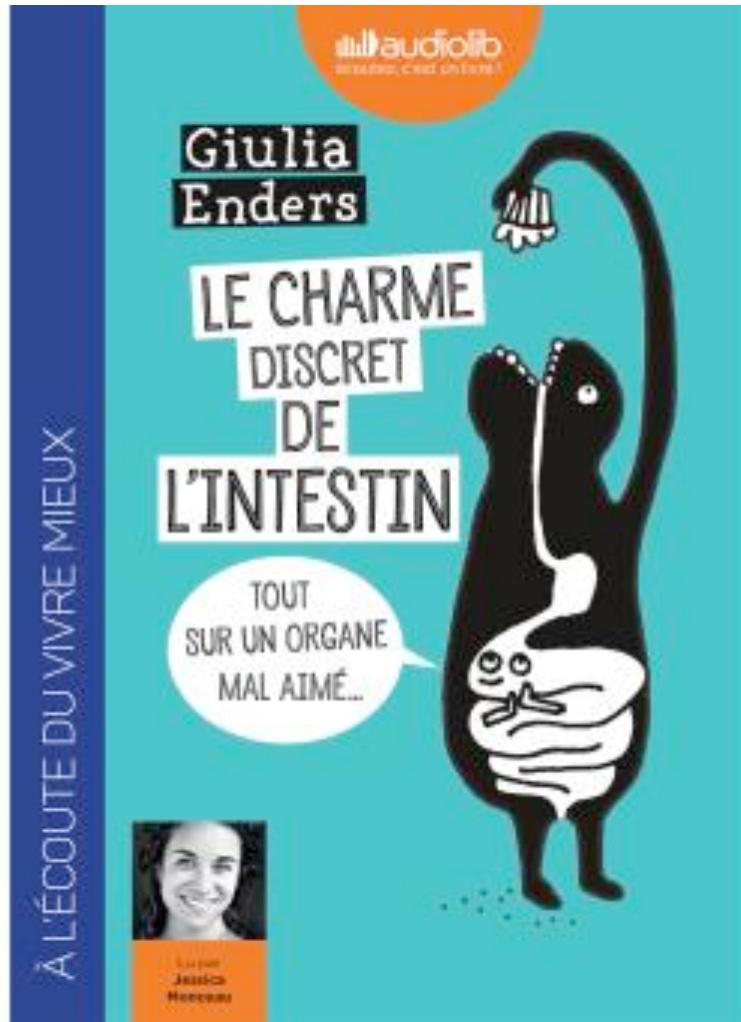


Studying bacterial communities by genomic methods

Gut microbiome dynamics and antimicrobial resistance ecology

Stu



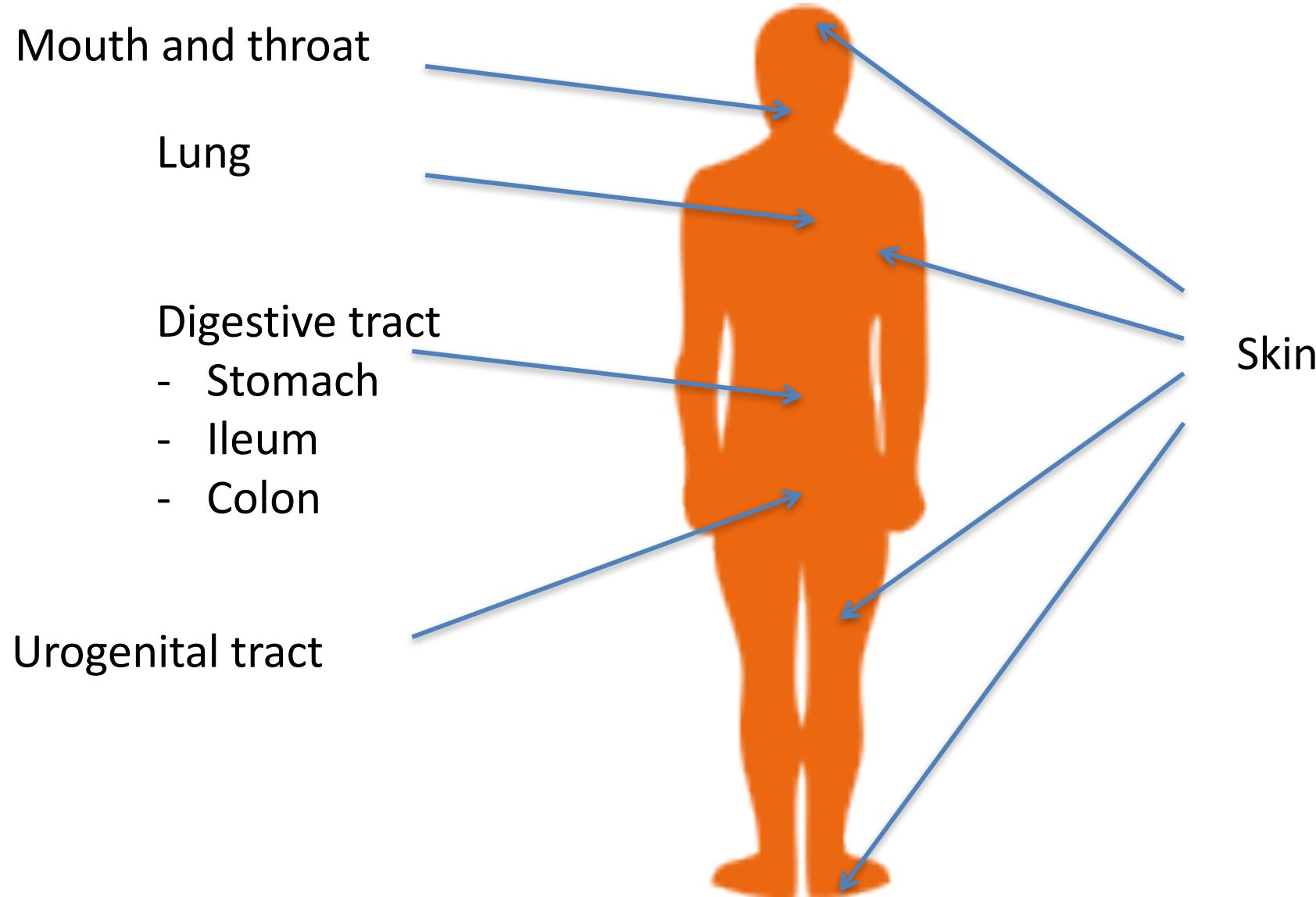
unities by
Is

cs and
cology

Outline

- Gut microbiome and health
- Methods to analyse the gut microbiome and its resistome
- Gut microbiome diversity
- The impact of antibiotics on the gut microbiome
- The resistome dynamics

Human associated microbiomes



Gut microbiome – few numbers

- $3 \cdot 10^{10}$ bacteria / g of feces (for an adult)
- $3.8 \cdot 10^{13}$ bacteria in total – total mass of 0.2 Kg
(estimate of $3 \cdot 10^{13}$ human cells)
- About 1000 species but variable numbers
- Dominated by Bacteroidetes (Gram negative) and Firmicutes (Gram positive)
- >70 % cultivable



Factors that shape the gut microbiome

- Diet
- Geography
- Age
- Health / Disease
- Medical intervention
- Host genetics
- (co)Evolutionary history

Functions of the microbiome

- Metabolic functions in the digestion
- Production of molecules, vitamins, short chain fatty acids
- Detoxification (bile salts hydrolase), antioxidants
- Barrier effect against pathogens (*C. difficile*)
- Train the immune system

Q. How the immune system distinguishes commensals from pathogenic bacteria?

Q. Notions of dysbiosis and of pathobiont

Impact of gut microbiota on health and disease

- Gut microbiome and disease from the digestive tract (Inflammatory bowel disease, Crohn, colitis)
- Gut microbiota and metabolic diseases: diabetes obesity
- Gut microbiota and immune related disease (asthma, allergies)
- Gut microbiota and cardiovascular disease
- Gut microbiota and neurological disease (autism) – “the brain-gut axis”

.....

- ⇒ These conclusions are mostly based on correlations
- ⇒ Some experiments in animal models
- ⇒ Effect / benefit of faecal transplantation

Methods to analyze the gut microbiome

- Barcoding by sequencing the 16S rRNA
 - Composition of the microbiome in Operational Taxonomic Units (OTU)
- Whole metagenome sequencing
 - Composition of the microbiome and functional capacity (metabolic, virulence, antibiotic resistance)

DNA sequencing (Illumina)

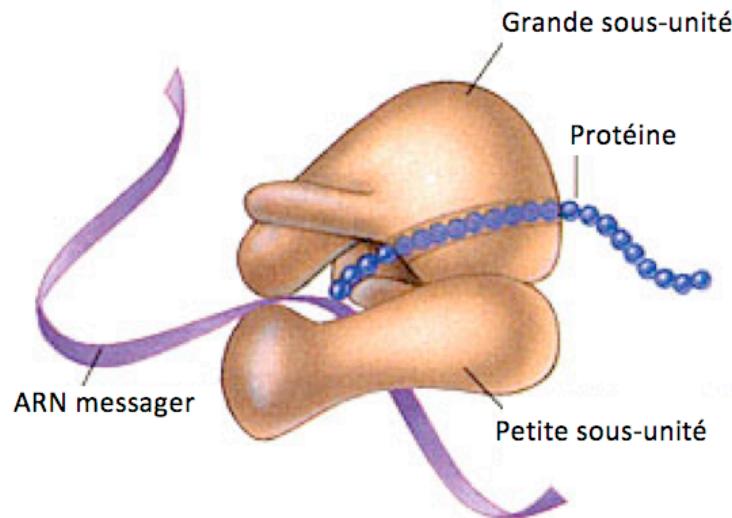


Sequence FastQ file

```
@M02475:33:00000000-AJE0A:1:1101:18082:1506 1:N:0:1
ATAAGAACCTATCCAAAAGAAATTATCATATTGCATTATCTGACAATTCTTATGGGATAGGTTCTTAGTGT
+
-A,6<--CFF<FFFE<88,,,,CF9FE,E,CF,@,CE,CCF9,<,,CE@CFF,C,,.,.,.,<CCFG,,C,
@M02475:33:00000000-AJE0A:1:1101:8515:1527 1:N:0:1
CGTTATTATCGCGTTACTCGCTTTCGAATCGAACCTTCATTGACAACGTGCCAGACTCGTCTTCTAT
+
6-AC-CE9FC,C7FC,@CC,8@CFEC+,;@,6,+7;C,;6;,C,<,,.,.;+,,,6,,,::,<,8:@6.,
@M02475:33:00000000-AJE0A:1:1101:9390:1553 1:N:0:1
AATTAGGTTCATTCACCGCAGCGGCCGCCAGCTTCAGATGAGCGTTCTCGATCAGTCAGACCGTCGCCAA
+
--AC@9@FGF9FGG9EE7@++@7+@@@+@C7+CEFE9,,C,,.;+@FC@@FC,,,:@,,CC,,,CC+BC+:B,+
```

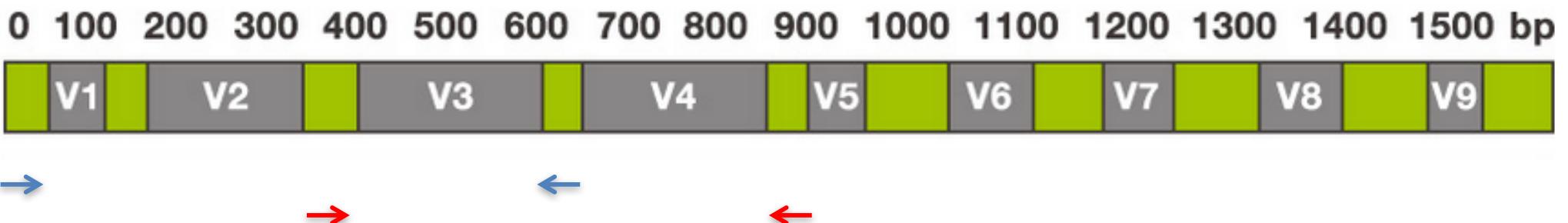
HiSeq 3000

16S rRNA sequencing

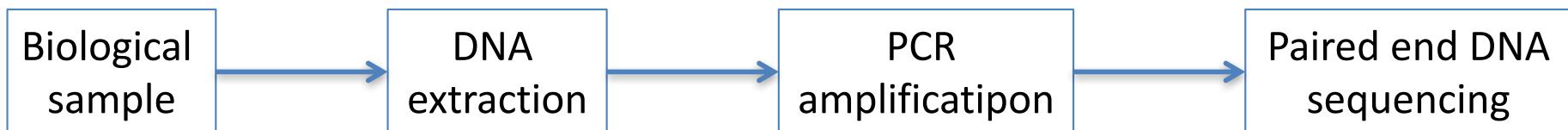


The ribosome a **universal** machine for protein synthesis

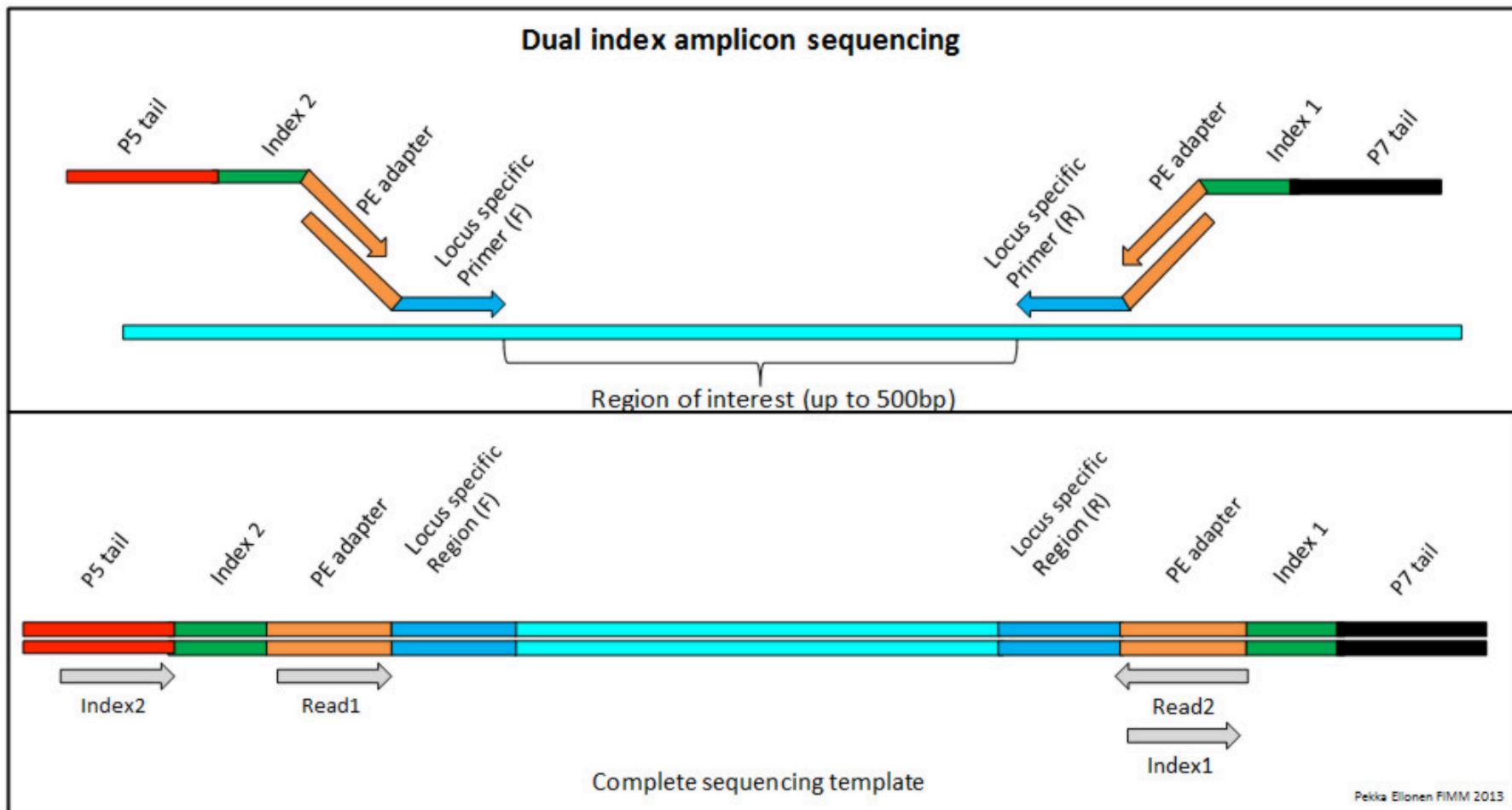
Three RNA molecules : 23S, **16S** et 5S (16S 1600 nucleotides) and Proteins



- Principle: PCR amplification of variable regions by using primers corresponding to conserved regions



PCR amplifications



- ⇒ Two PCR steps: (1) amplification of the diversity – (2) multiplexing
- ⇒ Sequencing of more than 10000 molecules per sample
- ⇒ Analysis of the diversity

Bioinformatics analysis pipeline

Preprocessing

- Initial FastQ file
- Filter low quality reads
- Trim sequence for primer and quality
- Assemble the pair-end reads (Binning)
- FastQ file of assembled reads

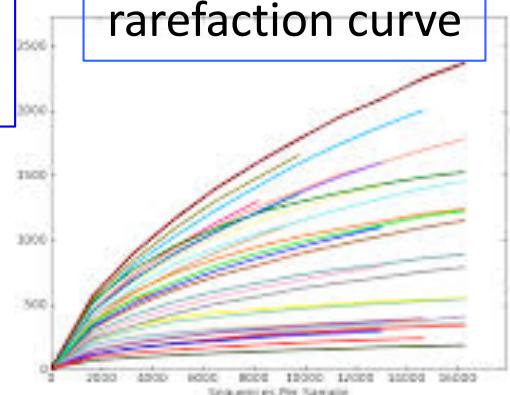
Taxonomic Classification

- Cluster reads into operational taxonomic unit (OTU)
- Assign taxonomy to cluster (OTU). Ribosomal sequence classifier

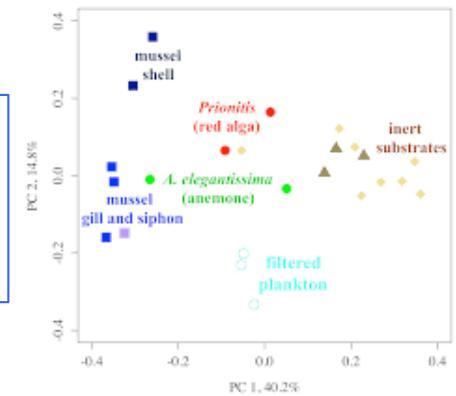
Excel file with taxa and frequency

Diversity and Visualization

α -diversity rarefaction curve



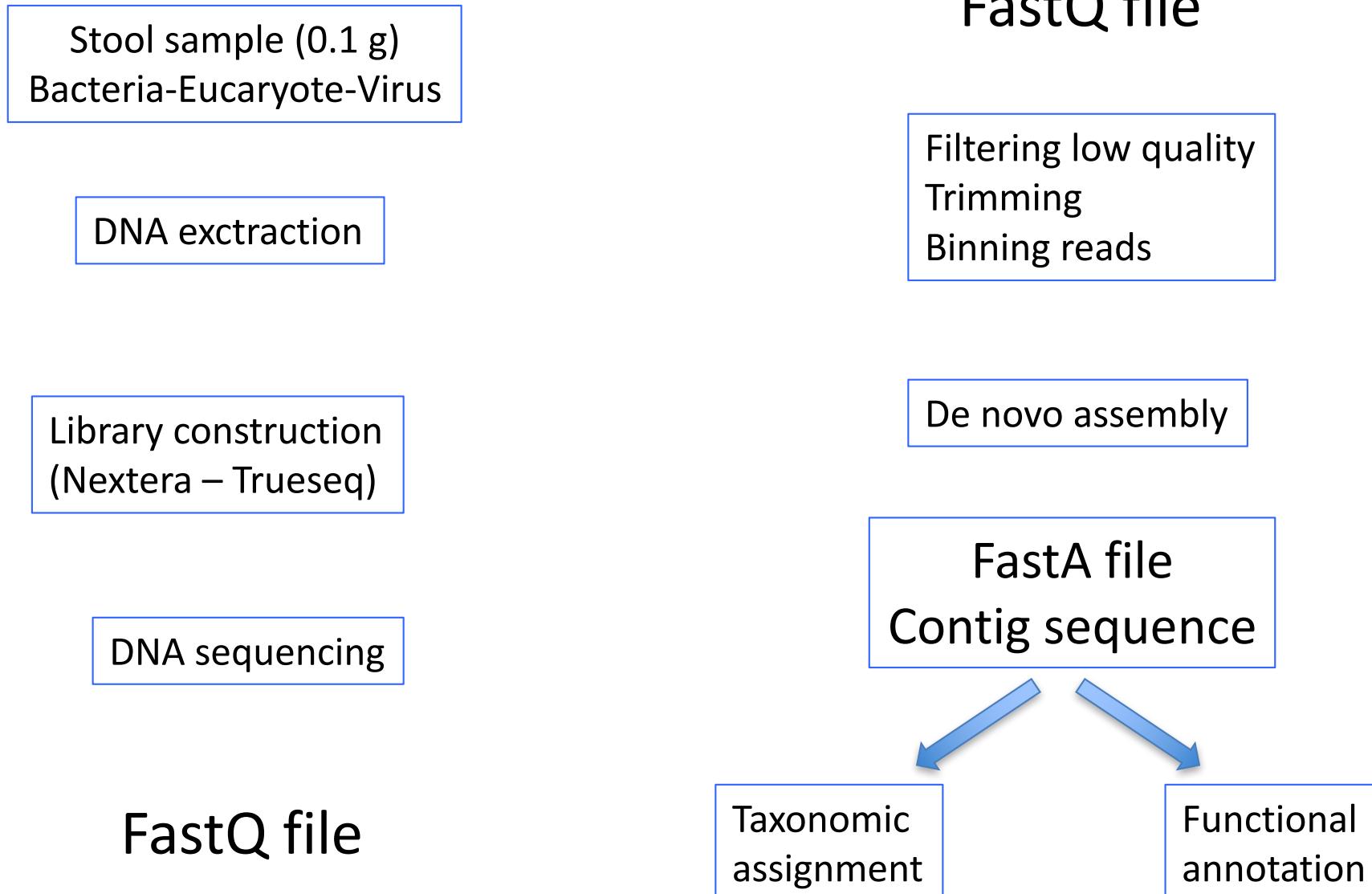
β -diversity Principal coordinate analysis



Two key steps

- Cluster reads into operational taxonomic unit (OTU)
 - Quality of the sequence
 - Region
 - Similarity criteria
 - Opportunity for innovation
 - Assign taxonomy to cluster (OTU). Ribosomal sequence classifier
- ⇒ Functional hypotheses based on OTUs composition
- ⇒ Metabolic reconstruction

Whole metagenome shotgun



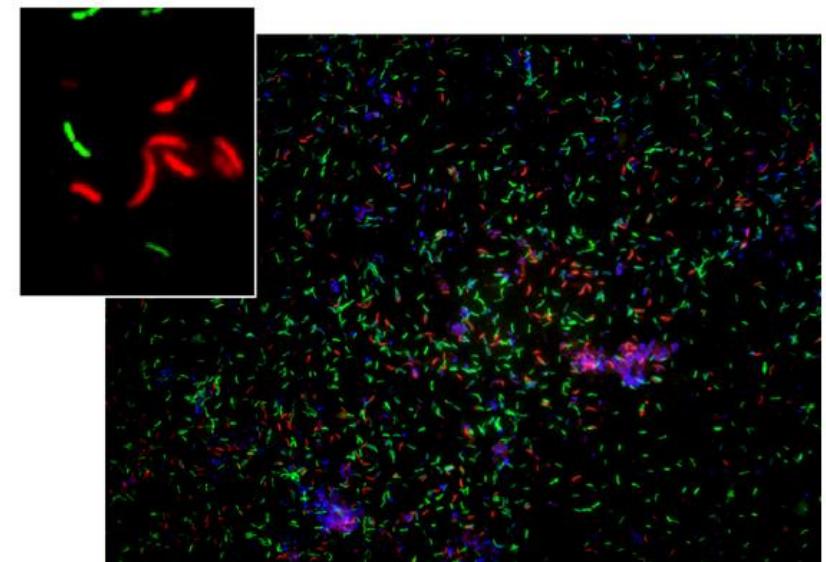
- Metagenome assembly:
 - 10 millions clusters 2×100 bases= 2 Gbases
 - 400 x E. coli
 - For species present at 1%: 4 x coverage
 - Similar sequence => problems
 - Software: Metaspades
- Taxonomic assignments
 - On reads or contigs
 - Based on K-means (Krakken) or by similarity
- Annotation of contigs and pathway enrichments

Relative / absolute quantification

Culture on plate: only cultivable bacteria
- Selective medium for specific species

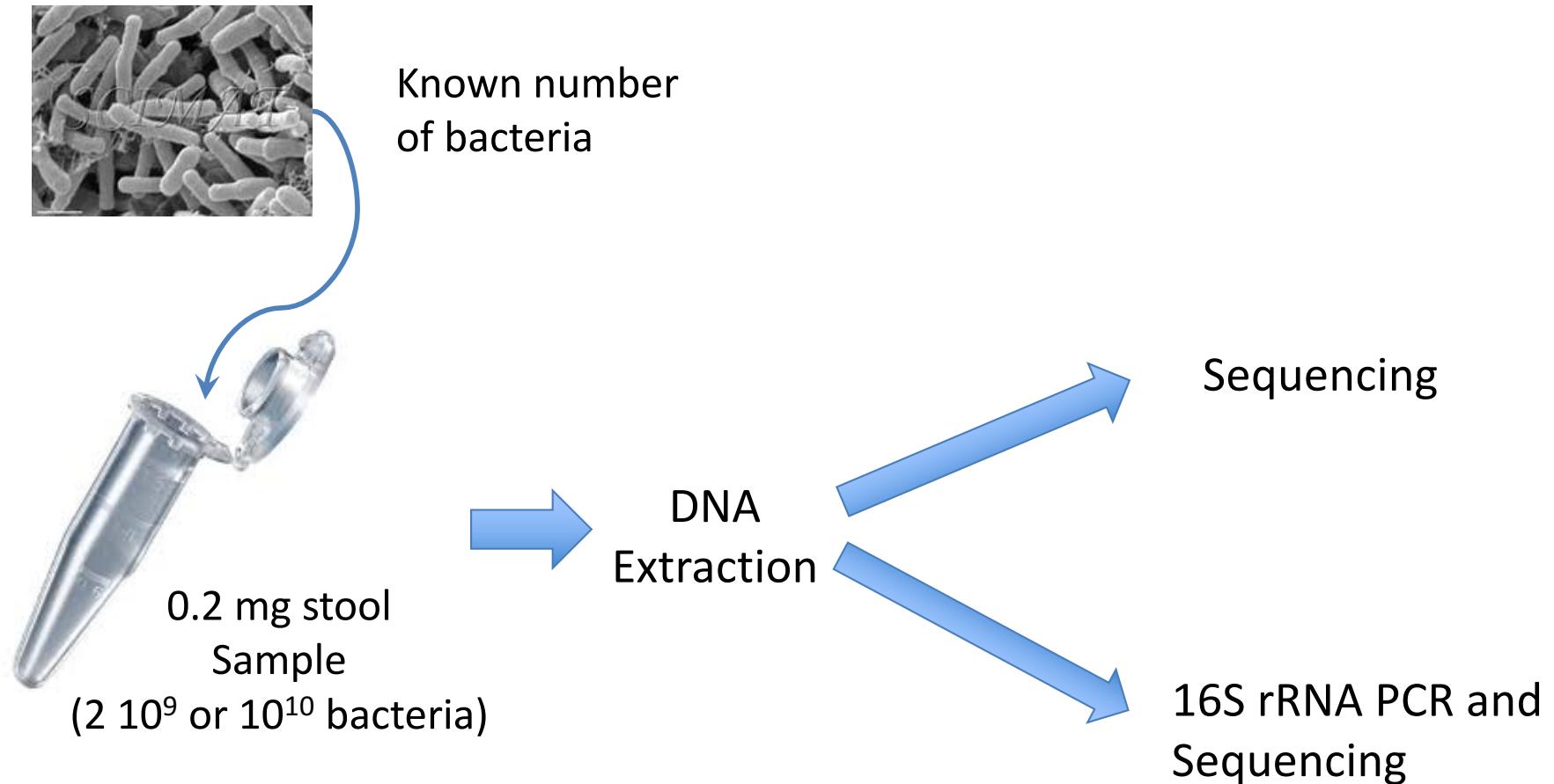


Fluorescent *in situ* hybridization
rRNA probes
Different specificities



Inclusion of internal standard (Spike)

Spiking stool samples



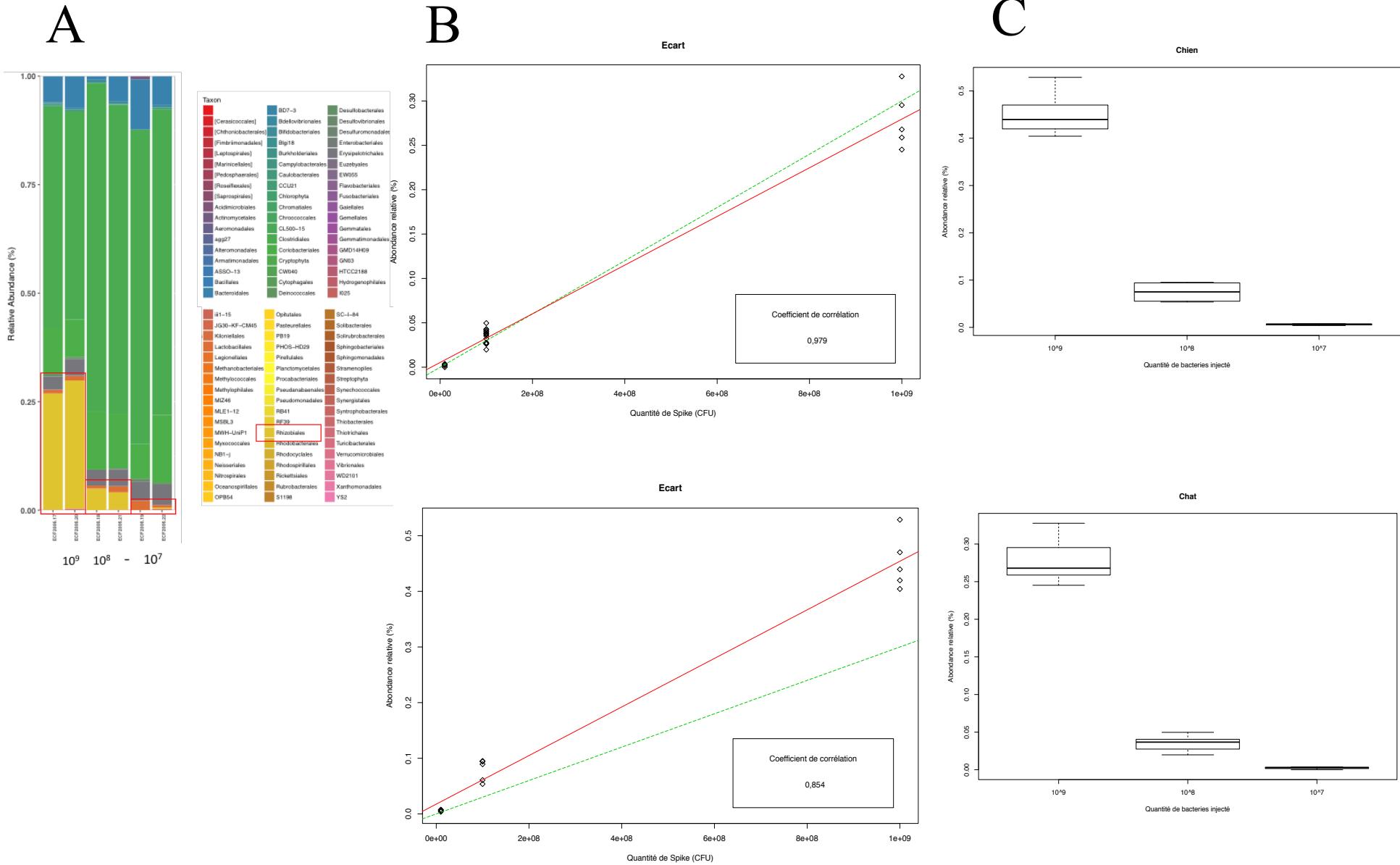
Spiking stool samples

- Choice of the bacteria
 - Missing in the analyzed sample
 - Easy to cultivate
 - Easy to lyze (DNA extraction)
- ⇒Agrobacterium
- Absolute quantification

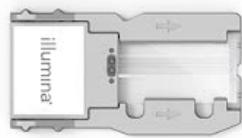
$$[\text{species A}] = [\text{Agro}] \times \text{reads}_{\text{speciesA}} / \text{reads}_{\text{Agro}}$$

$$[\text{Bacteria}] = [\text{Agro}] \times \text{reads} - \text{reads}_{\text{Agro}} / \text{reads}_{\text{Agro}}$$

Spiking the samples



Choice of the strategy and the sequencer



16S rDNA

5 10^6 clusters

200 samples
25 000 sequences

Extensive study
Only taxonomic info.



Whole metagenome shotgun

680 Gbases (2 x 150 b)
1 lane: 85 Gbases
17 000 *E. coli*

15 sample /lane
10 X coverage for
species present at
1%

Fewer samples
Taxonomic and
functional inferences
Lower depth

The resistome

Antibiotics families and their targets



Fluoroquinolone



Macrolide
Aminoglycosides
Tetracycline



Rifampicin
e

Metabolism

DNA

RNA

Proteins

Paroi bactérienne

Penicillin
Cephalosporine
Glycopeptides
Colistin



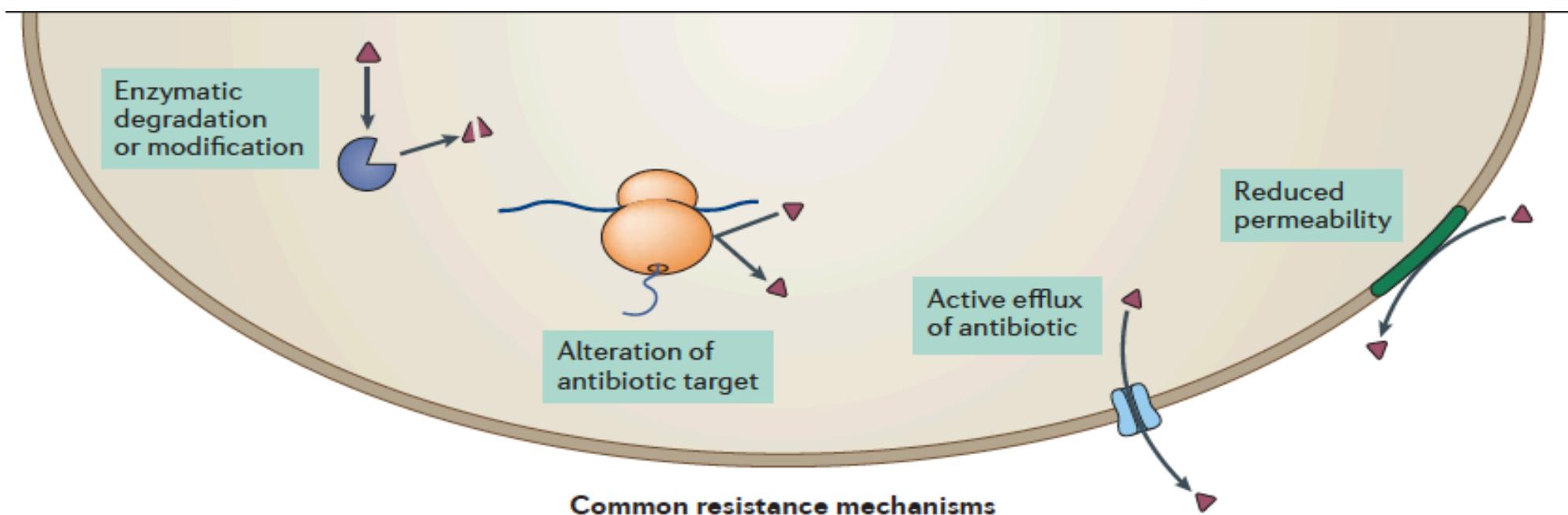
Diversity of resistance mechanisms

β -lactamase
Methylase
Acetylase
...

Ribosome binding
(TetQ), Lsa
Methylase
Mutations

Tetracycline: TetL

Aminoglycoside
Porin genes
Mutations



⇒Mutations in core genome genes
⇒Genes acquired by horizontal transfer

Characterization of the resistome

- Functional cloning
 - Shotgun library in an expression vector
 - Transformation of a test strain
 - Selection on different antibiotics

⇒ Issues: expression in *E. coli*
- In silico prediction
 - Analysis of metagenomic data
 - Search for putative resistance gene by similarity
(known or similar to known gene)

⇒ Issues: similarity threshold, functionality



Diversity of the microbiome and impact of antibiotics

Gut microbiome in urbanized and pre-agricultural populations

ARTICLE

Received 22 Nov 2013 | Accepted 14 Mar 2014 | Published 15 Apr 2014

DOI: 10.1038/ncomms4654

OPEN

Gut microbiome of the Hadza hunter-gatherers

Stephanie L. Schnorr^{1,*}, Marco Candela^{2,*}, Simone Rampelli², Manuela Centanni², Clarissa Consolandi³, Giulia Basaglia², Silvia Turroni², Elena Biagi², Clelia Peano³, Marco Severgnini³, Jessica Fiori², Roberto Gotti², Gianluca De Bellis³, Donata Luiselli⁴, Patrizia Brigidi², Audax Mabulla⁵, Frank Marlowe⁶, Amanda G. Henry¹ & Alyssa N. Crittenden⁷

ARTICLE

Received 19 Aug 2014 | Accepted 4 Feb 2015 | Published 25 Mar 2015

DOI: 10.1038/ncomms7505

OPEN

Subsistence strategies in traditional societies distinguish gut microbiomes

Alexandra J. Obregon-Tito^{1,2,3,*}, Raul Y. Tito^{1,2,*}, Jessica Metcalf⁴, Krishnavasan Sankaranarayanan¹, Jose C. Clemente⁵, Luke K. Ursell⁴, Zhenjiang Zech Xu⁴, Will Van Treuren⁴, Rob Knight⁶, Patrick M. Gaffney⁷, Paul Spicer¹, Paul Lawson¹, Luis Marin-Reyes⁸, Omar Trujillo-Villarroel⁸, Morris Foster⁹, Emilio Guija-Poma², Luzmila Troncoso-Corzo², Christina Warinner¹, Andrew T. Ozga¹ & Cecil M. Lewis¹

Combining 16S rDNA and metagenome sequencing

ARTICLE

Received 22 Nov 2013 | Accepted 14 Mar 2014 | Published 15 Apr 2014

DOI: 10.1038/ncomms4654

OPEN

Gut microbiome of the Hadza hunter-gatherers

Stephanie L. Schnorr^{1,*}, Marco Candela^{2,*}, Simone Rampelli², Manuela Centanni², Clarissa Consolandi³, Giulia Basaglia², Silvia Turroni², Elena Biagi², Clelia Peano³, Marco Severgnini³, Jessica Fiori², Roberto Gotti², Gianluca De Bellis³, Donata Luiselli⁴, Patrizia Brigidi², Audax Mabulla⁵, Frank Marlowe⁶, Amanda G. Henry¹ & Alyssa N. Crittenden⁷

CellPress

Current Biology
Article

Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota

Simone Rampelli,¹ Stephanie L. Schnorr,^{2,*} Clarissa Consolandi,³ Silvia Turroni,¹ Marco Severgnini,³ Clelia Peano,³ Patrizia Brigidi,¹ Alyssa N. Crittenden,⁴ Amanda G. Henry,² and Marco Candela^{1,*}

Combining 16S rDNA and metagenome sequencing

ARTICLE

Received 22 Nov 2013 | Accepted 14 Mar 2014 | Published 15 Apr 2014

DOI: 10.1038/ncomms4654

OPEN

Gut microbiome of the Hadza hunter-gatherers

Stephanie L. Schnorr^{1,*}, Marco Candela^{2,*}, Simone Rampelli², Manuela Centanni², Clarissa Consolandi³, Giulia Basaglia², Silvia Turroni², Elena Biagi², Clelia Peano³, Marco Severgnini³, Jessica Fiori², Roberto Gotti², Gianluca De Bellis³, Donata Luiselli⁴, Patrizia Brigidi², Audax Mabulla⁵, Frank Marlowe⁶, Amanda G. Henry¹ & Alyssa N. Crittenden⁷

CellPress

Current Biology
Article

Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota

Simone Rampelli,¹ Stephanie L. Schnorr,^{2,*} Clarissa Consolandi,³ Silvia Turroni,¹ Marco Severgnini,³ Clelia Peano,³ Patrizia Brigidi,¹ Alyssa N. Crittenden,⁴ Amanda G. Henry,² and Marco Candela^{1,*}

7000 reads +/- 2500 reads / sample

12 Millions 2 x 100 bases reads / sample

400 x coverage *E. coli* genome

Gut microbiome in urbanized and pre-agricultural populations



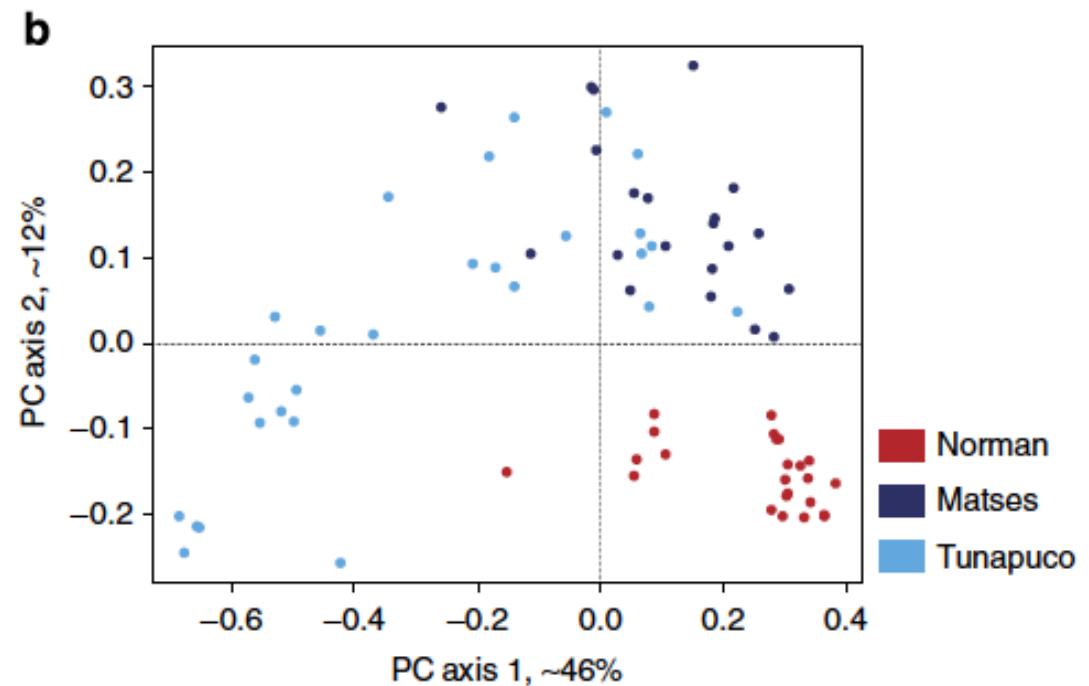
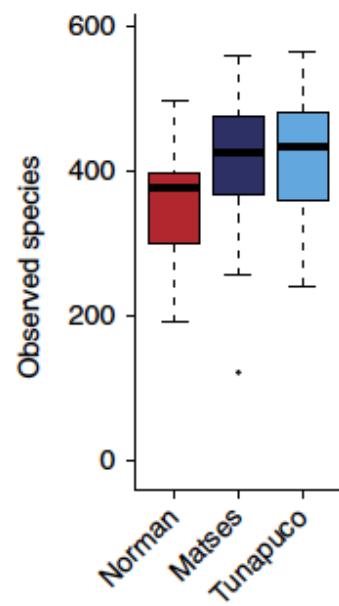
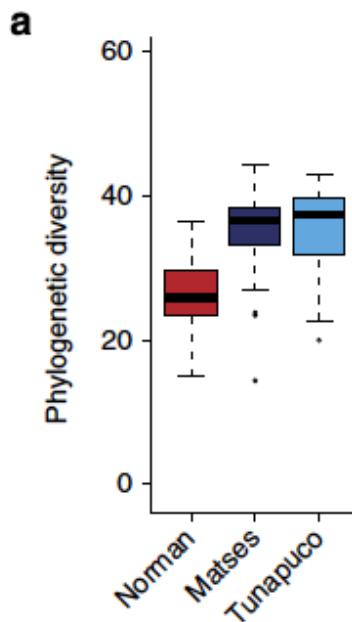
Hadza hunter-gatherers (27) /
Italian healthy (16)



Matses hunter-gatherers (25) **Urban from Oklahoma (23)**
Tunapuco traditional agricultural community (31)



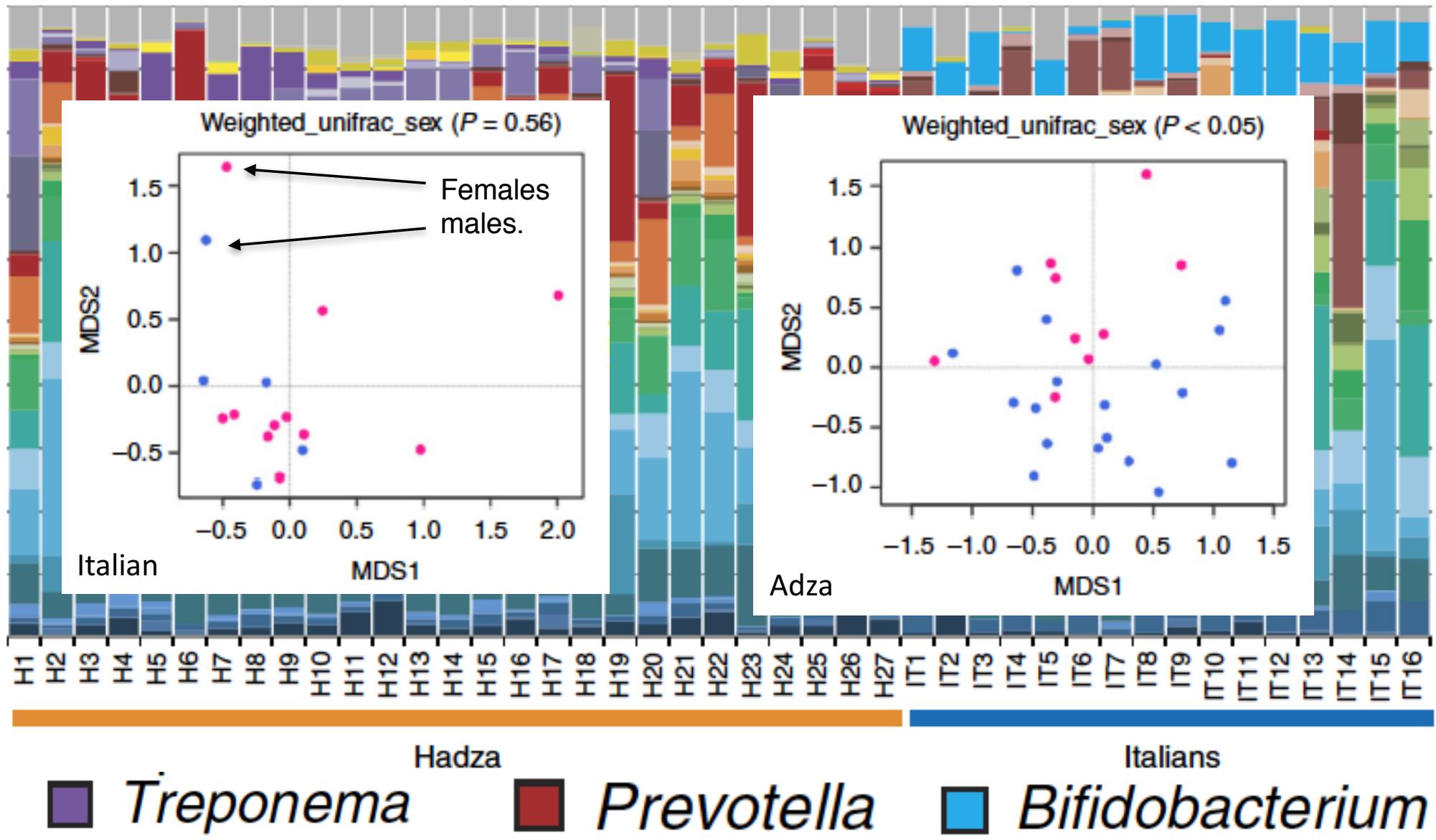
Decreased diversity in urbanized population



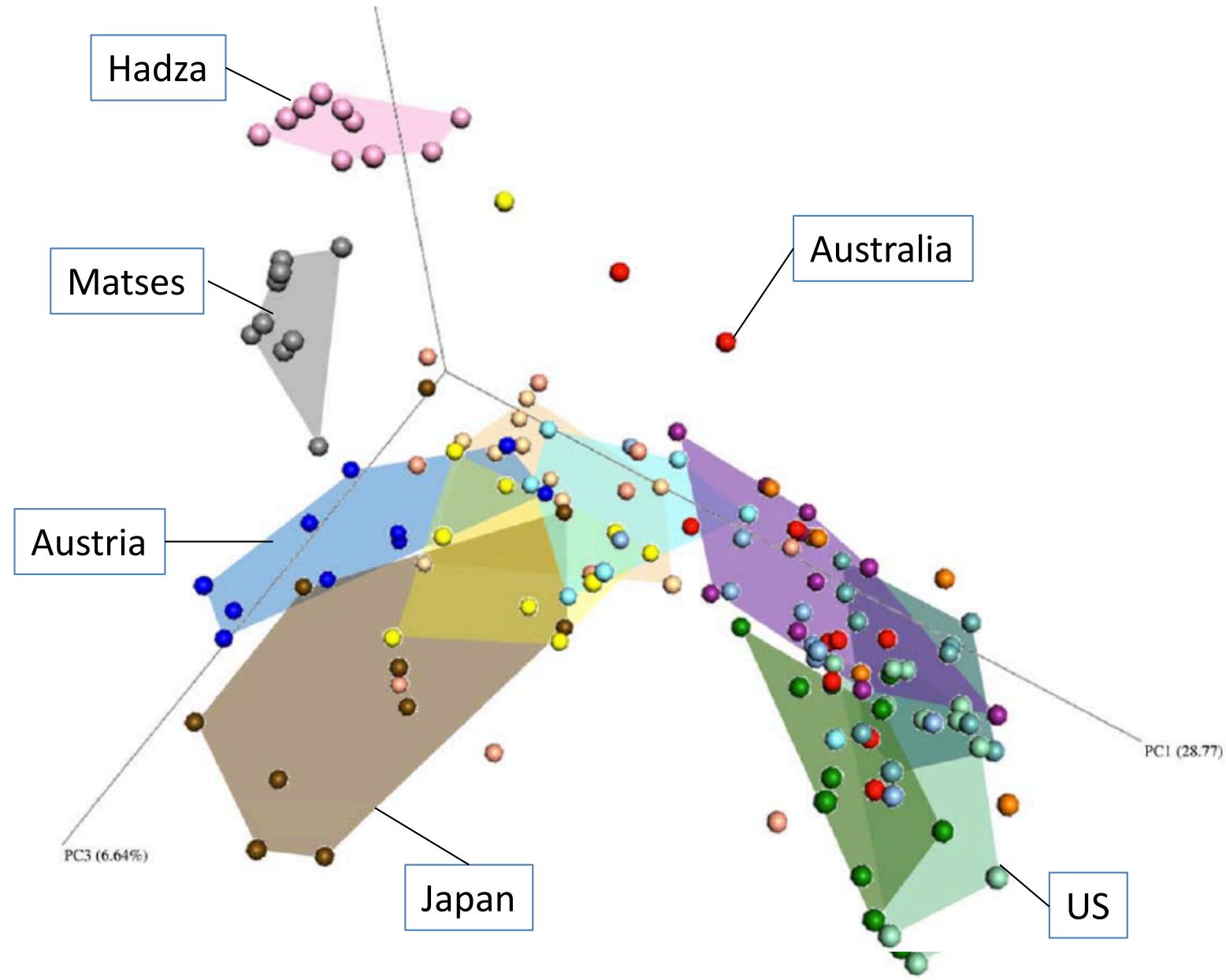
US population cluster compared to preagricultural and traditional populations

- Excess actinobacteria
- loss of treponema

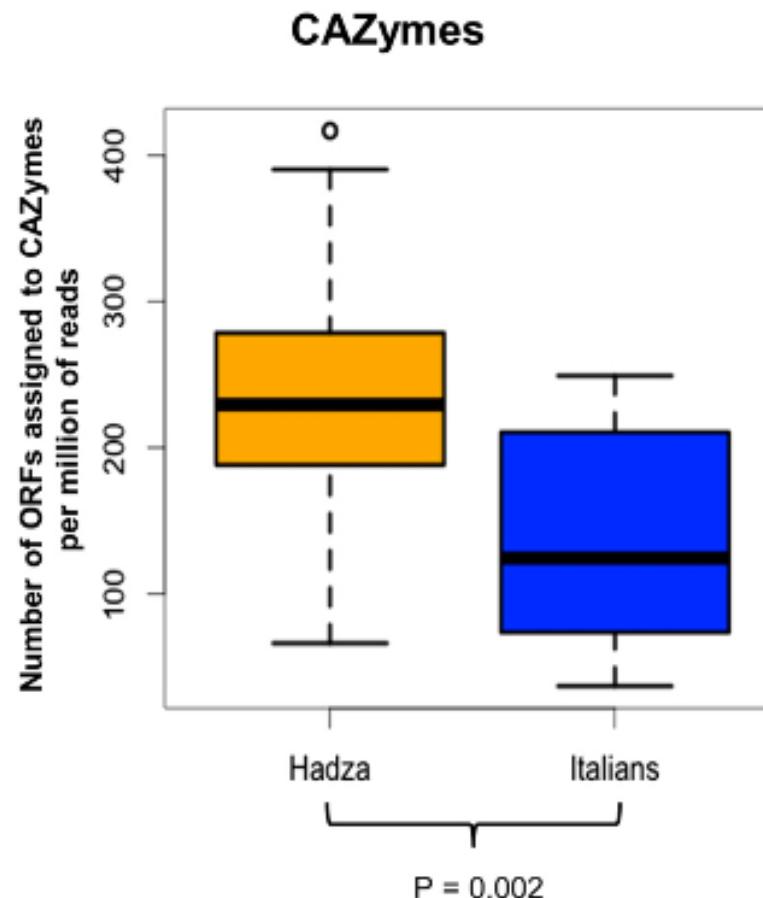
Comparison of Hadza and Italian microbiomes



Gut microbiome composition and populations

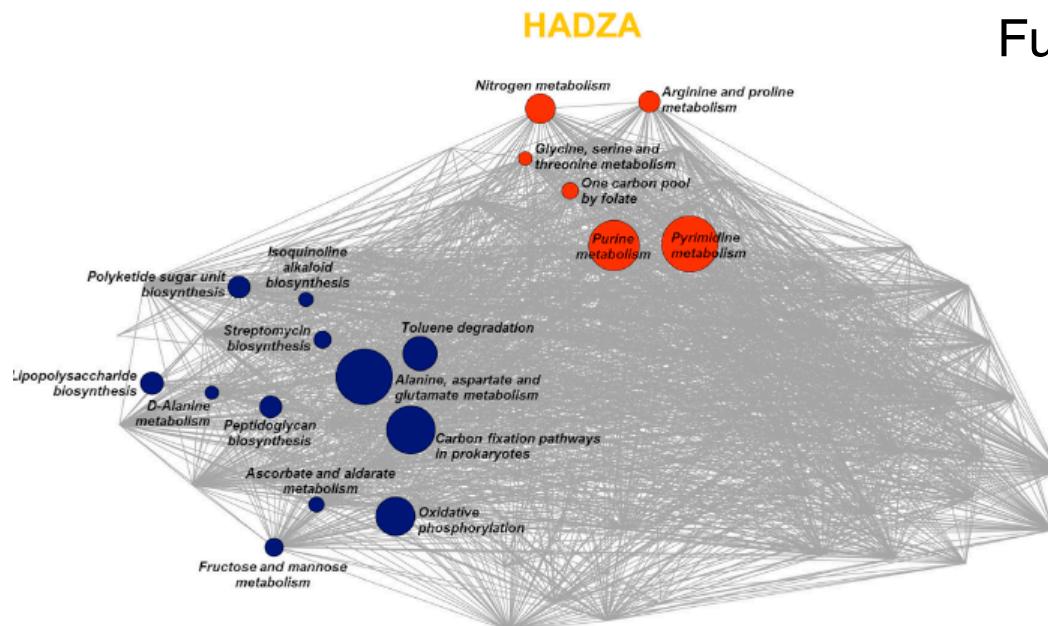


Different metabolic capabilities

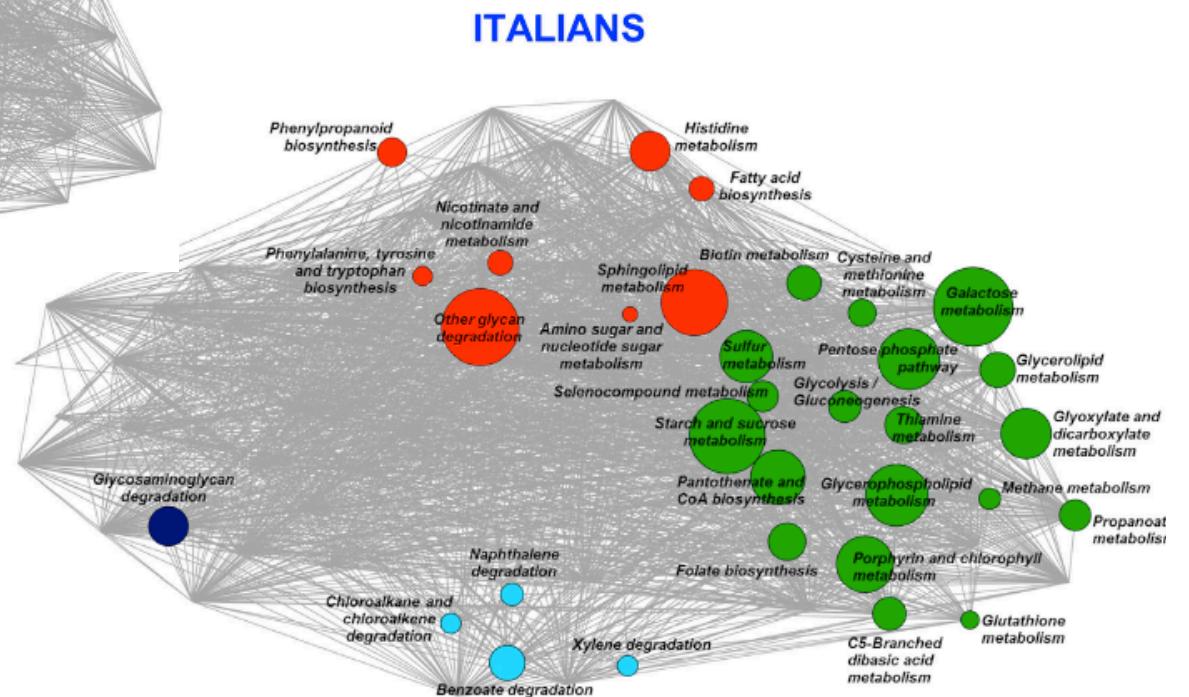


Enriched in branched-chain amino acid degradation
and aromatic amino acid biosynthesis.

Metabolic Potential of Hadza and Italian Metagenomes



Functional Microbial Co-abundance Pathways

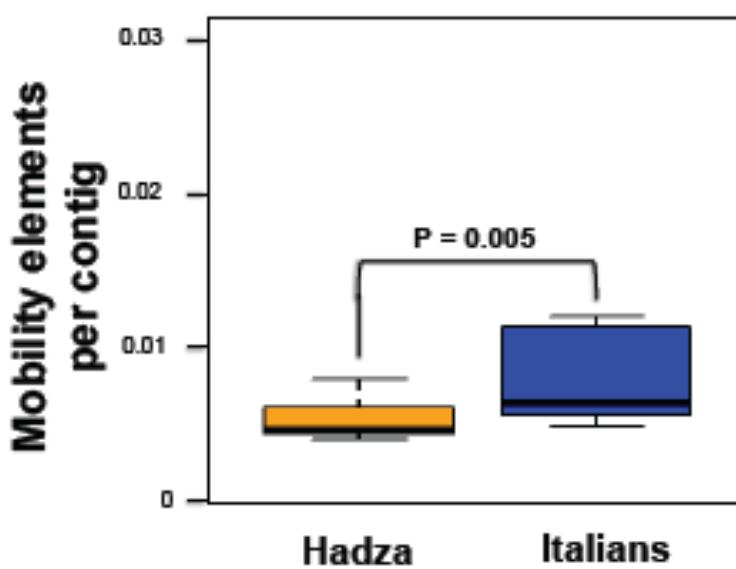
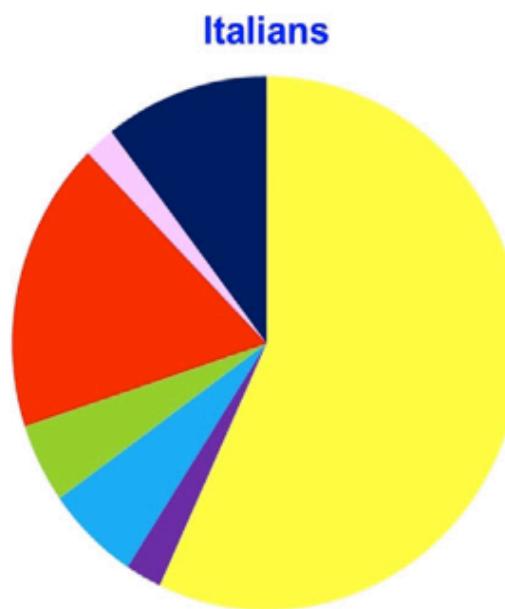
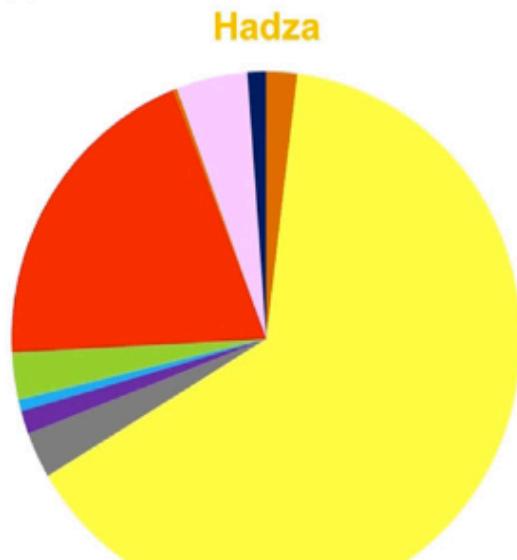


Natural history of the gut microbiome:
⇒ Relation with life style and diet
⇒ Ad hoc explanation?

Xenobiotic metabolism
Simple carbohydrates
Amino-acids

Antibiotic resistance genes

B



Despite lower antibiotic consumption Hadza microbiomes contain AMR genes but a smaller number of MGEs

- acridine
- tetracycline
- multidrug
- polypeptide
- macrolide
- aminoglycoside
- vancomycin
- beta-lactam antibiotic
- glycopeptide
- lincosamide
- chloramphenicol

Impact of antibiotics in adults

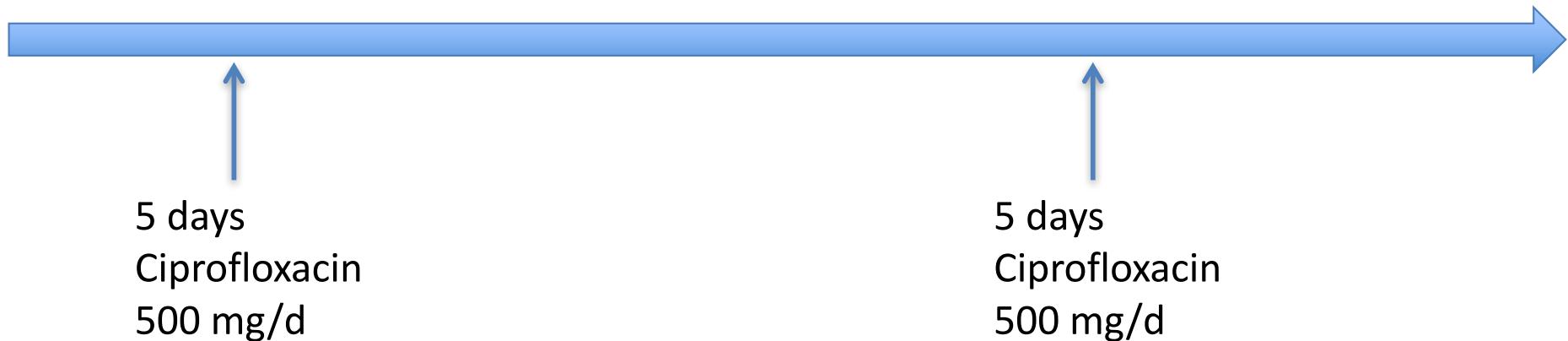
Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation

Les Dethlefsen^a and David A. Relman^{a,b,1}

^aDepartment of Microbiology and Immunology and Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305; and ^bVeterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304

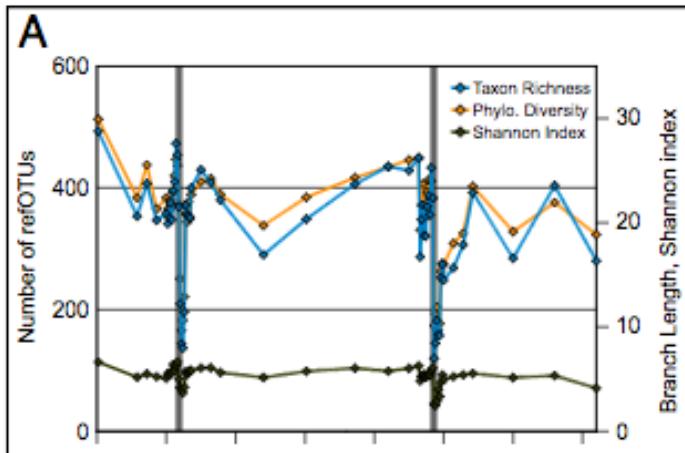
Impact of antibiotics in adults

- Three healthy volunteers



52 – 56 samples / subject (more frequent sampling before and after treatment)
DNA extraction sequencing of 16S rRNA (V1-V2-V3; >6000 sequences/sample)

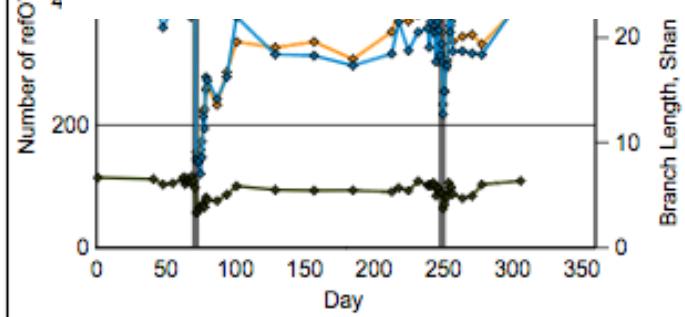
Effect of antibiotics on α -diversity



Decrease of α -diversity following each antibiotics course

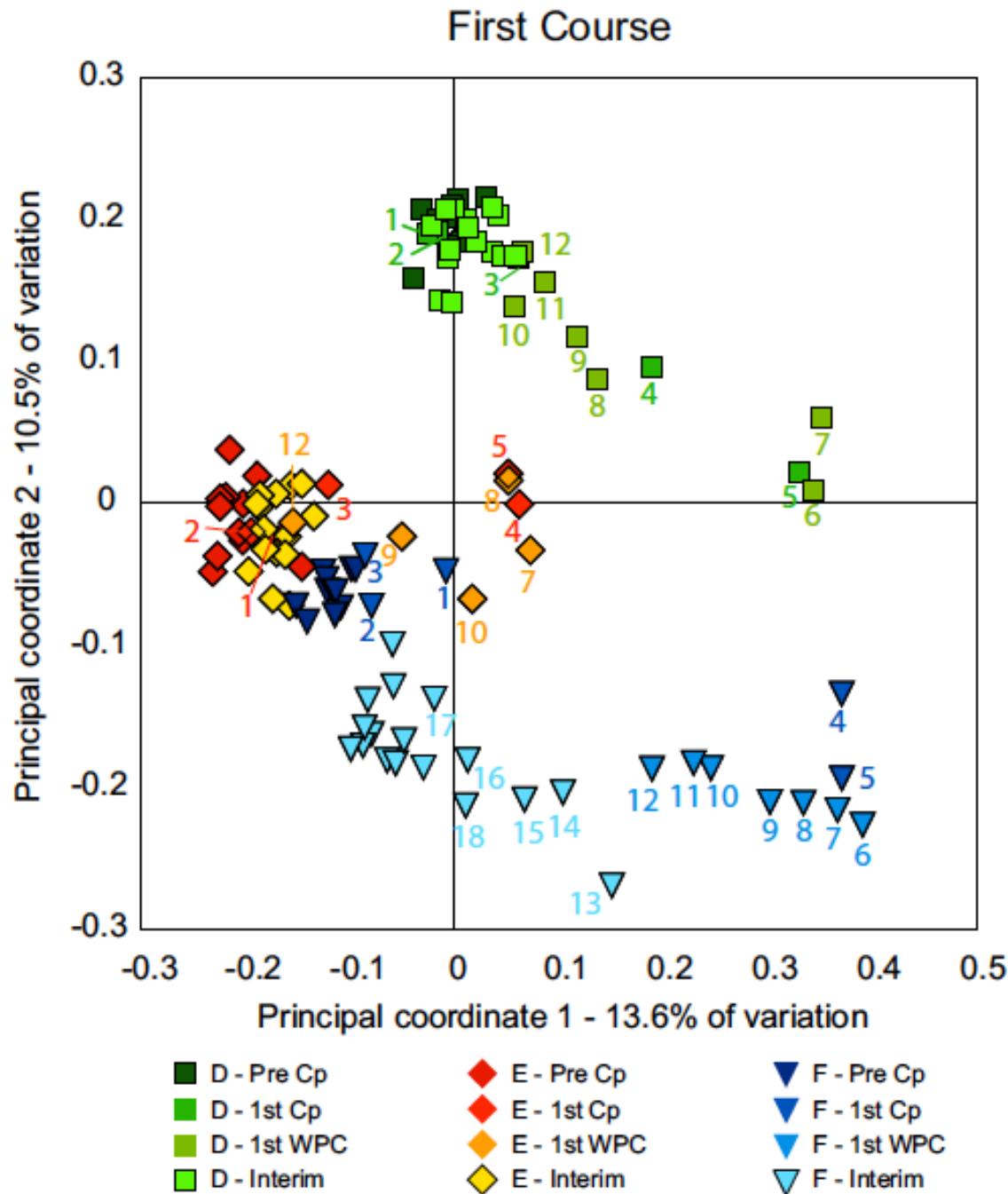
B

Subject	First Cp		Second Cp		Second Cp	
	1 d pre cfu/mL (low/high Cp)*	1 d post cfu/mL (low/high Cp)*	4 wk post cfu/mL (low/high Cp)*	1 d pre cfu/mL (low/high Cp)*	1 d post cfu/mL (low/high Cp)*	4 wk post cfu/mL (low/high Cp)*
D	1.3×10^4 (34%/0%)	1.4×10^5 (87%/61%)	2.1×10^4 (90%/6%)	2.7×10^5 (17%/0.4%)	6.2×10^4 (61%/3%)	1.6×10^5 (21%/2%)
E	1.6×10^4 (41%/2%)	2.2×10^5 (92%/92%)	3.3×10^4 (3%/0.3%)	4.8×10^4 (7%/6%)	7.5×10^3 (53%/13%)	7.8×10^4 (2%/0.6%)
F	8.3×10^6 (133%/0%)	4.7×10^4 (97%/42%)	4.1×10^5 (43%/4%)	1.1×10^6 (37%/0%)	2.6×10^4 (59%/10%)	3.4×10^5 (72%/1%)

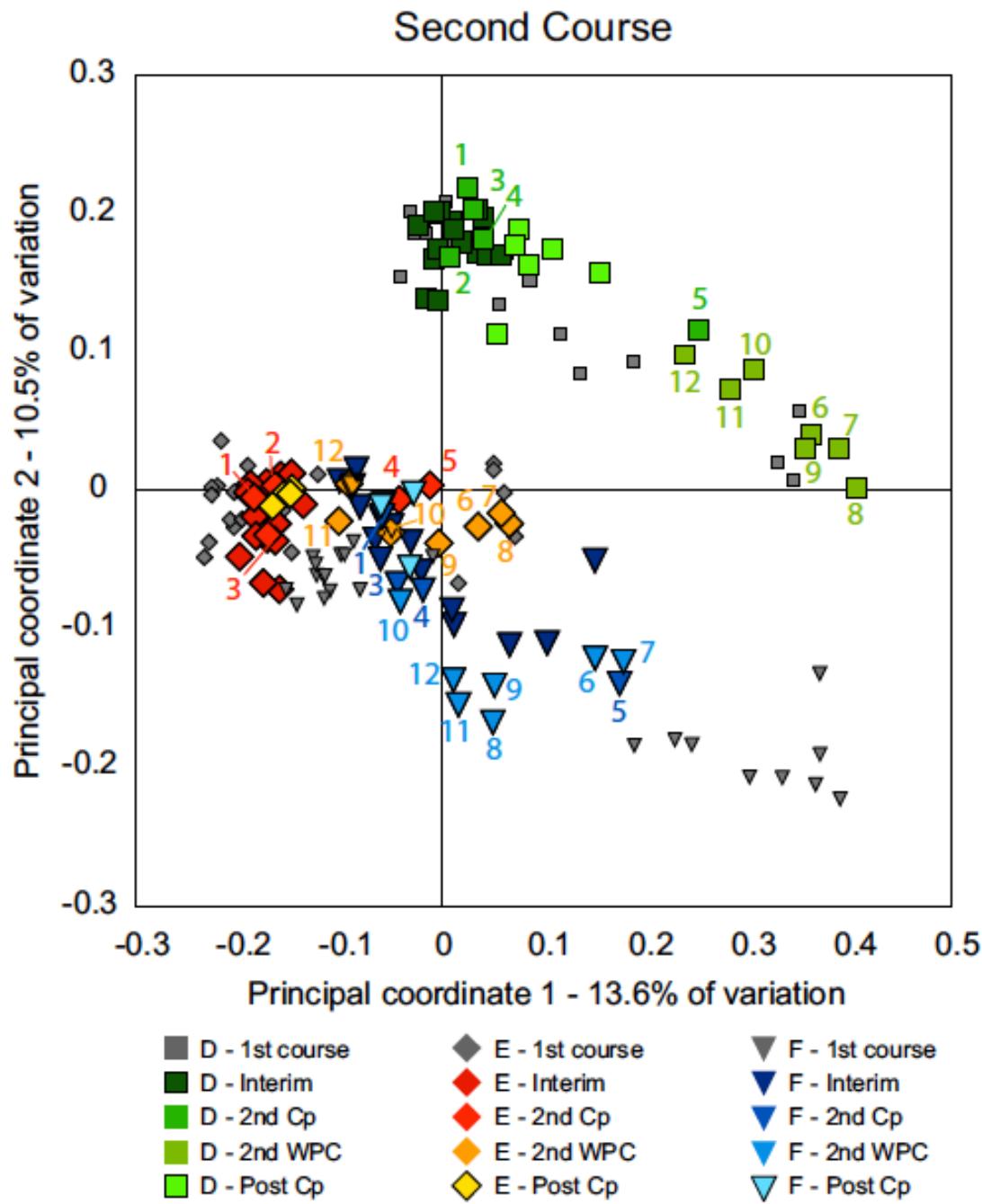


Effect on total counts (aerobic bacteria)
Effect on % of ciprofloxacin resistant bacteria

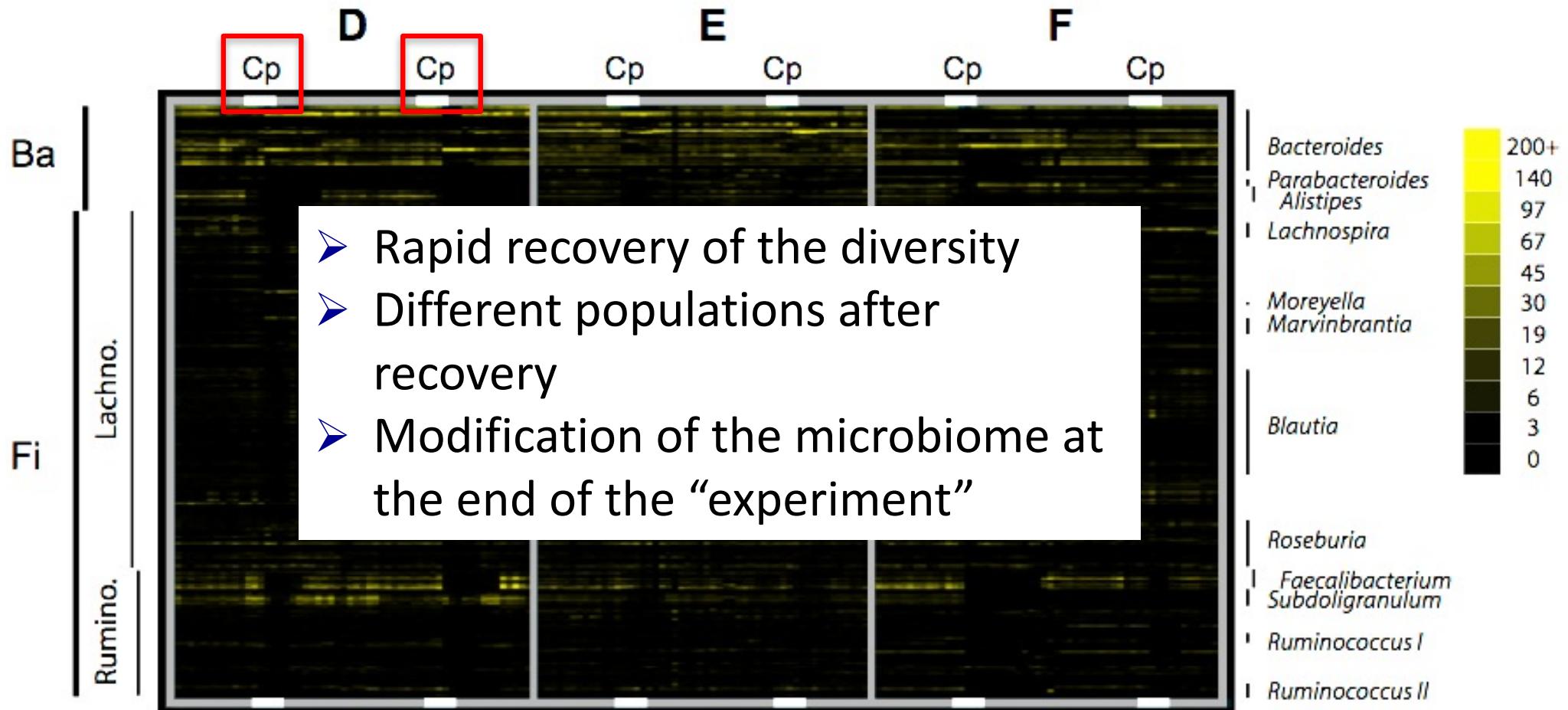
Recovery following Cp treatment



Recovery following Cp treatment



Heat map - abundance of refOTUs



Different patterns of modification
⇒ Decrease, increase and disappearance
⇒ Possibility of clonal replacement in the same genera.

Gut colonization and impact of antibioprophylaxis

MICROBIOME

Antibiotics, birth mode, and diet shape microbiome maturation during early life

Nicholas A. Bokulich,¹ Jennifer Chung,¹ Thomas Battaglia,¹ Nora Henderson,¹ Melanie Jay,^{1,2}
Huilin Li,³ Arnon D. Lieber,¹ Fen Wu,^{1,2} Guillermo I. Perez-Perez,^{1,4} Yu Chen,^{1,2} William Schweizer,⁵
Xuhui Zheng,⁴ Monica Contreras,¹ Maria Gloria Dominguez-Bello,¹ Martin J. Blaser^{1,4,6*}

43 Babies over 2 years

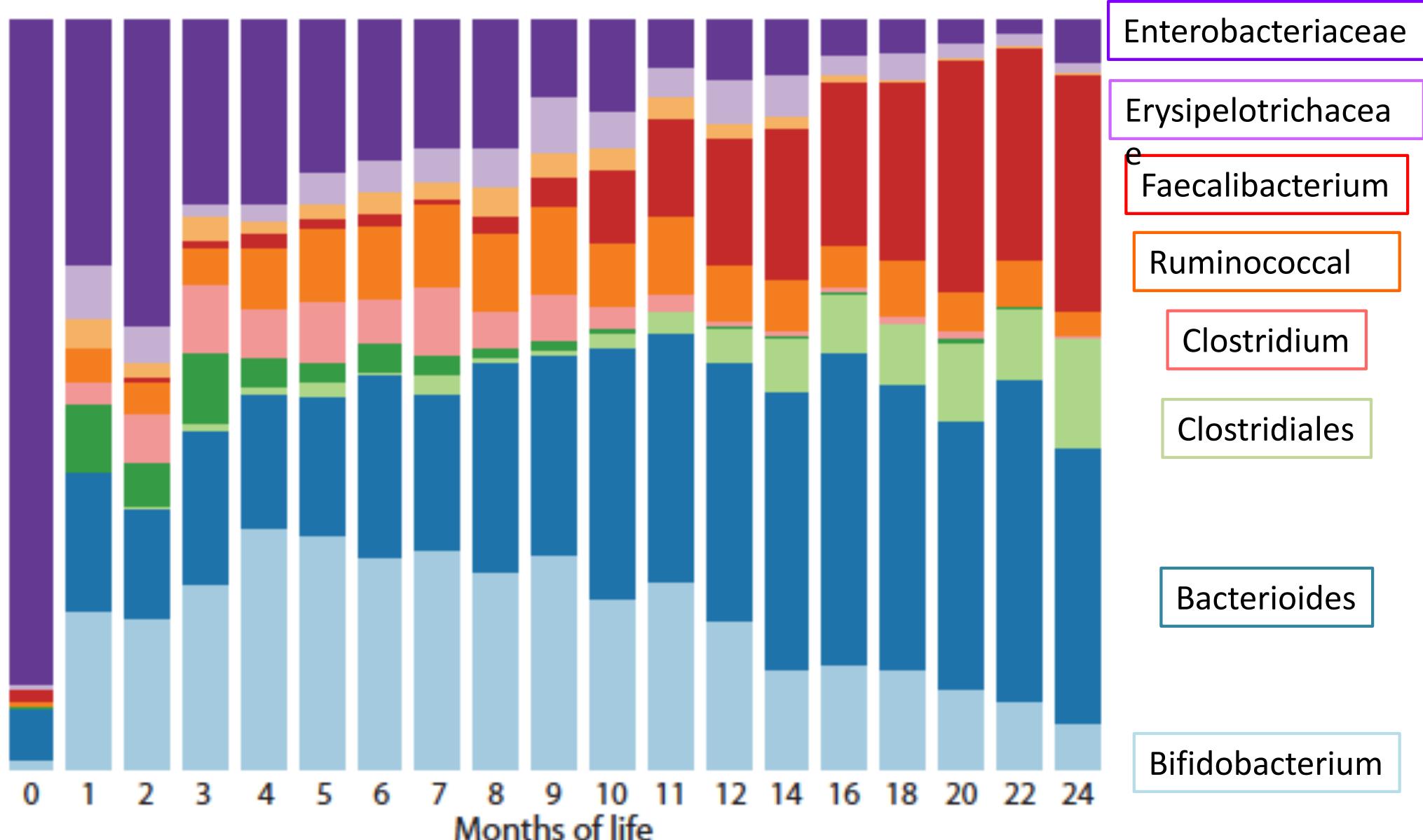


- vaginal delivery (24) - Breast milk-dominant (20); Formula-dominant (4)
- Cesarean section (n = 19) - Breast milk-dominant (11); Formula-dominant (8)

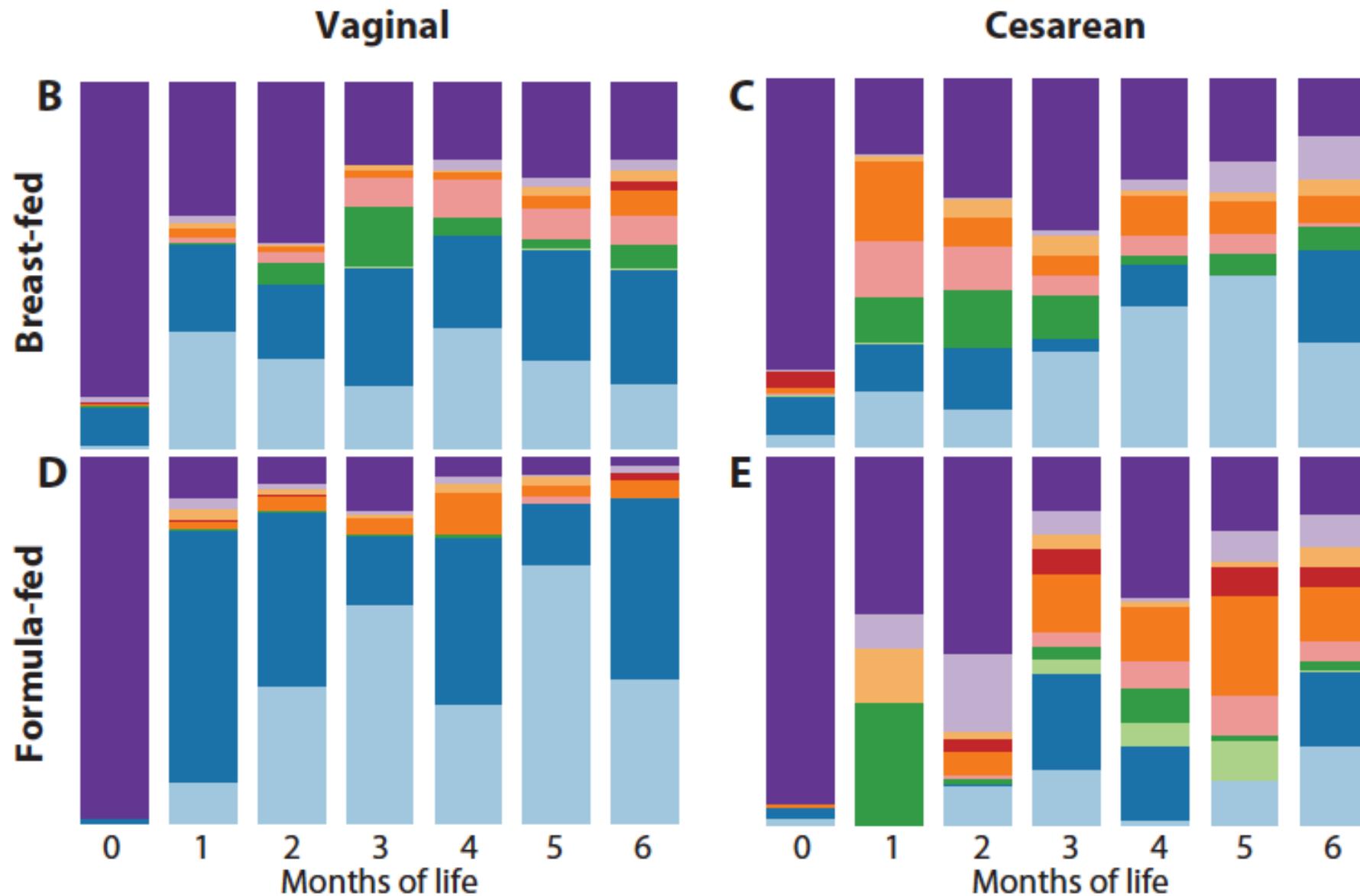
19 stool samples from the baby

4 stool samples from the mother (pre and postpartum)

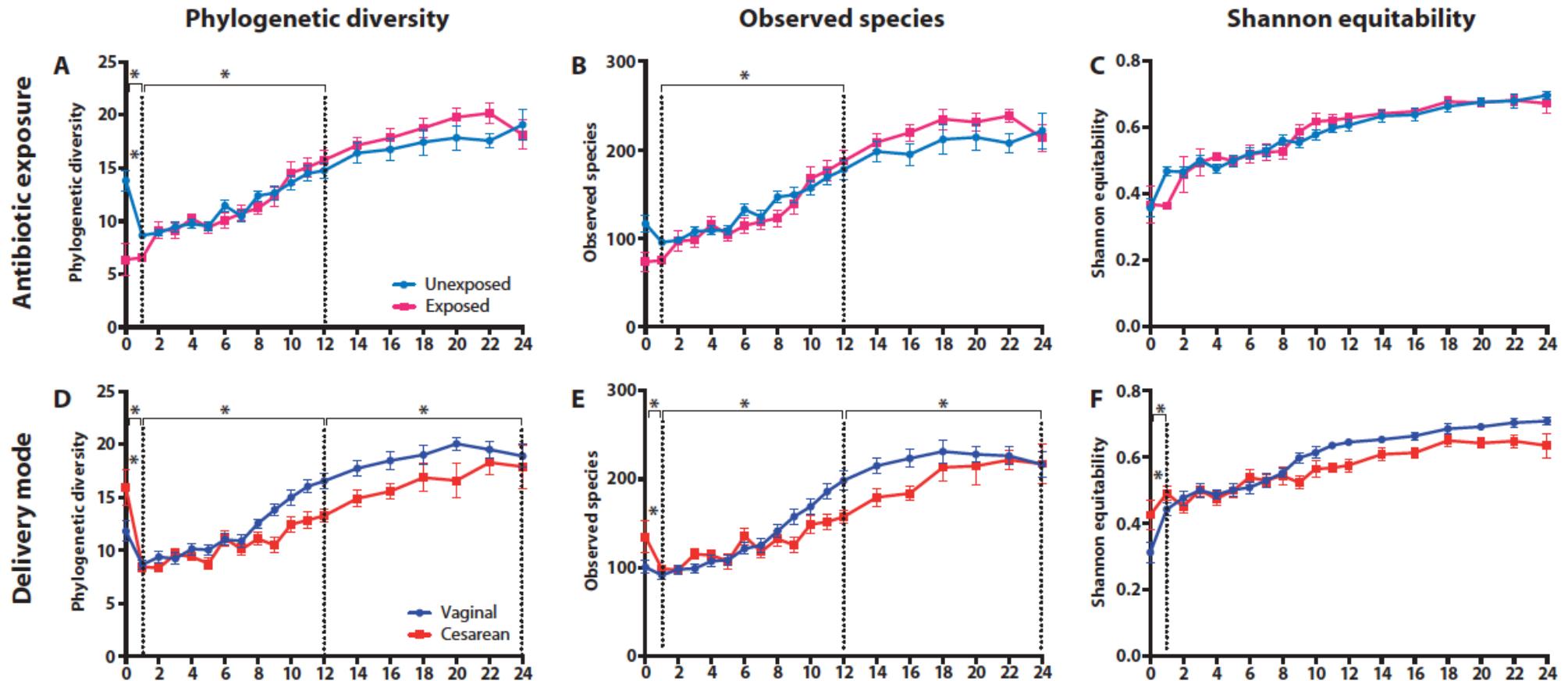
Evolution of the gut microbiome: 2 first years of life



Differences between babies groups



Effect of caesarean-section and antibiotics on α -diversity



⇒ Delay in diversification and lower diversity following C-section
⇒ Effect of antibioprophylaxis only during the first month

The gut resistome Two meta analyses

RESEARCH ARTICLE

Abundance profiling of specific gene groups using precomputed gut metagenomes yields novel biological hypotheses

Konstantin Yarygin^{1,2*}, Alexander Tyakht^{1,2}, Andrey Larin¹, Elena Kostryukova^{1,2},
Sergei Kolchenko^{2,3}, Vilgelm Bitner^{2,3}, Dmitry Alexeev^{1,2}

- 784 metagenomes from healthy populations worldwide and patients with inflammatory bowel diseases and obesity
- Method: gene coverage matrix obtained by pre-mapping the metagenomic reads to a global gut microbial catalogue (ARDB for antibiotics).

ARTICLE

Received 21 Feb 2013 | Accepted 13 Jun 2013 | Published 23 Jul 2013

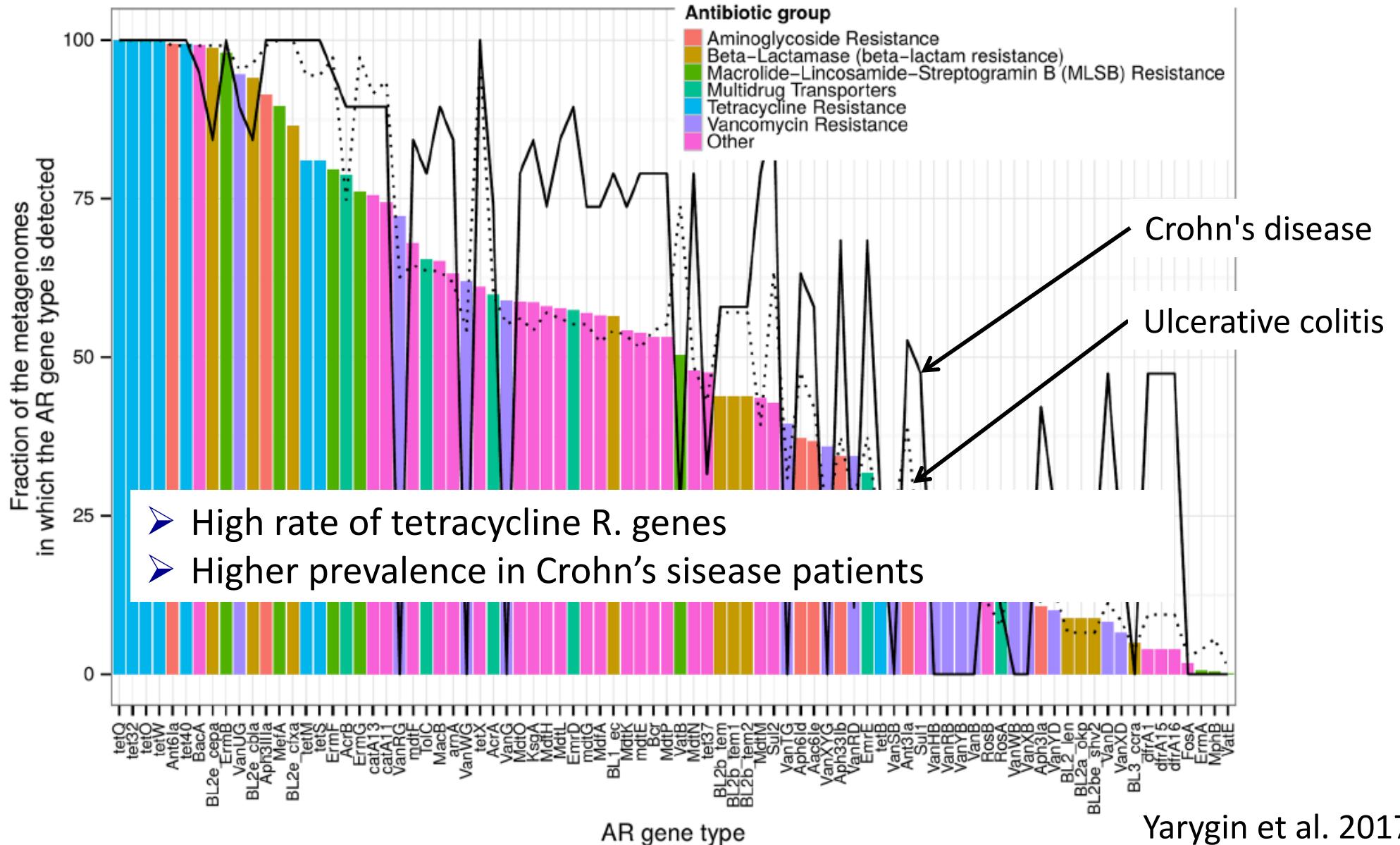
DOI: 10.1038/ncomms3151

Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota

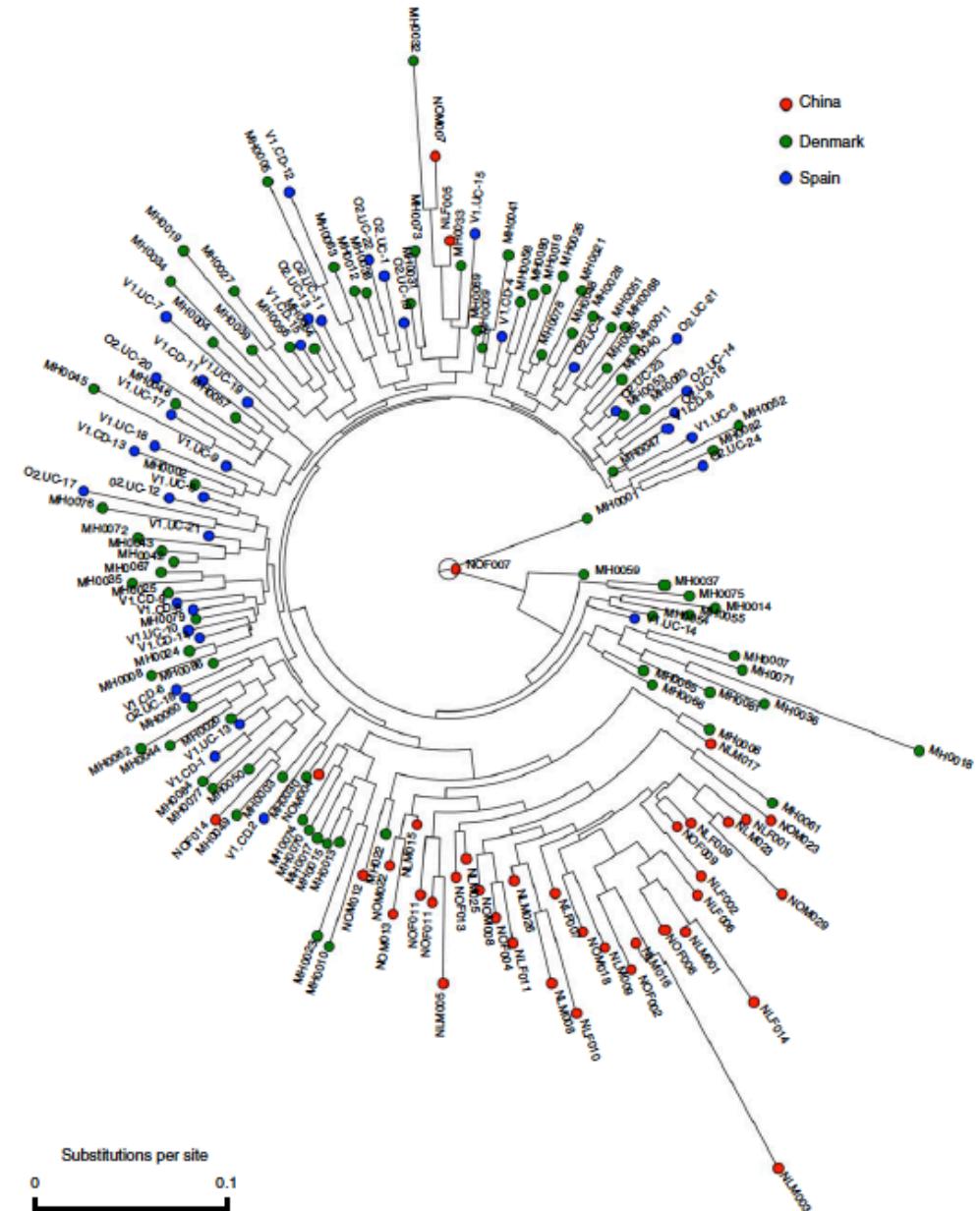
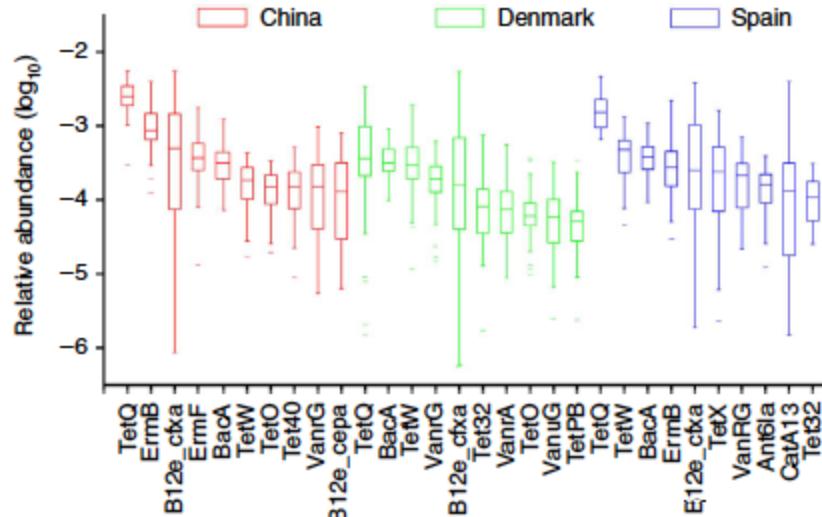
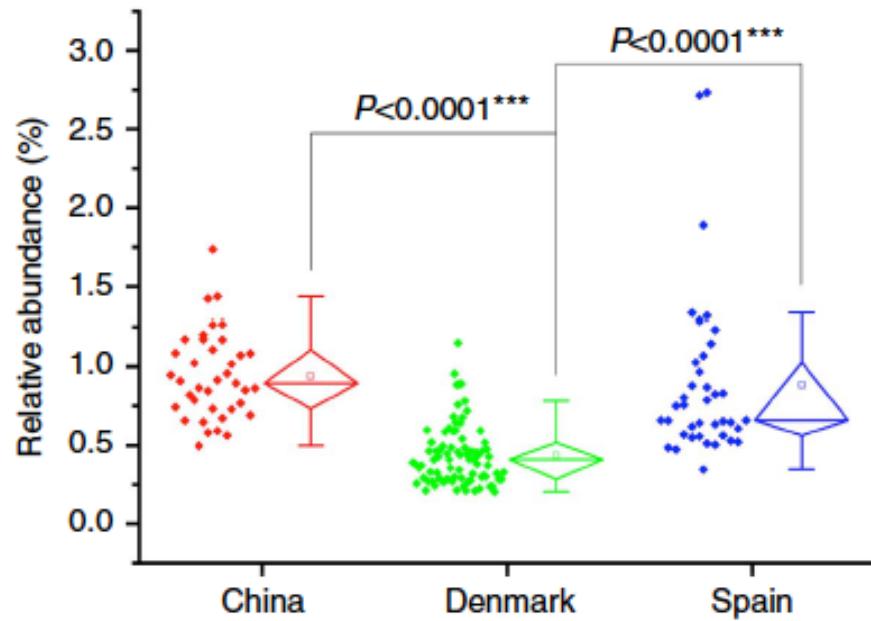
Yongfei Hu^{1,*}, Xi Yang^{1,*}, Junjie Qin², Na Lu¹, Gong Cheng¹, Na Wu¹, Yuanlong Pan¹, Jing Li¹, Liying Zhu³, Xin Wang³, Zhiqi Meng³, Fangqing Zhao⁴, Di Liu¹, Juncai Ma¹, Nan Qin⁵, Chunsheng Xiang⁵, Yonghong Xiao⁵, Lanjuan Li⁵, Huanming Yang², Jian Wang², Ruifu Yang⁶, George F. Gao^{1,7}, Jun Wang² & Baoli Zhu¹

- 162 metagenomes 85 Danish, 39 Spanish and 38 Chinese
- BLAST search against ARDB

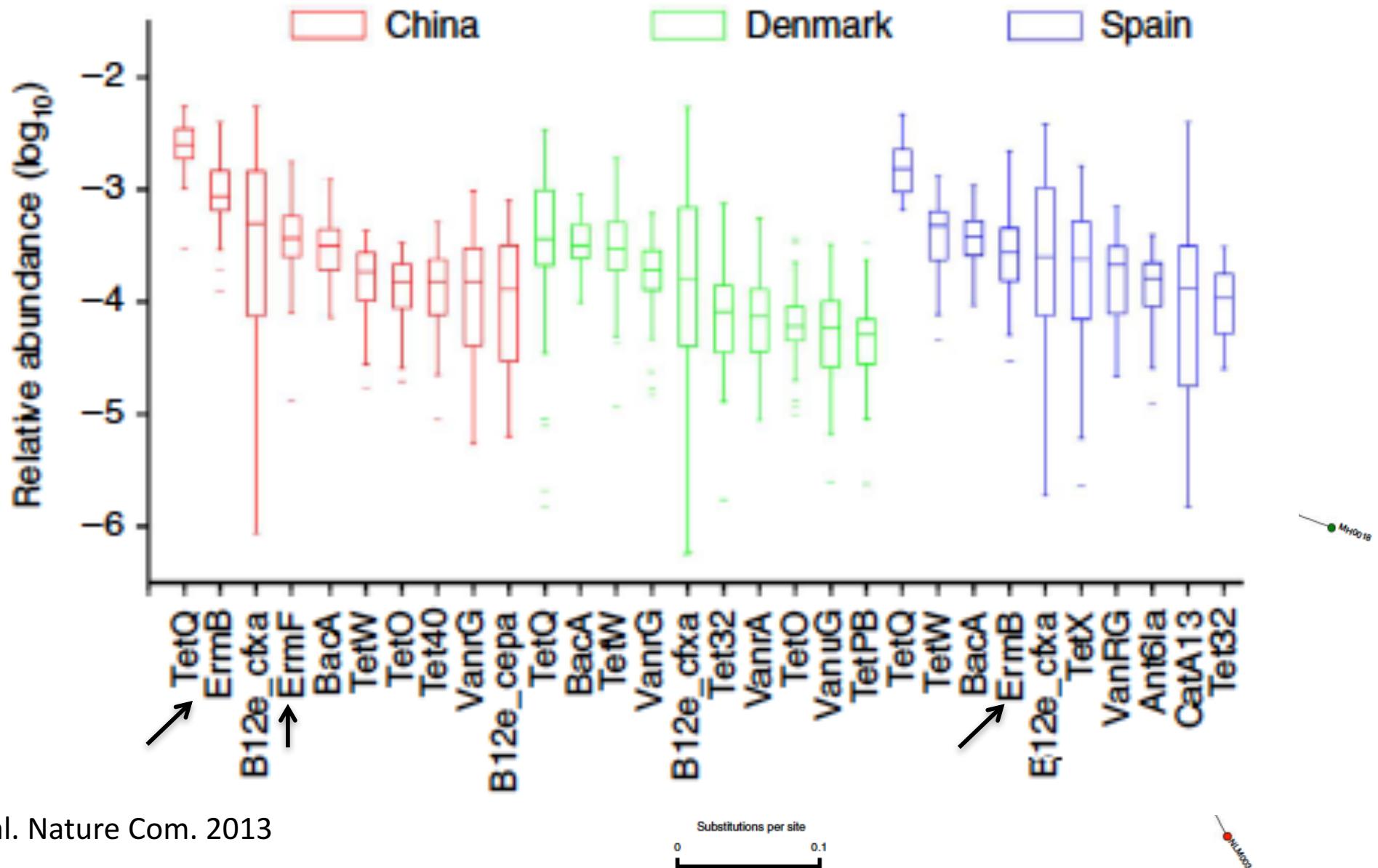
Prevalence antibiotic resistance genes



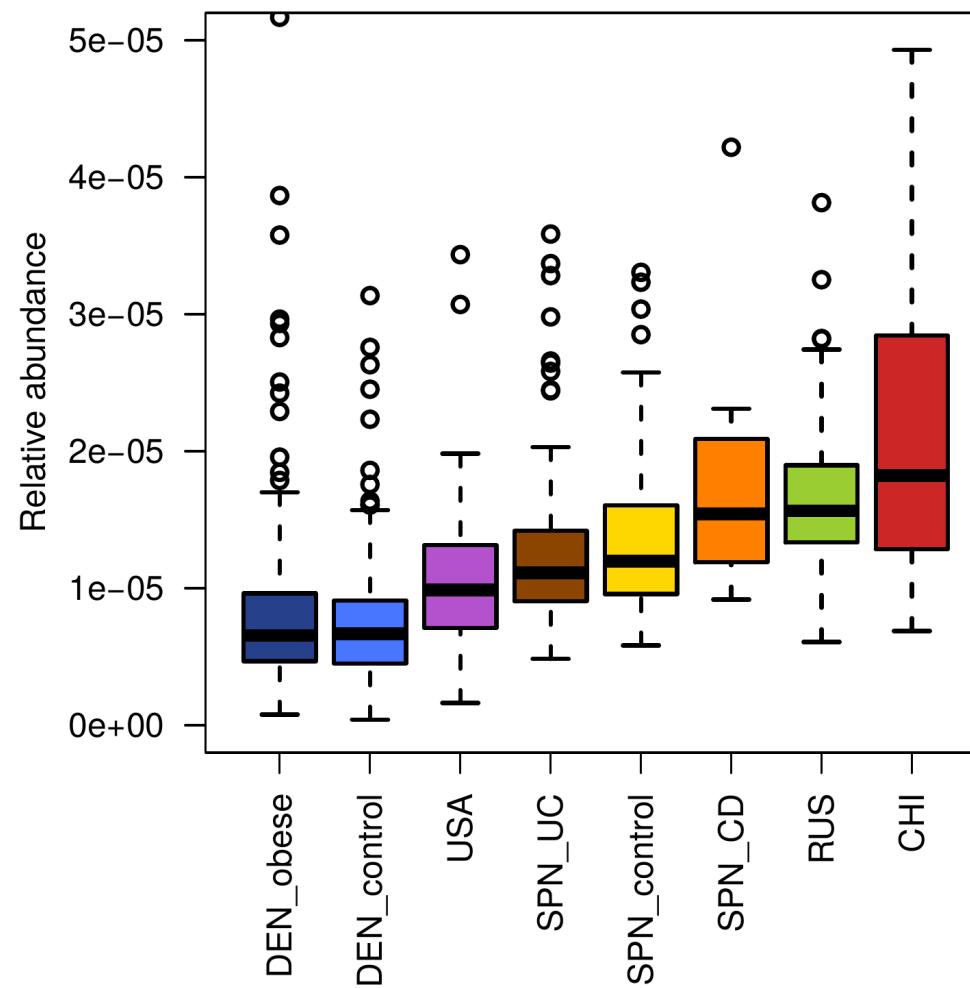
Analysis of 162 metagenomes



Analysis of 162 metagenomes



Variation between countries and health status



- Tetracycline resistant is the most abundant class in microbiomes

Q. What is the impact of tetracycline on bacterial populations?

Bacteroides

Heterogeneity in resistant fecal *Bacteroides fragilis* group collected from healthy people

Microbial. Pathogenesis 2016

T. Narimani ^a, M. Douraghi ^b, P. Owlia ^c, A. Rastegar ^a, M. Esghaei ^d, B. Nasr ^e, M. Talebi ^{a,*}

^a Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

^b Department of Microbiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

^c Molecular Microbiology Research Center(MMRC), Shahed University, Tehran, Iran

^d Department of Virology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

^e Department of Microbiology, Isfahan University of Medical Sciences, Isfahan, Iran

177 Bacteroides isolates from stool samples from healthy individuals in Iran

Bacteroides species	Number of isolates (%)	Te (%)	
<i>B. fragilis</i>	53	44 (83)	100% of the cases <i>tet(Q)</i> gene
<i>Non B. fragilis</i>	124	108 (87)	
Total	177 (100)	152 (86)	

Molecular biology, genetics and biotechnology

The prevalence of antibiotic resistance genes in *Bacteroides fragilis* group strains isolated in different European countries

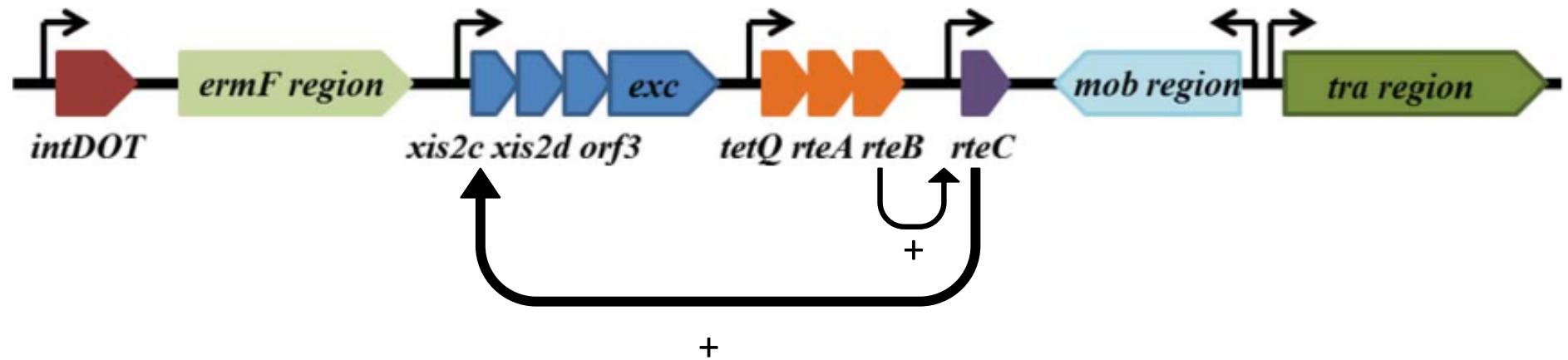
Zsuzsa Eitel, József Sóki*, Edit Urbán, Elisabeth Nagy on behalf of ESCMID Study Group on Anaerobic Infection¹

161 isolates: 78.9% (*B. fragilis*) and 84.8% (non-fragilis) TcR and expressing *tet(Q)*

Also a high rate of ampicillin res. (*cepA* gene)

Genetic support of the *tet(Q)* gene

Always carried by a conjugative transposon CTnDOT



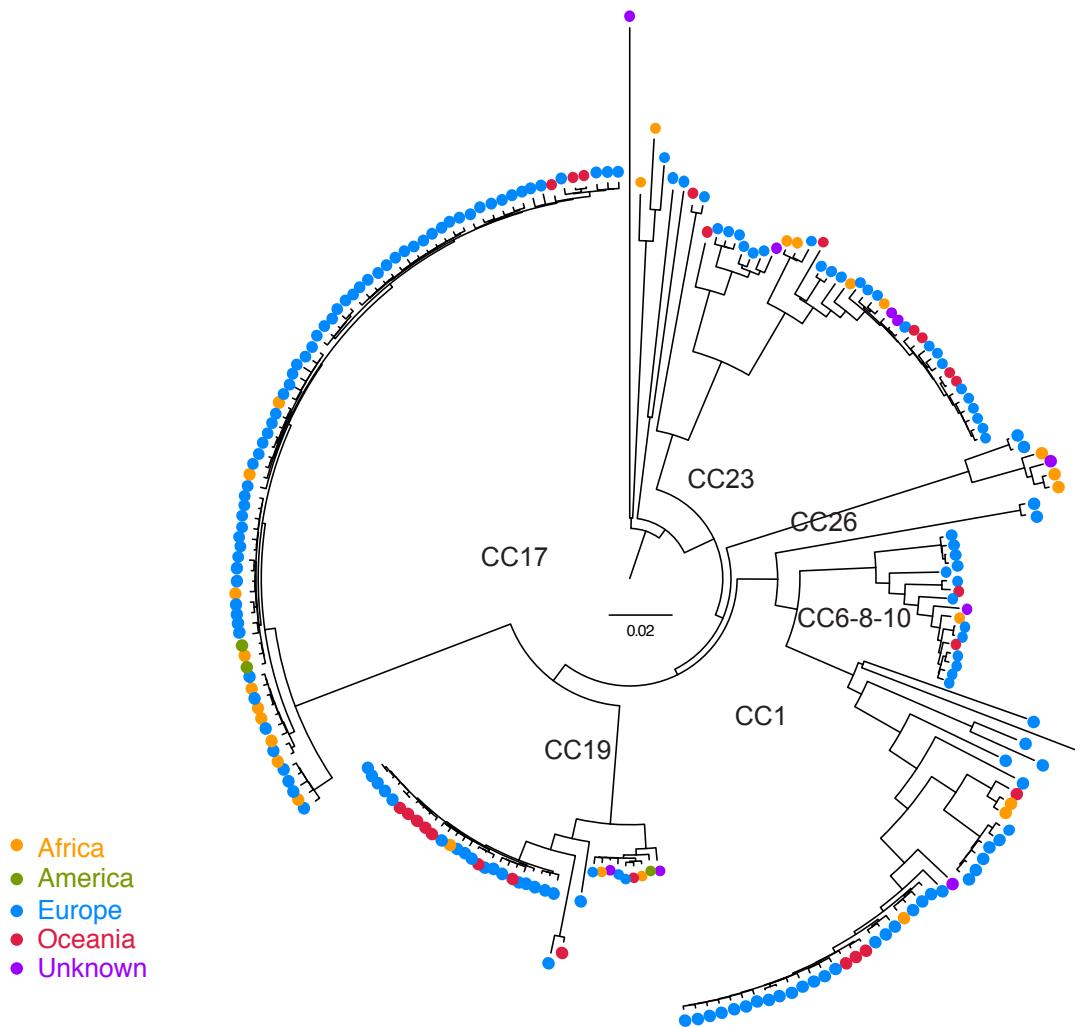
⇒ Tetracycline activates the transcription of the two component system *rteAB* and indirectly excision and conjugation

⇒ High frequency of exchange in the gut

Q. why exclusively this gene and this transposon?

In *Prevotella* in an IS21-family transposon

Replacement of GBS population by TcR clones



- ⇒ Other species with high rate of TcR: *Streptococcus suis*: 80% resistant
- ⇒ Other species with 30 to 60% resistant
- ⇒ Tetracycline had probably a profound effect on microbiomes composition

Conclusions

- Microbiome composition is diverse but with a conserved core microbiome
- Antibiotic resistance genes are present in all metagenomes analysed
- The use of antibiotics have an impact on metagenome composition and health
- Other factors contribute to its composition (lifestyle, diet)
- Some technical limitations: experimental: choice and number of samples, sequencing depth, bioinformatics: thresholds, significance.

Challenges and new concepts in antibiotics research

March 19 - 21, 2018

Institut Pasteur, Paris, France

SPEAKERS

Frank Møller AARESTRUP, Technical University of Denmark, DK
Dan ANDERSSON, Uppsala University, SW
Michel ARTHUR, Université Pierre et Marie Curie, FR
Nathalie BALABAN, The Hebrew University of Jerusalem, IL
David BIKARD, Institut Pasteur, Paris, FR
Martin BLASER, New York University School of Medicine, US
Sebastian BONHOEFFER, ETH Zürich, SW
Priscille BRODIN, Institut Pasteur de Lille, FR
Stewart COLE, (keynote) EPFL, Lausanne, CH
Jean-Marc GHIGO, Institut Pasteur, Paris, FR
Sophie HELAINE, Imperial College London, UK
Roy KISHONY, Technion Israel Institute of Technology, IL
Kim LEWIS, (keynote), Northeastern University, US
Sharon PEACOCK, (keynote), University of Cambridge, UK
Marie-Cécile PLOY, University of Limoges, FR
Nassos TYPAS, European Molecular Biology Laboratory, DE
Nora VAZQUEZ-LASLOP, University of Illinois, US
Kamini WALIA, Indian Council of Medical Research, IN
Tim WALSH, Heath Park Hospital, UK
Pei ZHOU, Duke University Medical Center, US

<http://www.amr-2018.org/>



SCIENTIFIC COMMITTEE

Carmen BUCHRIESER, Institut Pasteur, FR
Bruno COIGNARD, Santé Publique France, FR
Pascale COSSART, Institut Pasteur, FR
Hilde de REUSE, Institut Pasteur, FR
Philippe GLASER, Institut Pasteur, FR
Ivo GOMPERTS BONECA, Institut Pasteur, FR
Didier GUILLEMOT, Institut Pasteur, FR
Didier MAZEL, Institut Pasteur, FR
Thierry NAAS, Paris Saclay, FR
Lulla OPATOWSKI, Paris Saclay, FR
Thierry PLANCHENAULT, Institut Pasteur, FR
Philippe SANSONETTI, Institut Pasteur, FR
Kathleen VICTOIR, Institut Pasteur, FR

