

# Clostridioides difficile PCR RIBOTYPE 002 INFECTION IN THE NETHERLANDS IS NOT ASSOCIATED WITH INCREASED DISEASE SEVERITY

## Background:

*Clostridioides difficile* PCR ribotype (RT) 002 is the third most prevalent RT in the Netherlands. In 2019, a sudden increase in *C. difficile* infections (CDI) with a clonal strain of RT002 was reported in Ireland. In retrospect, a significant increase in CDI due to RT002 was detected from 6.5% between May'16- May'17 to 11.9% between May'17 - May'18 in the Netherlands (Fig. 1).

## Aim of the study

Our aim was to analyze and characterize CDI due to RT002, to compare the strains with the Irish data and compare clinical characteristics with hypervirulent (HV) RTs (RT027 and RT078) and non HV RTs

## Methods

### Clinical data

Setting Dutch general hospitals and university medical centers, using the national *C. difficile* surveillance data  
 Period May 2009- May 2019  
 Inclusion Hospitalized patients with CDI due to RT002

### Antibiotic phenotype

AST MIC Test Strip (bioMérieux, Liofilchem)

### Molecular typing

NGS Illumina Novaseq 6000 (Genome Scan B.V.)  
 WGS typing cgMLST (Ridom®SeqSphere v5.1)

## Results

### Clinical characteristics (Table1)

- Prevalence of severe CDI in RT002 is similar as in non-HV RT (23% vs 21%)
- Complicated CDI course in RT002 is lower in comparison with RT027 (14% vs. 22%), but similar to RT078 and non-HV RT
- There is no difference in CDI-related mortality between all groups
- RT002 had more community onset of CDI in comparison with RT027, RT078 and non-HV RTs (43% vs. 28%, 38% and 38%, resp.) and less antibiotic usage (63% vs. 78%, 71% and 70%, resp.)

### AST

- All strains were susceptible for metronidazole and vancomycin (MIC of 0.06 mg/L and <0.06-0.06mg/L)

### WGS

- No clonal expansion was found in the Netherlands and no genetic relatedness was found with the Irish strain (Fig. 2)
- A T349I amino acid change was found in *vanS*, this resulted not in a higher MIC for vancomycin (Fig. 3a)
- Analysis of the trehalose genes showed no L172I mutation in *treR* and no secondary metabolism cluster (Fig 3b)

Table 1: Comparison of clinical characteristics of patients with RT002 vs Others ribotypes (Others includes RT001, RT005 and RT014/020/295)

|                                     | RT002 (n=495)       | Others (n=5372)     | RT027 (n=123)        | RT078/226 (n=852)   | RT014/020/295 (n=460) | RT001 (n=765)         | RT005 (n=324)       |
|-------------------------------------|---------------------|---------------------|----------------------|---------------------|-----------------------|-----------------------|---------------------|
| Age                                 | 67,38 (65,66-69,11) | 67,06 (66,55-67,57) | 70,078 (67,24-72,91) | 68,55 (67,46-69,64) | 66,31 (65,23-67,40)   | 71,62 (70,46-72,79) * | 67,71 (65,63-69,90) |
| Men                                 | 230 (46,46)         | 2625 (48,89)        | 65 (52,85)           | 422 (49,53)         | 546 (46,83)           | 377 (49,41)           | 153 (47,22)         |
| Severe CDI                          | 109 (23,19)         | 1038 (20,61)        | 32 (29,36)           | 215 (26,77)         | 207 (18,73) *         | 116 (16,34) *         | 72 (23,61)          |
| Dehydration and/or hypoalbuminaemia | 62 (13,19)          | 527 (10,48)         | 16 (14,68)           | 116 (14,48)         | 109 (9,87)            | 47 (6,62) *           | 34 (11,15)          |
| Bloody diarrhoea                    | 23 (4,89)           | 242 (4,81)          | 6 (5,50)             | 33 (4,11)           | 41 (3,71)             | 30 (4,23)             | 18 (5,90)           |
| Pseudomembraneous colitis           | 22 (4,68)           | 178 (3,53)          | 7 (6,42)             | 46 (5,73)           | 32 (2,90)             | 21 (2,96)             | 8 (2,62)            |
| Fever and leucocytosis              | 40 (8,51)           | 345 (6,86)          | 11 (10,09)           | 88 (10,99)          | 70 (6,34)             | 41 (5,77)             | 34 (11,15)          |
| Complicated course                  | 62 (14,39)          | 574 (12,64)         | 22 (22,45) *         | 112 (15,75)         | 94 (9,38) *           | 102 (16,48)           | 35 (12,92)          |
| Overall mortality                   | 57 (13,23)          | 508 (11,19)         | 19 (19,39)           | 105 (14,77)         | 83 (8,28) *           | 93 (15,03)            | 32 (11,81)          |
| CDI mortality                       | 16 (3,71)           | 118 (2,60)          | 5 (5,10)             | 29 (4,08)           | 19 (1,90) *           | 27 (4,38)             | 8 (2,95)            |
| Community onset                     | 212 (43,09)         | 2014 (37,94) *      | 34 (28,33) *         | 323 (38,18)         | 447 (38,67)           | 184 (24,44) *         | 131 (40,68)         |
| CDI last 8 weeks                    | 83 (25,08)          | 873 (25,50)         | 15 (23,08)           | 153 (28,65)         | 204 (27,35)           | 126 (24,95)           | 56 (28,57)          |
| Antibiotic use                      | 274 (63,13)         | 3270 (70,01) *      | 80 (77,67) *         | 531 (71,47) *       | 718 (69,64) *         | 524 (78,92) *         | 202 (72,66) *       |

An asterisk (\*) represents a p<0.05, when comparing with RT002.

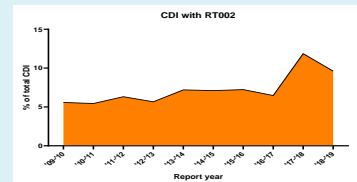


Figure 1: incidence of CDI due to RT002 between '09-'19 in the Netherlands

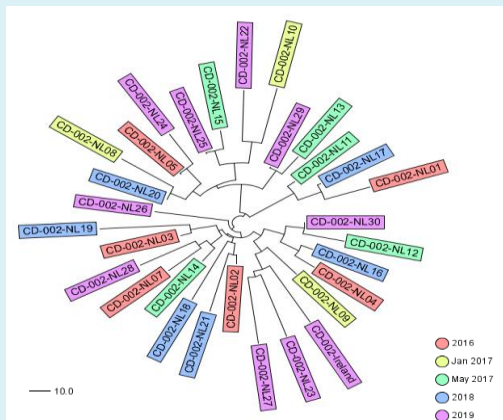


Figure 2: allele difference-based neighbor joining tree of Dutch and Irish RT002 (CD-002-Ireland) strains. Strains from May-Dec 2016, Jan-Apr 2017, May-Dec 2017, Jan-Apr 2018 and May 2018-Apr 2019 are colored red, yellow, green, blue and purple, resp.

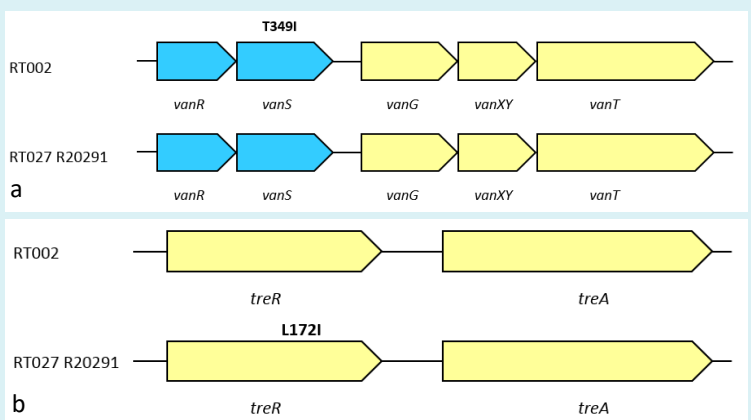


Figure 3: schematic demonstration of the vanco-operon (above) and the primary trehalose cluster (below). (a) RT002 with the T349I mutation, possibly associated with enhanced vancomycin MIC. (b) RT002 did not have the L172I mutation associated with increased metabolism of trehalose.

## Conclusions

- The increase in CDI incidence due to RT002 in the Netherlands is not caused by clonal expansion of one strain
- The Dutch RT002 are not genetically related to the Irish strain.
- CDI associated with RT002 has a similar CDI severity, complicated course and CDI-related mortality as non-HV strains

