ASSOCIATION OF CLASSICAL MICROBIOLOGY AND TARGETED METAGENOMIC ANALYSIS TO EVALUATE THE PRESENCE OF *CLOSTRIDIUM DIFFICILE* IN A BELGIAN NURSING HOME

C. Rodriguez, B. Taminiau, N. Korsak, V. Avesani, J. Van Broeck, M. Delmée, G. Daube

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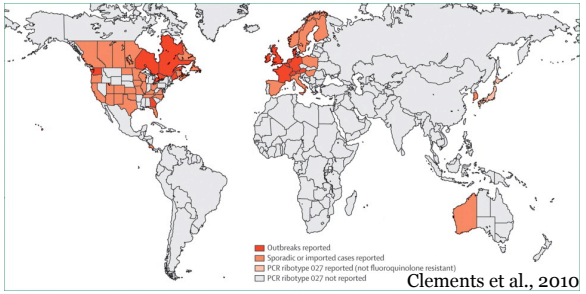
Background

- Since toxigenic *C. difficile* was recognized as the major cause of antibiotic-associated diarrhea and pseudomembranous colitis in 1978, many outbreaks have been documented

- In the last years, an enhanced virulence and increased antibiotic resistance of *C. difficile* strains (PCR-ribotype 078/NAP-1/B1) has been observed



Clements et al., 2010

- There are emerging data on the occurrence of *C. difficile* infection in the community: non-hospitalized and younger patients with absence of other traditional risk factors

Hypothesis about a potential risk of foodborne infections linked to *C. difficile*

https://www.health.harvard.edu

Background

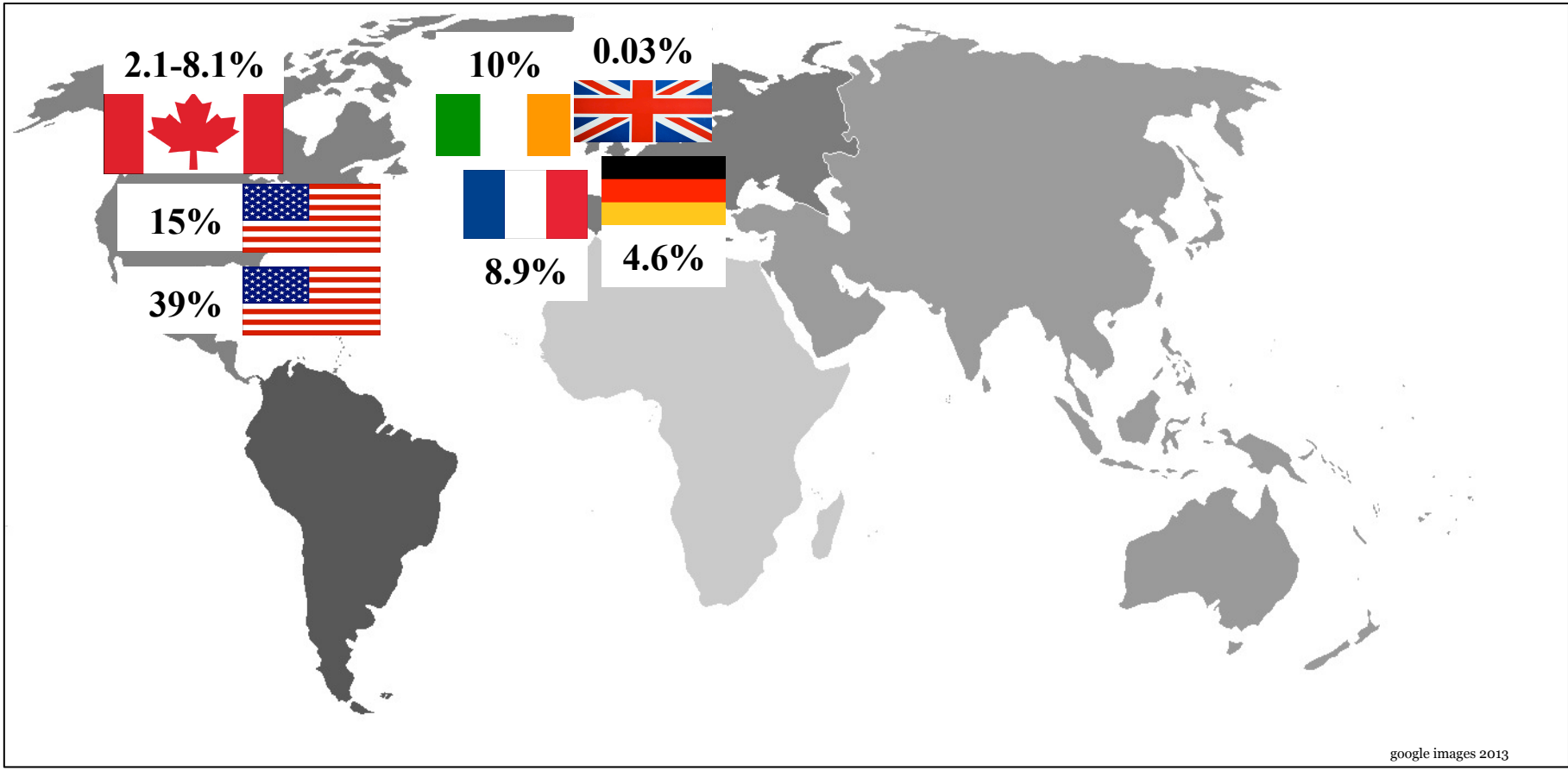
- Patients with serious illnesses and prolonged hospitalizations are at particular risk, as people above 65 years of age

- The increased risk of acquiring *C. difficile* in the elderly may be de to age-related changes in intestinal flora, immune senescence or the presence of underlying diseases

- There is not much data describing the prevalence and molecular epidemiology of *C. difficile* in nursing homes in absence of an epidemic situation

Arvand, M., et al., 2012. High prevalence of *Clostridium* difficile colonization among nursing home residents in Hesse, Germany. Plosone, 7, e30183.

Clostridium difficile Infection in Nursing homes



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Birgand, G., et al., 2010. Investigation of a large outbreak of *Clostridium diff*icile PCR-ribotypes 027 infections in northern France, 2006-2007 and associated clusters in 2008-2009. Eurosur, 24, 1-6.

Marwick, C.A., et al 2013. Community-associated *Clostridium difficile* infection among older people in Tayside, Scotland, is associated with antibiotic exposure and care home residence: cohort study with nested case-control. J Antimicrob Chemoth, 3.

Mylotte, J.M., et al., 2012. Surveillance for *Clostridium difficile* infection in nursing homes. J Am Geriatr Soc, 61, 122-5.

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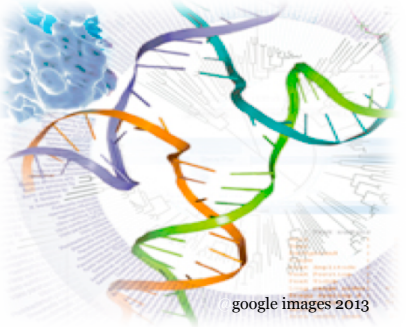
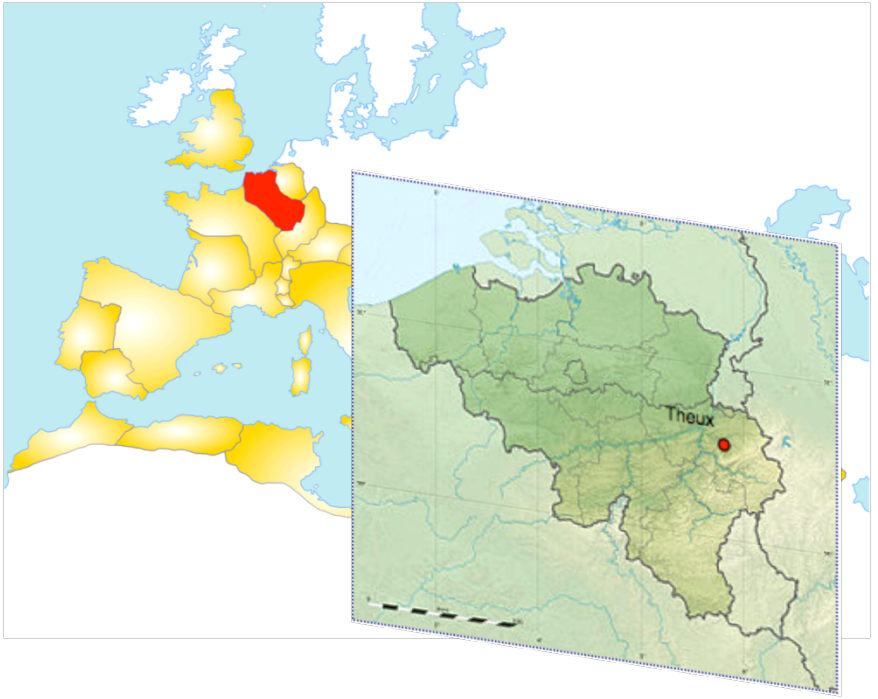


Objectives

- To evaluate and follow the prevalence of *C. difficile* in a Belgian nursing home
- To establish a relationship between other intestinal bacterial populations and *C. difficile* colonization
- To evaluate the global evolutions of the total microflora and the relation with the *C. difficile* presence

Study design



Capacity: 110 beds

- 34 nursing home
- 61 nursing home and long-term care
- 15 day centers

Employees

- 73

Study design

STOOL SAMPLES

From March to June 2013

- During a 4-month period, stool samples from a group of 23 elderly care home residents were collected weekly

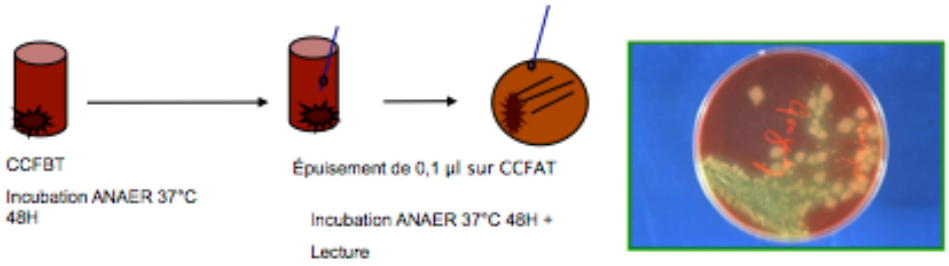
- Two samples per person were collected: the first sample was cultivated and examined for *C. difficile* by classical microbiological methods and the second one was used to study the microbial biodiversity of the faeces content by amplicon sequencing coupled to microbial metagenomic analysis .

Methodology

- **Direct and enrichment culture**

  Home-made cycloserine cefoxitin fructose taurocholate

  (Delmée et al., 1987. Epidemiology and prevention of *Clostridium difficile* in a leukaemia unit. E J Clin Microbiol, 6, 623-27)



- ***C. difficile* latex agglutination rapid test Kit DR 1107A Oxoid**
- **Detection of a species-specific internal fragment of *tpi*, detection of genes for toxin B, toxin A and binary toxin (*cdtA*) by PCR et Genotype Cdiff test system**

  (Lemée et al., 2004. Multiplex PCR targeting *tpi* (triose phosphate isomerase), *tcdA* (toxin A), and *tcdB* (toxin B) genes for toxigenic culture of *Clostridium difficile*. J Clin Microbiol, 42, 5710-14)
  (Antikainen et al., 2009. Detection of virulence genes of *Clostridium difficile* by multiplex PCR. Acta Phat, Microbiol Inmuno Scand, 117, 607-13)

- **Cytotoxicity assay using confluent monolayer MRC-5 cells**

  Cytotoxic activity was confirmed using a specific *C. difficile* antitoxin kit (T500, TechLab, USA)

  (Rodriguez et al., 2012. *Clostridium difficile* in young farm animals and slaughter animals in Belgium. Anaerobe, 18, 621-625)

- **PCR-ribotyping**

  (Bidet et al.,1999. Development of a new PCR-ribotyping method based on ribosomal RNA gene sequencing. FEMS Microbiol Letters, 175, 261-66)

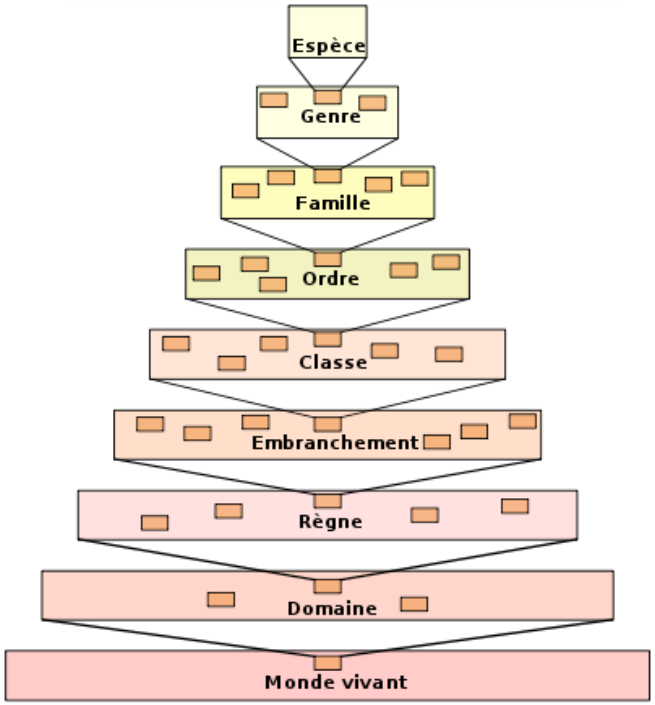
- **Targeted Metagenomic analysis**

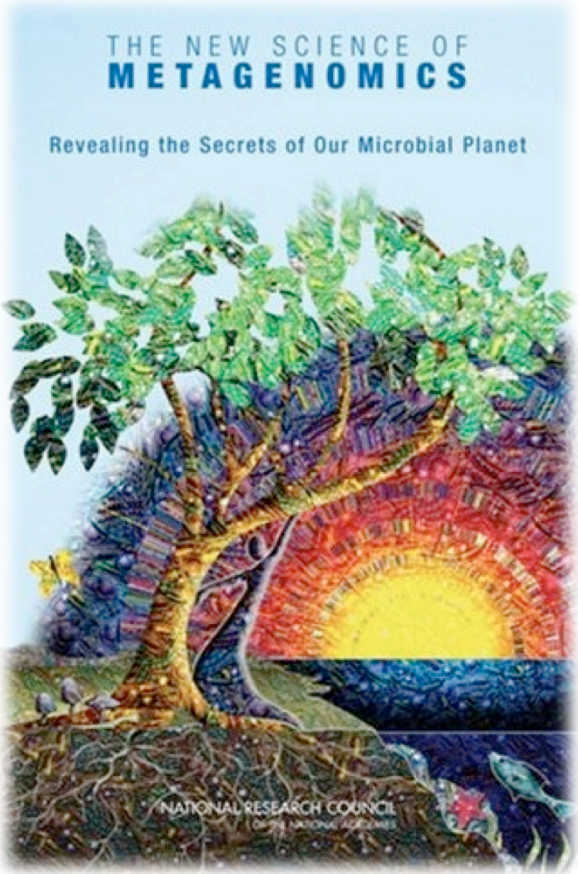
Metagenomics

Targeted Metagenomics

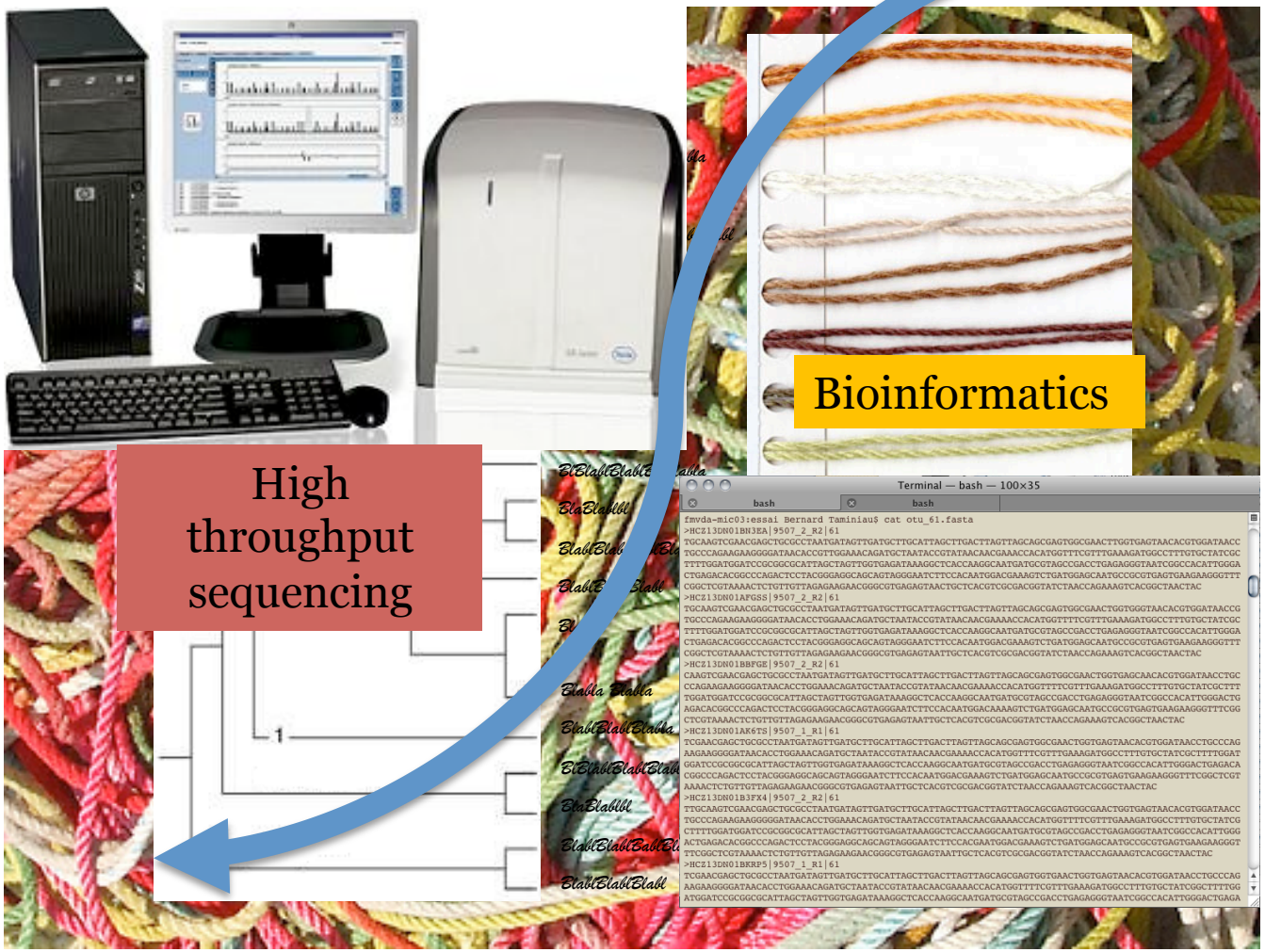
- Who is in there

16S rDNA as the target

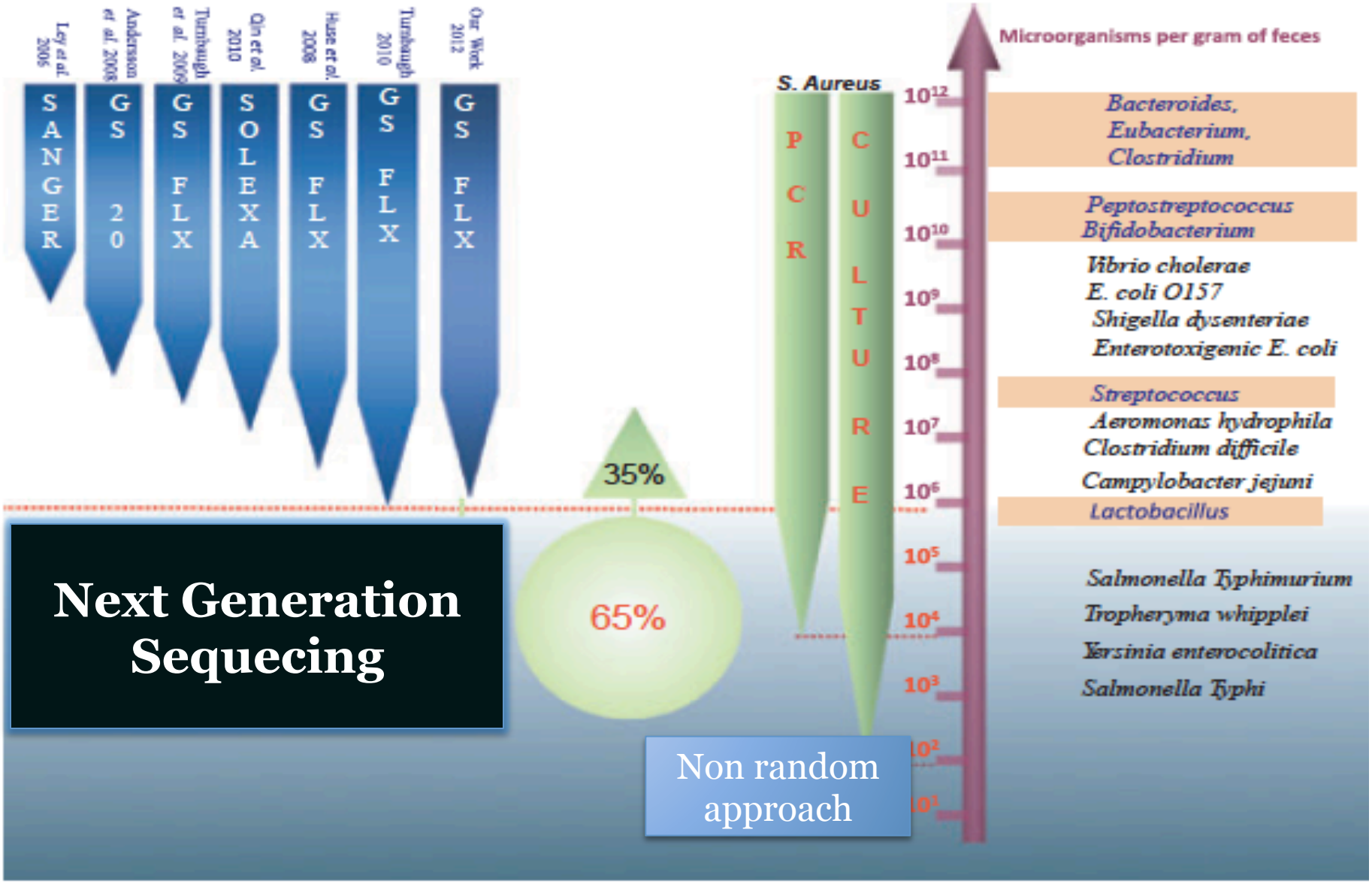




Metagenomics



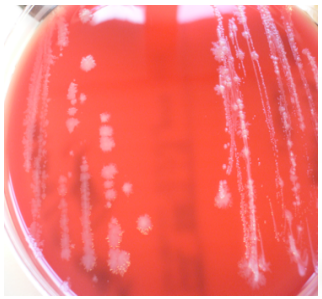
Metagenomics



Results: Prevalence of *C. difficile* in nursing home residents

C. difficile recovery:

- 7/23 (30.4%) residents were (at least one week) positive for *C. difficile*
- *C. difficile* was detected in 13/30 (43.3%) episodes of diarrhea
- 4/13 (30.7%) residents positives for *C. difficile* had previously received an antibiotic therapy



Resident	Week																
N°	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
1		D										D/AB		AB			
2																	
3																	
4				D	D												
5	AB/P											D					
6					AB												
7	AB					AB											
8				H	H	H	H				P						
9																	
10																	
11																	
12	D		D				D	D		D	D						
13																	
14																	
15	D	D	D	D		D	D	D		D	D/P	D/P	P	P			
16		D/AB	AB	AB			X	X	X	X	X	X	X	X	X	X	X
17	D/AB	AB	AB		AB												
18							D										
19		D	D														
20																	
21				D	D	D				D					P		
22										X	X	X	X	X	X	X	X
23																	
24			AB/P		AB												

Sample not available | Negative | Positive after direct culture | Positive results after 3 days of enrichment

D diarrhea AB antibiotic P probiotic H hospitalization X death or resident outside of the study

Resident	Week															
N°	01	02	03	05	06	07	08	09	10	11	12	13	14	15	16	17
1	020	020							020	020	020		020		020	
2																
3																
4																
5																
6																
7																
8																
9																
10																
11																
12																
13	UCL36	UCL36														
14																
15	027	027	027		027	027	027	027	027	027	027	027	027			027
16																
17																
18				027		027		027			027			027		027
19	UCL46	UCL46	027			027				027		UCL36				
20																
21																
22																
23										UCL36						
24			UCL36	UCL36			UCL36									

Sample not available | Negative | Positive after direct culture | Positive results after 3 days of enrichment

Analyzed in metagenomics

Patient N°	Week 01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
1		D										D/AB/P		AB			
2																	
3																	
4				D	D												
5	AB/P											D					
6					AB												
7	AB					AB											
8				H	H	H	H				P						
9																	
10																	
11																	
12	« D »		« D »				« D »	« D »		« D »	« D »						
13																	
14																	
15	D	D	D	D		D	D	D		D	D/P	D/P	P	P			
16		D/AB	AB	AB			X	X	X	X	X	X	X	X	X	X	X
17	D/AB	AB	AB		AB												
18							D										
19		D	D														
20																	
21				D	D	D				D					P		
22										X	X	X	X	X	X	X	X
23																	
24			AB/P		AB												

Sample not available | Negative | Positive after direct culture | Positive results after 3 days of enrichment

D diarrhea AB antibiotic P probiotic H hospitalization X death or resident outside of the study

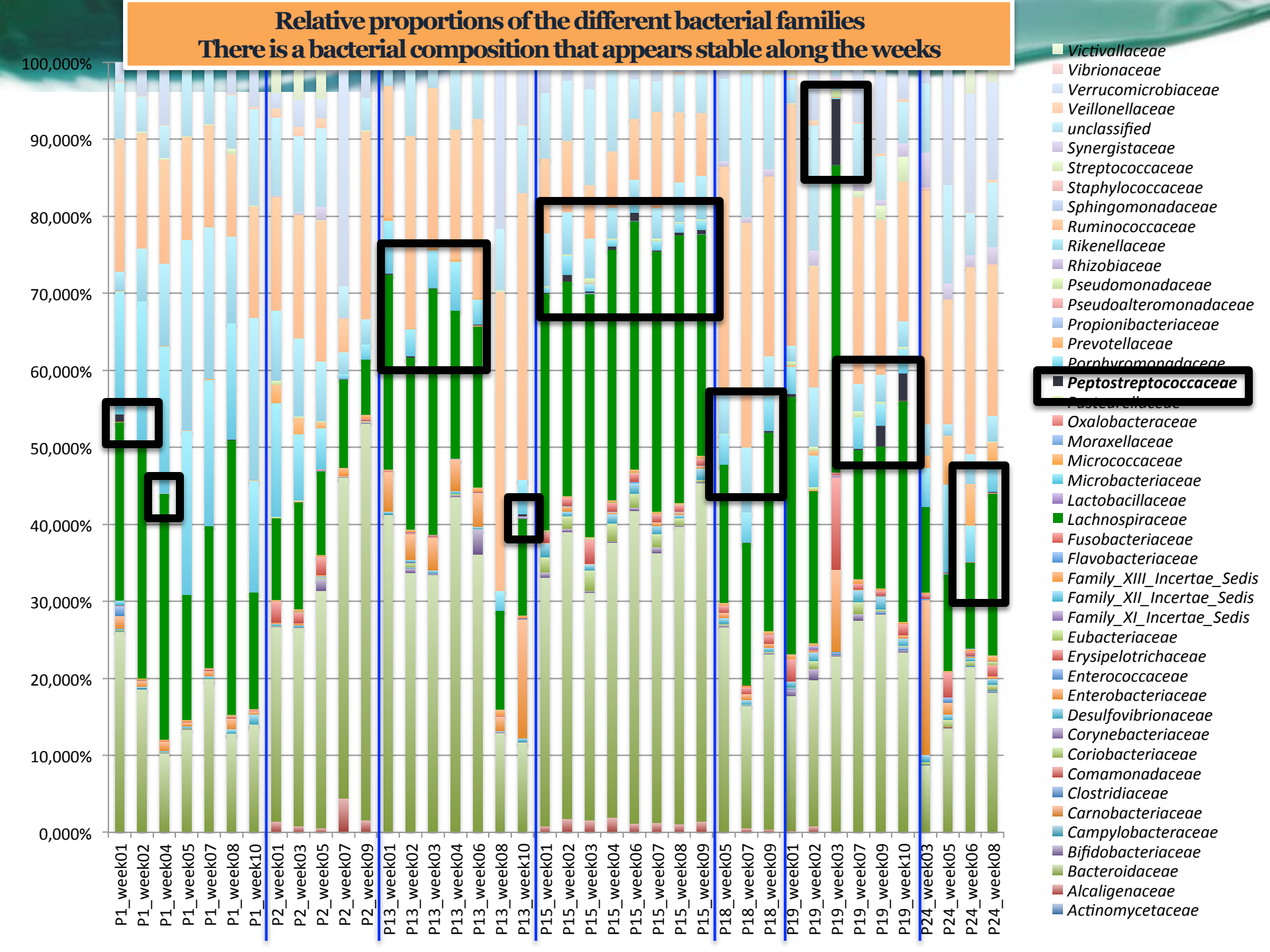
The results so far:

80 samples sequenced and analyzed: 6300 OTUs

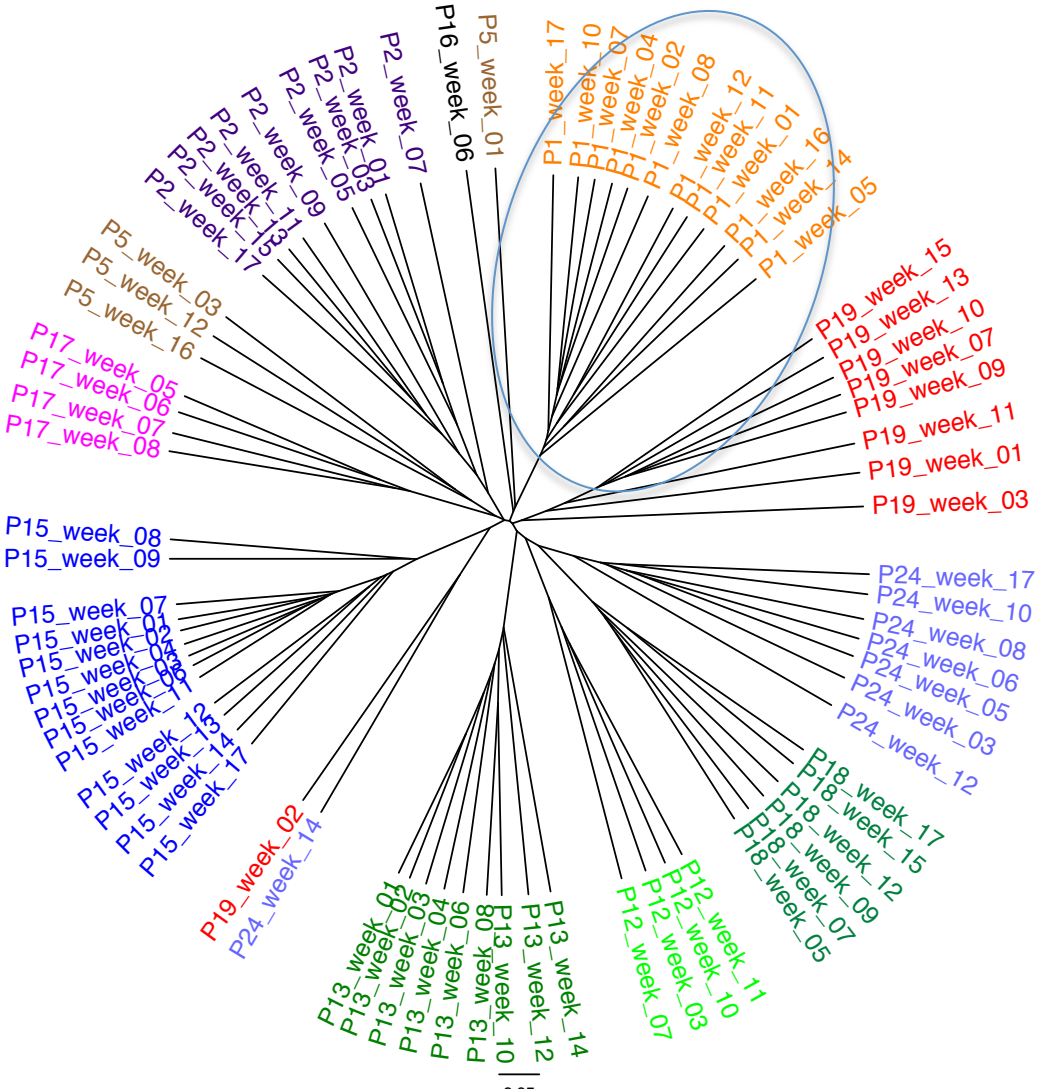
Positive detection of *Clostridium difficile* :

C. difficile detection		
n	**Microbiology**	**Amplicon sequencing**
20	+	+
36	-	-
19	+	-
5	-	+

Relative proportions of the different bacterial families
There is a bacterial composition that appears stable along the weeks



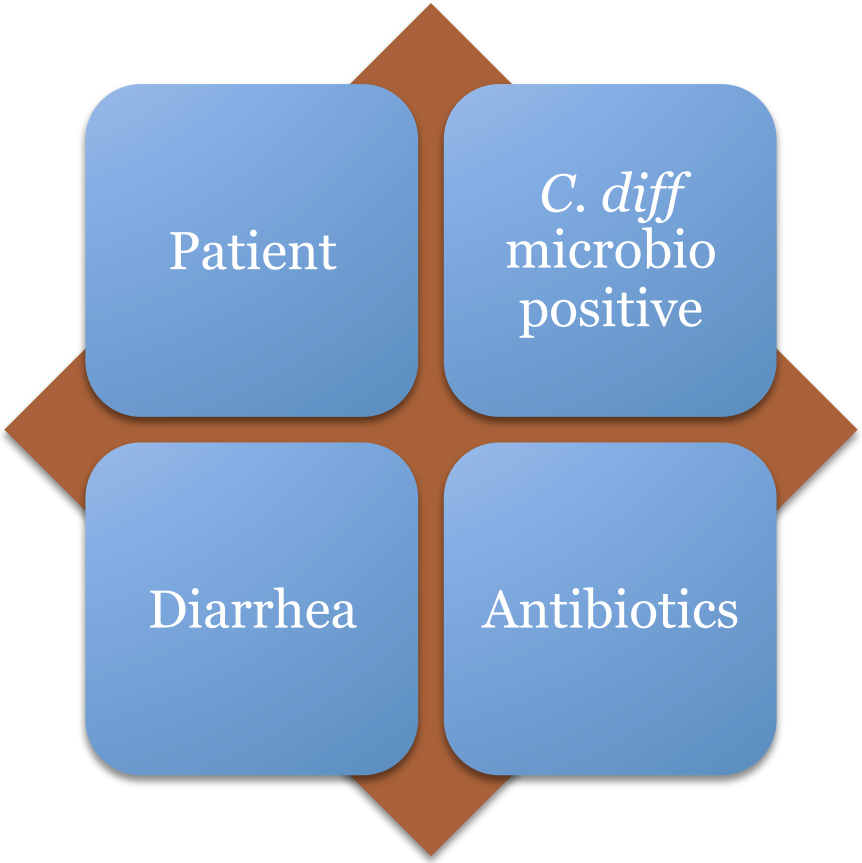
Phylotype tree based on population distribution – Braycurtis dissimilarity index



- Study the phylotype composition of the samples
- This tree reflects how many samples have the same bacterial content or not
- Almost all the samples are clustered in a sub-tree corresponding to a single resident
- Each resident has his own bacterial imprint which is stable during the entire study

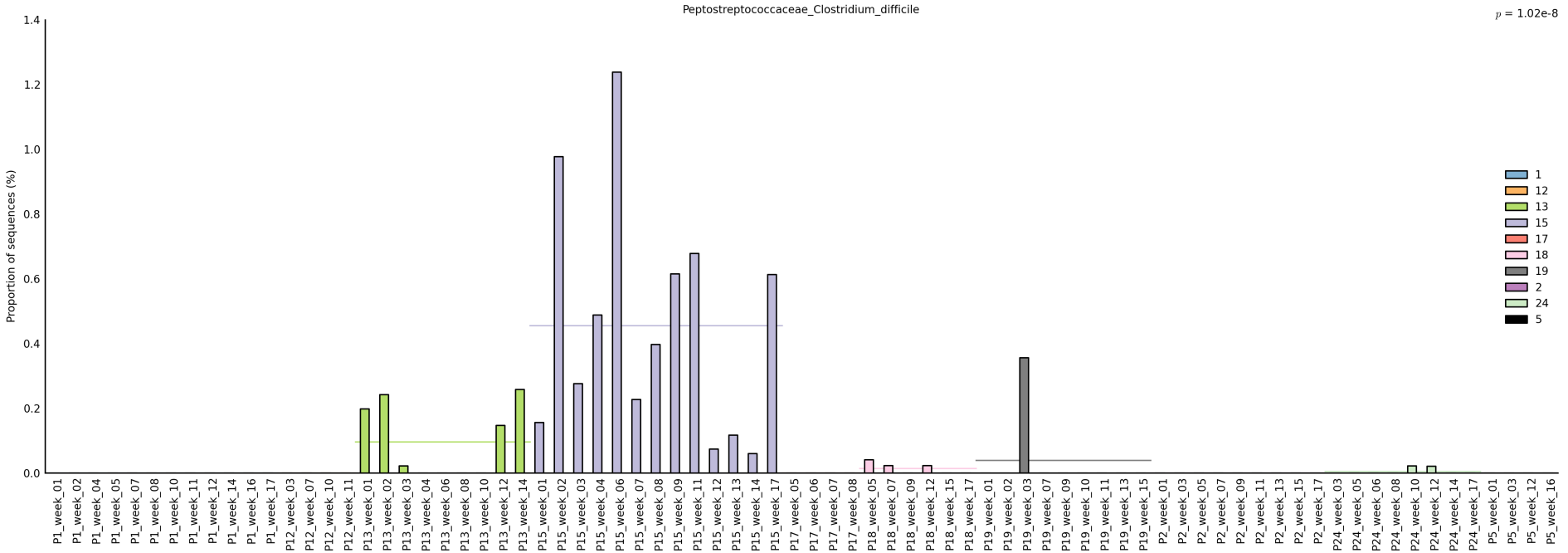
Abundance and Correlation Analysis

- ANOVA (AMOVA)
- Principal component analysis
- METASTATS
- UNIFRAC
- PARSIMONY



We can analyze the results using or combaining different criteria and using different test

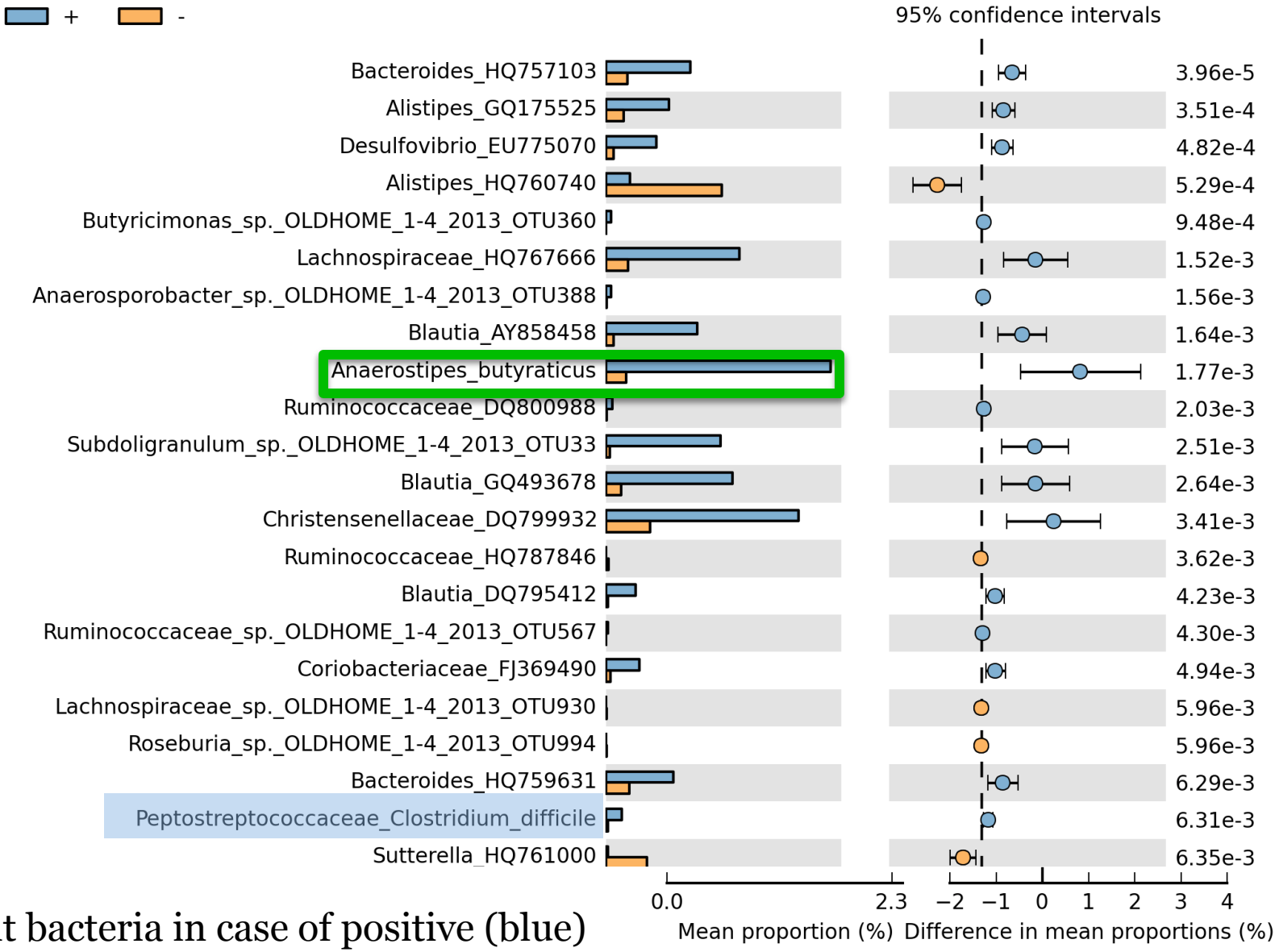
C. difficile abundance



13 15 18 19 24

resident

- Proportion of sequences of *C. difficile* detected for each resident each week
- Residents positive for *C. difficile* by classical microbiology showed an important proportion of *C. difficile* sequences



Different bacteria in case of positive (blue) and negative (orange) residents in relation with *C. difficile*

The story so far

- *C. difficile* prevalence of 30.4% in a Belgian nursing home
- The most common PCR-ribotype identified was 027
- Residents have all their microbiota print
- Metagenomics analysis can't substitute targeted procotocols
- But It offers a global picture of the microbiota context:
  - With correlations
  - Identifications
  - Follow up

ACKNOWLEDGEMENTS

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Eléonore Lyeza

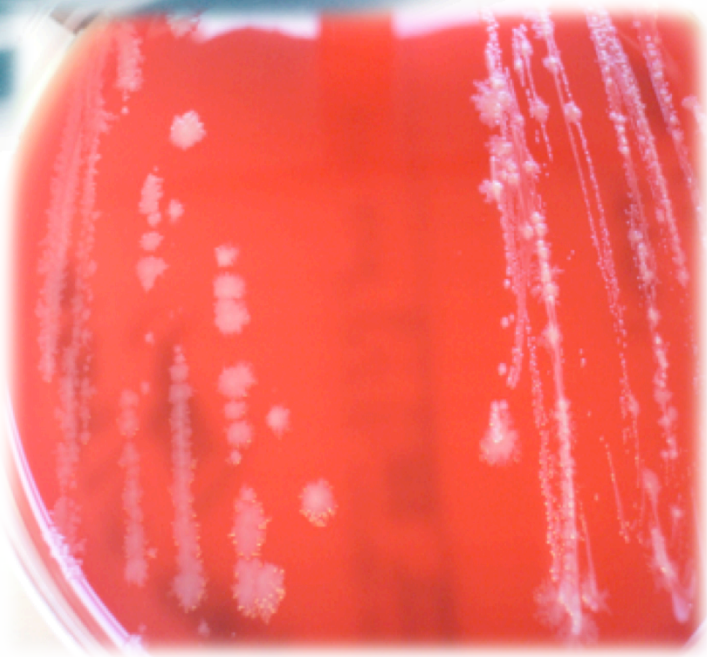
DDA
Département
Sciences des
Denrées alimentaires

METAGENOMIC ANALYSIS

Dr. Bernard Taminiau

bernard.taminiau@ulg.ac.be

Next generation sequencing technique



Classical microbiology

THANK YOU VERY MUCH