

# SUBTYPING *CLOSTRIDIUM DIFFICILE* PCR-RIBOTYPE 018 STRAINS BY ANALYSIS OF VIRULOME, RESISTOME, SNPs, wgMLST AND MLVA

Gateau Cécile<sup>1</sup>, Couturier Jeanne<sup>1,2,3</sup>, Barbut Frédéric<sup>1,2,3</sup>.

<sup>1</sup>National Reference Laboratory for *C. difficile*, Saint-Antoine Hospital, AP-HP, Paris France; <sup>2</sup>Department of Microbiology, Saint-Antoine hospital, AP-HP, Paris, France; <sup>3</sup>INSERM UMR S1139, University Paris Descartes, Paris, France.

## Background- Objective

*Clostridium difficile* PCR-Ribotype (RT) 018 is an emerging RT associated to severe infections and outbreaks with a transmission index 10-fold higher than that of RT078 strains. As for other common RT i.e 001, 027 or 014, we need more discriminant methods to subtype some specific RT to better understand transmission mechanisms or to investigate outbreaks. We compared MLVA (Multi-Locus VNTR [Variable Number Tandem Repeat] Analysis), wgMLST (whole genome Multi Locus Sequence Typing), SNPs, virulome and resistome for subtyping RT018 strains.

## Materials and Methods

### Strains :

- 31 RT018 strains including
  - 17 strains from a well-documented outbreak in a geriatric unit (GU) in Strasbourg in 2017, France (all resistant to moxifloxacin, erythromycin and binary toxin-negative)
  - 2 RT018 strains isolated in the same ward two years before (CD15-230, CD15-235)
  - 12 epidemiologically unrelated strains from other French healthcare facilities (HCF)

**MLVA** : seven tandem repeat loci (A6, B7, C6, E7, F3, G8, H9) were amplified by PCR. The genetic relationship between two strains was assessed by calculating the summed tandem repeat differences (STRD). Strains with an STRD  $\leq 10$  were defined as genetically related and clonal complexes (CC) were defined by an STRD  $\leq 2$ .

**wgMLST** : WGS was performed on NextSeq instrument (Illumina).

Data of 8745 loci were analysed with BioNumerics® 7.6.3 software. The genetic relationship between two strains was assessed by calculating the number of different alleles. Strains with an allele difference  $\leq 200$  were defined as genetically related and clonal complexes were defined by an allele difference  $\leq 20$ .

**SNP** : analysis of SNP on core genome was performed using BioNumerics® 7.6.3 software. Strains with less than 10 SNPs belong to the same clonal complex those with 10-20 SNPs were considered genetically related. Strains were different if they display more than 20 SNP.

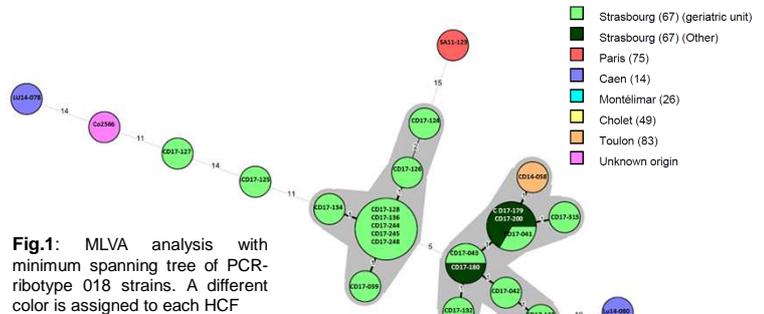
**BIOMERIEUX EPISEQ® CS Software** was used to characterized by the virulome and resistome of the different strains from data generated by WGS.

## Results

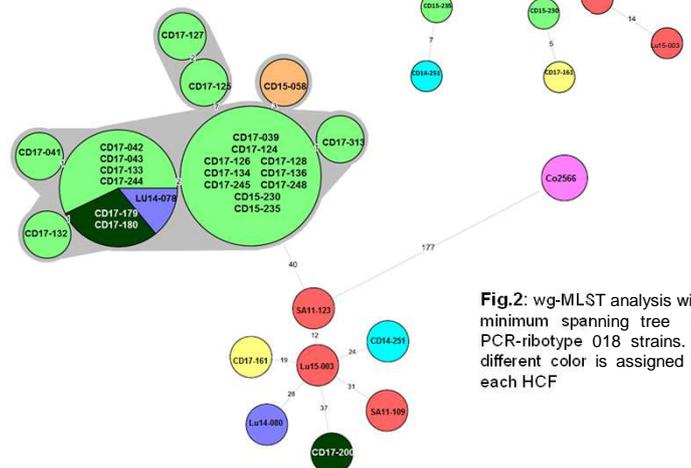
The MLVA analysis indicated that among the 31 *C. difficile* RT 018 strains, 19 are included in 2 clonal complexes (Fig.1). The first one comprised 9 strains (53%), all isolated in patients from the geriatric units and the second one included 10 strains (6 strains (35%) from the geriatric units and 4 strains (33%) from other HCF). Two 018 epidemic strains did not belong to CC (Table 1).

There is only 1 clonal complex for the wg-MLST that includes 17 epidemic strains of RT018 (100%) and 4 strains (33%) from other HCF (Fig.2, Table 1). Among the epidemic strains, 82.4 % (14/17) display the same resistome whereas 33.3% (4/12) only of the non epidemic strains had the same epidemic resistome pattern. Virulome was not discriminating.

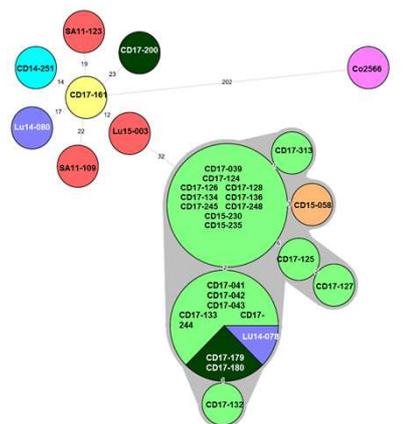
Phylogenetic tree generated by analysis of SNPs gave the same clusters as wgMLST (Fig.3).



**Fig.1:** MLVA analysis with minimum spanning tree of PCR-ribotype 018 strains. A different color is assigned to each HCF



**Fig.2:** wg-MLST analysis with minimum spanning tree of PCR-ribotype 018 strains. A different color is assigned to each HCF



**Fig.3:** SNP analysis of PCR-ribotype 018 strains. A different color is assigned to each HCF

Each circle represents a single MLVA-type (Fig.1) or wg-MLST type (Fig. 2), the size of the circle being proportional to the number of strains included. The numbers between the circles correspond to the number of STRDs between the MLVA-types (fig.1) or number of allele difference between the wg-MLST type (fig 2) or number of SNPs differences in the core genome (fig.3).

	MLVA				wg-MLST			cg-SNP		
	$\leq 2$ STRD Clonal complex		$>2$ STRD $\leq 10$ Genetically related	$>10$ STRD	$\leq 20$ allele difference Clonal complex	$>20$ allele difference $\leq 200$ Genetically related	$>200$ allele difference	$\leq 10$ SNP Clonal complex	$> 10$ SNP $\leq 200$ Genetically related	$>200$ SNP
	Cluster 1	Cluster 2		Cluster 1			Cluster 1			
% of strains RT 018 at Strasbourg (geriatric units)*	52.9 9/17	35.3 6/17	0 0/17	17.8 2/17	100 17/17	/	/	100 17/17	/	/
% of strains RT 18 in other French healthcare facilities	0 0/12	33.3 4/12	25 3/12	41.7 5/12	33.3 4/12	66.7 8/12	/	33.3 4/12	58.3 7/12	8.3 1/12

**Table 1:** Comparison of clonal complexes identified by MLVA and wg-MLST and SNP analysis. \*This percentage does not take account the 2 RT 015 strains isolated in 2015 in this geriatric unit.

## References

1. Barbanti F, Spigaglia P. Anaerobe. (2016) Characterization of *Clostridium difficile* PCR-ribotype 018: A problematic emerging type.
2. Senoh M et al. J Med Microbiol. (2015) Predominance of PCR-ribotypes, 018 (smz) and 369 (trf) of *Clostridium difficile* in Japan: a potential relationship with other global circulating strains?
3. Baldan R et al. J Clin Microbiol. (2015) *Clostridium difficile* PCR Ribotype 018, a Successful Epidemic Genotype.
4. Lee JH et al. J Med Microbiol. (2014) The changes of PCR ribotype and antimicrobial resistance of *Clostridium difficile* in a tertiary care hospital over 10 years.

## Conclusions

SNP and wgMLST analysis gave very similar results and the same classification of strains. All the epidemic strains belong to the same clonal complex. MLVA and wgMLST gave quite consistent information but the wgMLST better separated epidemic from non-epidemic strains. MLVA was able to discriminate RT018 isolated in 2015 from the epidemic clone in 2018.