

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



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

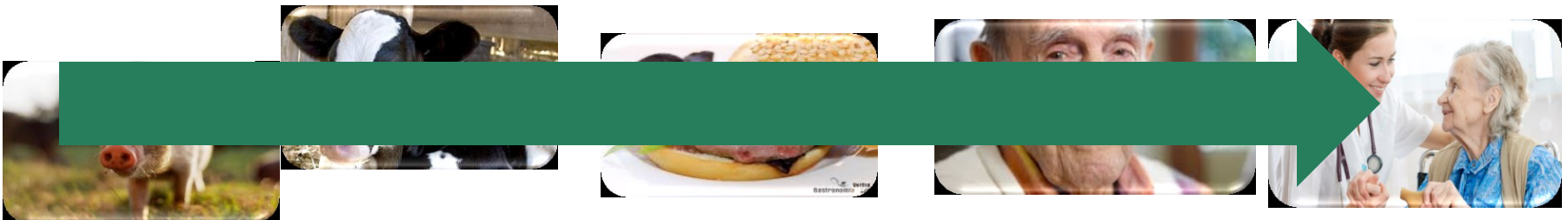
Gut Day 2013
7th november 2013
Groningen, the Netherlands



Background

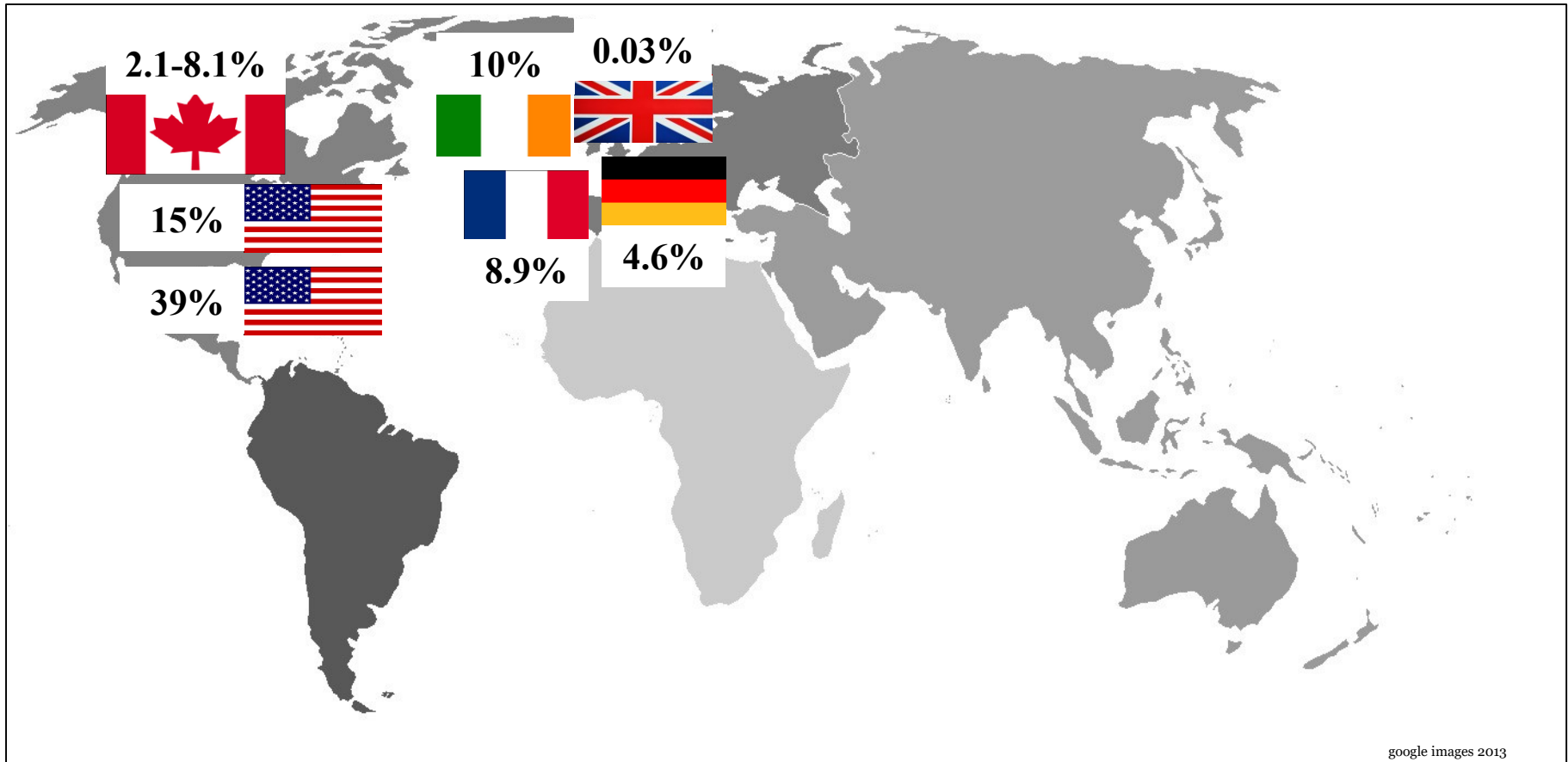
- Since toxigenic *C. difficile* was recognized as the major cause of antibiotic-associated diarrhea and pseudomembranous colitis in 1978, many outbreaks have been documented
- enhanced virulence and increased antibiotic resistance of *C. difficile* strains (PCR-ribotype 078/NAP-1/B1) has been observed for the last years
- 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 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*



Clostridium difficile Presence in Nursing homes

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



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

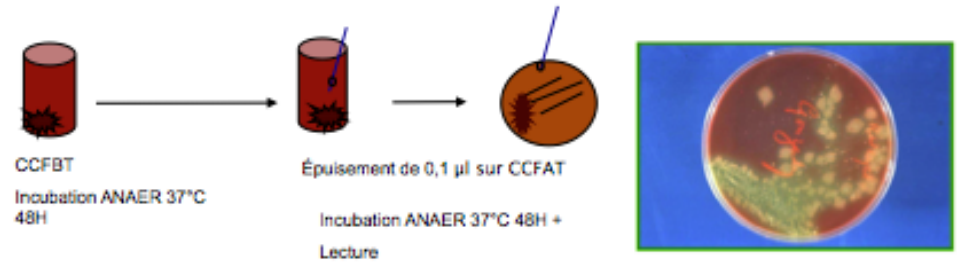
Study design

- During a 4-month period, stool samples from a group of 23 elderly care home residents were collected weekly (*From March to June 2013*)



- **Direct and enrichment culture**

Home-made cycloserine cefoxitin
fructose taurocholate



- **Detection of a species-specific internal fragment of *tpi*, detection of genes for toxin B, toxin A and binary toxin (*cdtA*) by PCR and Genotype Cdiff test system**
- **PCR-ribotyping**
- **Targeted Metagenomic analysis : 16S V1-V3 libraries**
 - Home-made bioinformatic pipeline

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



Analyzed in metagenomics

Patient	Week																	
	N°	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	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	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																
	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										

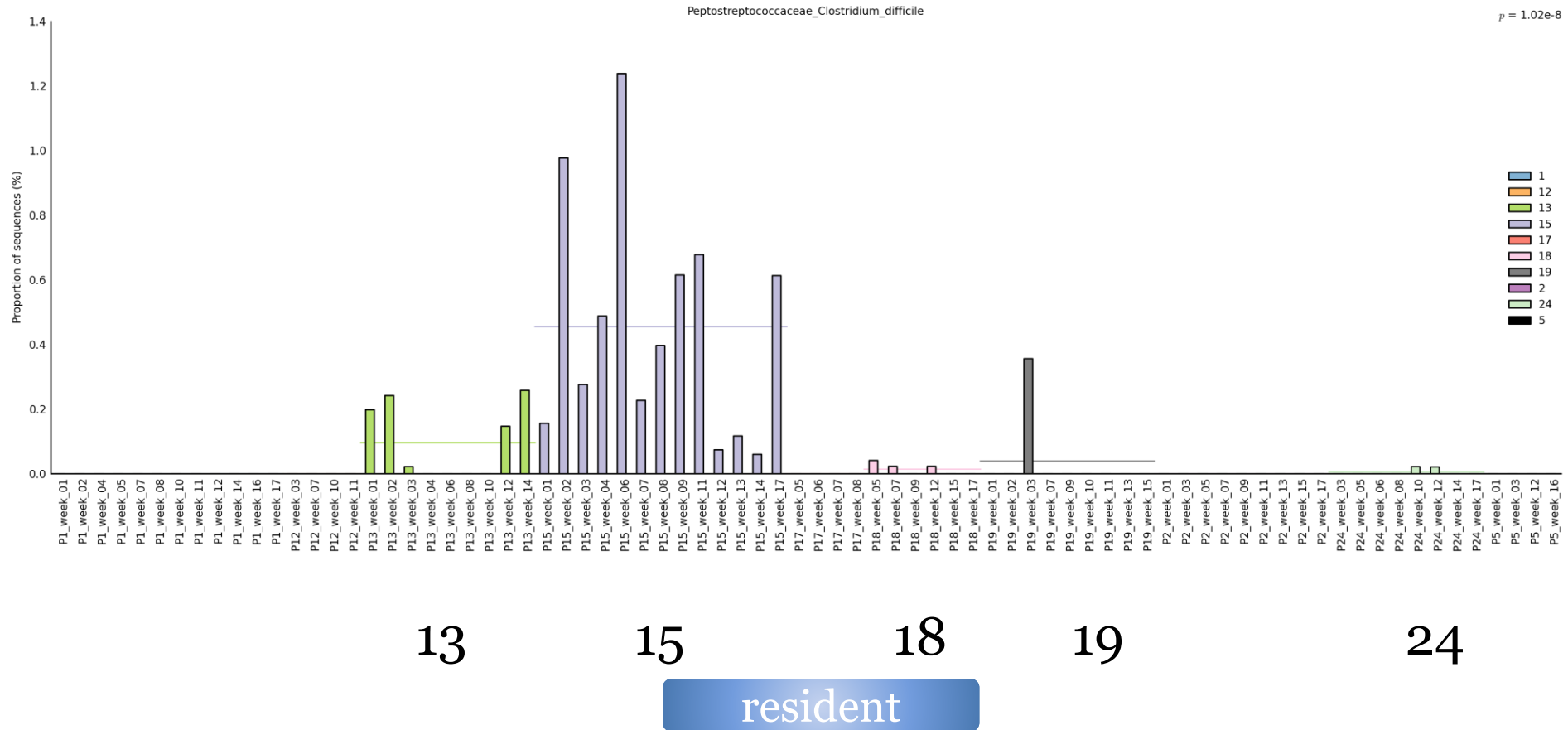
Sample not available

Negative

Positive after direct culture

Positive results after 3 days of enrichment

C. difficile abundance



- 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

The results so far:

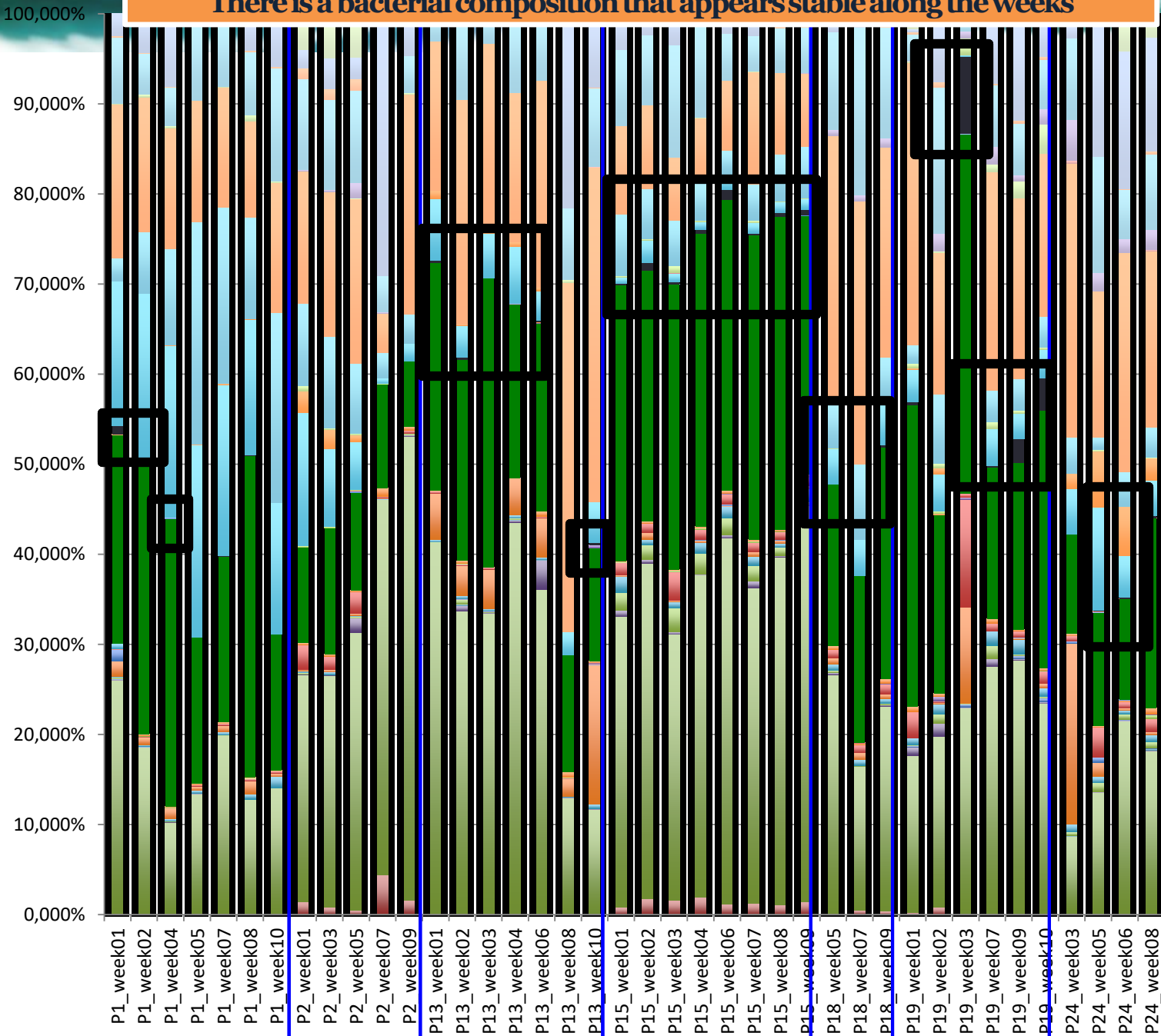
80 samples sequenced and analyzed: 6300 OTUs

Positive detection of *Clostridium difficile* :

<i>C. difficile</i> detection		
n	Microbiology	Amplicon sequencing
36	-	-
5	-	+

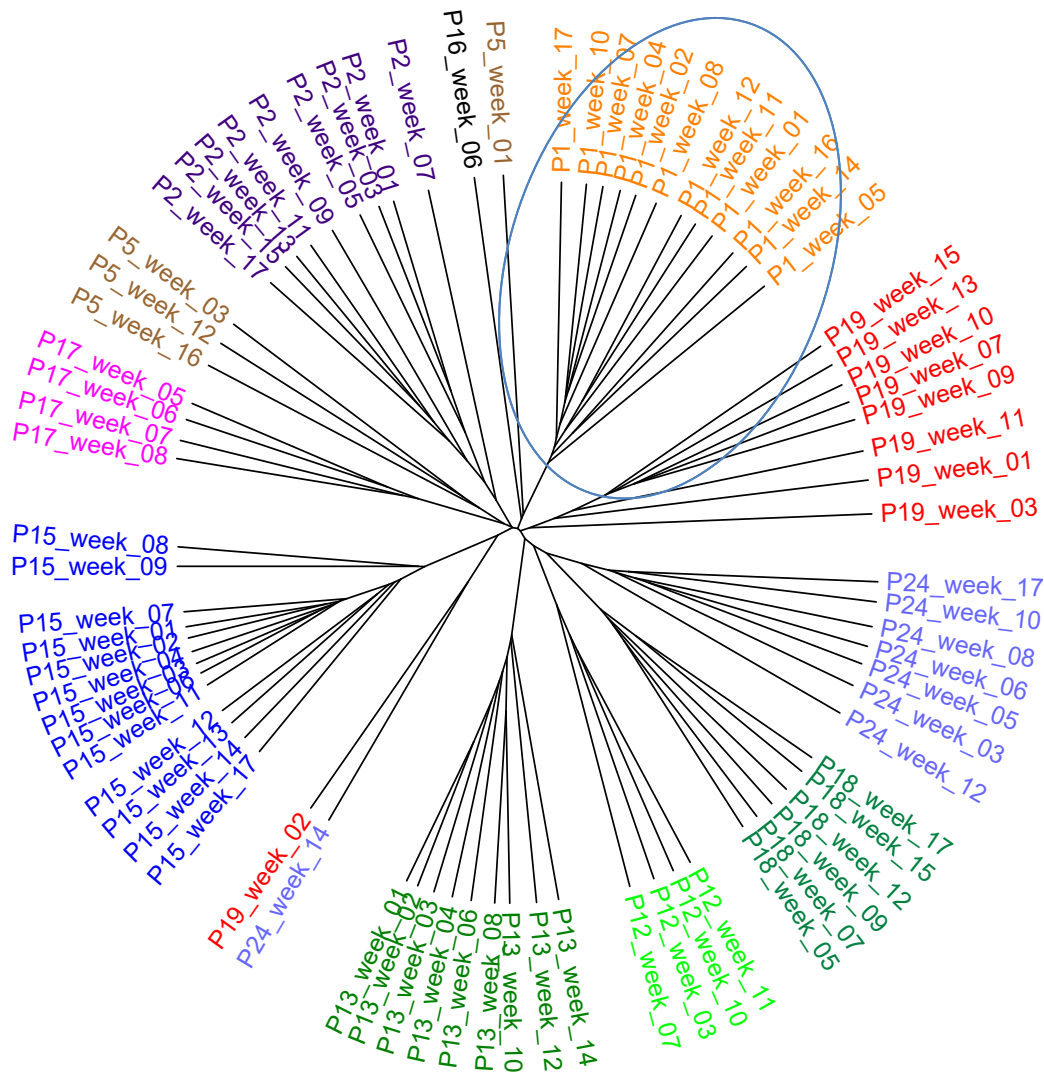
Relative proportions of the different bacterial families

There is a bacterial composition that appears stable along the weeks



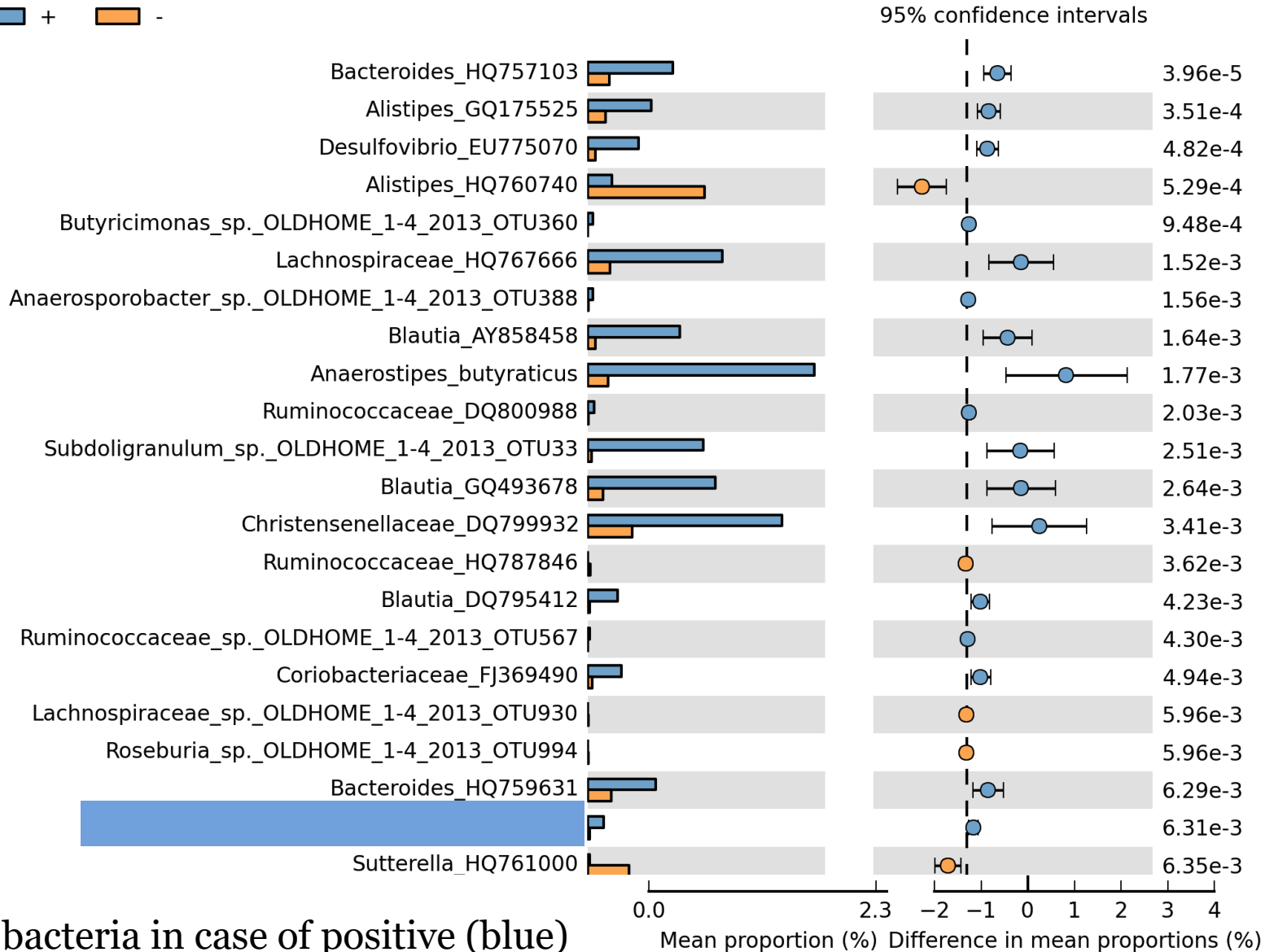
- *Victivallaceae*
- *Vibrionaceae*
- *Verrucomicrobiaceae*
- *Veillonellaceae*
- *unclassified*
- *Synergistaceae*
- *Streptococcaceae*
- *Staphylococcaceae*
- *Sphingomonadaceae*
- *Ruminococcaceae*
- *Rikenellaceae*
- *Rhizobiaceae*
- *Pseudomonadaceae*
- *Pseudoalteromonadaceae*
- *Propionibacteriaceae*
- *Prevotellaceae*
- *Porphyromonadaceae*
- ***Peptostreptococcaceae***
- *Oxalobacteraceae*
- *Moraxellaceae*
- *Micrococcaceae*
- *Microbacteriaceae*
- *Lactobacillaceae*
- *Lachnospiraceae*
- *Fusobacteriaceae*
- *Flavobacteriaceae*
- *Family_XIII_Incertae_Sedis*
- *Family_XII_Incertae_Sedis*
- *Family_XI_Incertae_Sedis*
- *Eubacteriaceae*
- *Erysipelotrichaceae*
- *Enterococcaceae*
- *Enterobacteriaceae*
- *Desulfovibrionaceae*
- *Corynebacteriaceae*
- *Coriobacteriaceae*
- *Comamonadaceae*
- *Clostridiaceae*
- *Carnobacteriaceae*
- *Campylobacteraceae*
- *Bifidobacteriaceae*
- *Bacteroidaceae*
- *Alcaligenaceae*
- *Actinomycetaceae*

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

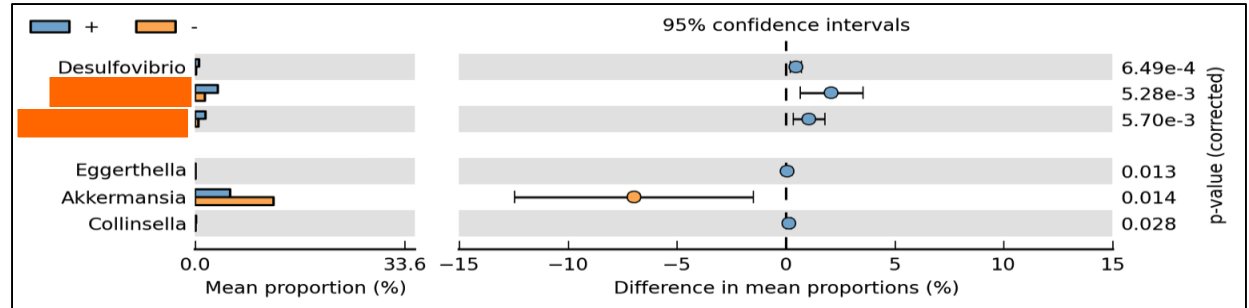
■ + ■ -



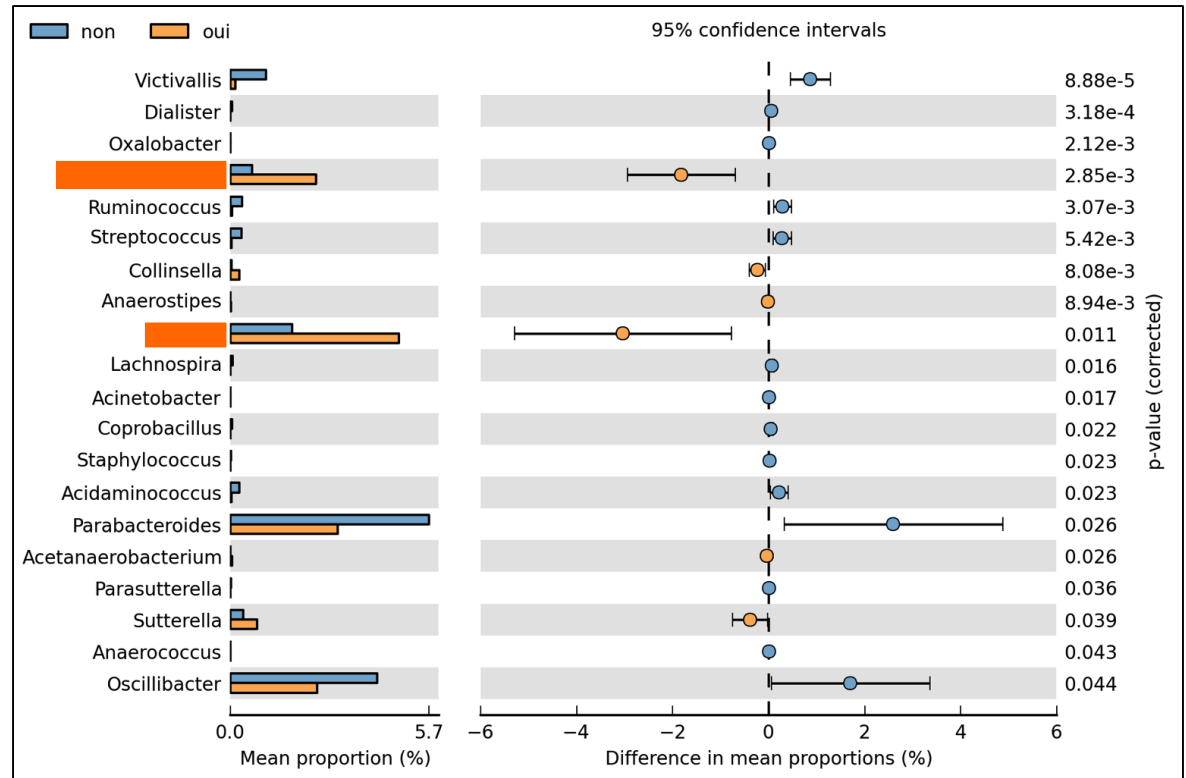
Different bacteria in case of positive (blue) and negative (orange) residents in relation with *C. difficile*

Statistical differences in genera abundance between

Cdiff positive (blue)
Cdiff negative (orange)



Diarrheic (orange)
Non diarrheic (blue)



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 protocols
- But It offers a global picture of the microbiota context:
 - With correlations
 - Identifications
 - Follow up

ACKNOWLEDGEMENTS



Nursing Home Sainte-Joséphine
(Theux-Belgium)



Prof. Georges Daube

Dr. Cristina Rodriguez

Dr. Nicolas Korsak



Prof. Michel Delmée

Véronique Avesani

Johan Van Broeck

Eléonore Lyeza

