

# ASSOCIATION OF CLASSICAL MICROBIOLOGY AND 16S rDNA METAGENETIC ANALYSIS TO EVALUATE THE PRESENCE OF *CLOSTRIDIUM DIFFICILE* IN A BELGIAN NURSING HOME

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## BACKGROUND

Increasing age, several co-morbidities, environmental contamination, antibiotic exposure and other intestinal perturbations appear to be the greatest risk factors for *C. difficile* infection (CDI). Therefore, elderly care home residents are considered particularly vulnerable.

## OBJECTIVES

The objective of this study was to evaluate and follow the prevalence of *C. difficile* in a Belgian nursing home. A *C. difficile* microbiological detection scheme was performed along with an overall microbial biodiversity study of the feces content by 16S rDNA metagenetic analysis.

## MATERIALS AND METHODS

<b>SAMPLING</b>	During a 4-month period, stool samples from a group of 23 elderly care home residents were collected weekly.
<b>CULTURE AND CHARACTERISATION</b>	Culture was performed in a selective medium CCFAT. An identification of the isolated colonies was done by detection of <i>tpi</i> , <i>tcdA</i> , <i>tcdB</i> and <i>cdtA</i> genes and by PCR ribotyping.
<b>METAGENETIC ANALYSIS</b>	The metagenetic analysis was targeted on the V1-V3 hyper-variable regions of 16S rDNA. The sequence reads were processed using MOTHUR software package. Taxonomical assignment of the populations beyond the genus level was performed with Silva data set and Blast algorithms. Richness and microbial diversity of the samples were evaluated using MOTHUR.

## RESULTS

Seven out of 23 (30.4%) residents were (at least one week) positive for *C. difficile*. The most common PCR-ribotype identified was 027

Table 1. Positive detection of *C. difficile*

<i>C. difficile</i> detection		
n	Microbiology	Amplicon sequencing
20	+	+
36	-	-
19	+	-
5	-	+

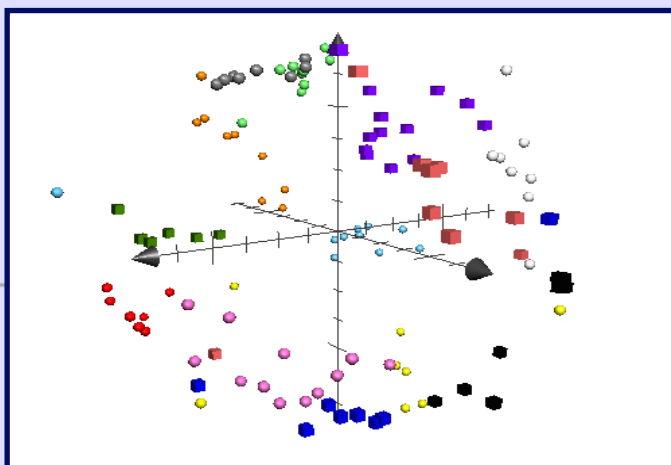


Figure 1. Non metric dimensional scaling representation of *C. difficile* positive (Boxes) and negative patients (Spheres)

- ❖ 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
- ❖ Residents' positives for *C. difficile* by classical microbiology showed an important proportion of *C. difficile* sequences

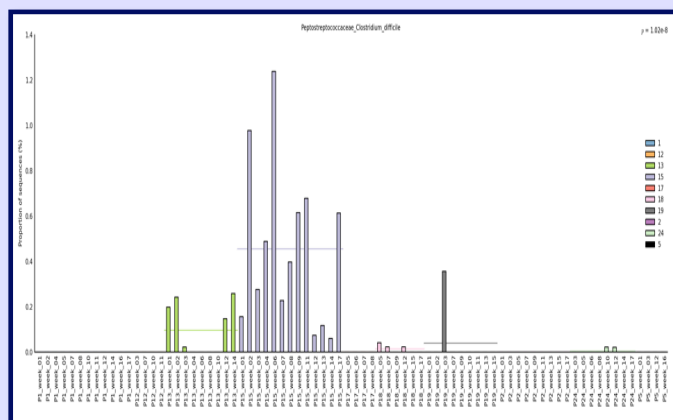


Figure 2. Proportion of sequences of *C. difficile* detected for each resident each week

## CONCLUSIONS

This unique association of classical microbiology protocol with pyrosequencing allowed to follow *C. difficile* in patients and to identify several other bacterial populations whose abundance is correlated with *C. difficile*. However, metagenetic sequencing can't substitute targeted protocols. It was not used as a diagnostic tool to detect *C. difficile* but rather to determine the identification and correlations of the major bacterial populations that are present in the gut microbiota.