

# Draft Genome Sequence of *Clostridium difficile* Belonging to Ribotype 018 and Sequence Type 17

E. Riccobono,<sup>a</sup> V. Di Pilato,<sup>b</sup> N. Della Malva,<sup>a</sup> S. Meini,<sup>c</sup> F. Ciralo,<sup>d</sup> F. Torricelli,<sup>e</sup> G. M. Rossolini<sup>a,f,g,h</sup>

Department of Experimental and Clinical Medicine, University of Florence, Careggi University Hospital, Florence, Italy<sup>a</sup>; Department of Surgery and Translational Medicine, University of Florence, Florence, Italy<sup>b</sup>; Internal Medicine Unit, Santa Maria Annunziata Hospital, Florence, Italy<sup>c</sup>; Office of the Chief Medical Officer, Santa Maria Annunziata and Serristori Hospitals, Florence, Italy<sup>d</sup>; Genetic Diagnostic Unit, Florence Careggi University Hospital, Florence, Italy<sup>e</sup>; Department of Medical Biotechnologies, University of Siena, Santa Maria alle Scotte University Hospital, Siena, Italy<sup>f</sup>; Clinical Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy<sup>g</sup>; Don Carlo Gnocchi Foundation, Florence, Italy<sup>h</sup>

***Clostridium difficile*, belonging to ribotype 018 (RT018), is one of the most prevalent genotypes circulating in hospital settings in Italy. Here, we report the draft genome of *C. difficile* CD8-15 belonging to RT018, isolated from a patient with fatal *C. difficile*-associated infection.**

Received 5 July 2016 Accepted 6 July 2016 Published 1 September 2016

**Citation** Riccobono E, Di Pilato V, Della Malva N, Meini S, Ciralo F, Torricelli F, Rossolini GM. 2016. Draft genome sequence of *Clostridium difficile* belonging to ribotype 018 and sequence type 17. *Genome Announc* 4(5):e00907-16. doi:10.1128/genomeA.00907-16.

**Copyright** © 2016 Riccobono et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to G. M. Rossolini, gianmaria.rossolini@unifi.it.

*Clostridium difficile* infection (CDI) is one of the most common hospital-acquired infections and the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis (1). Increasing CDI incidence rates have been mainly attributed to successful clones belonging to a few predominant ribotypes (RTs), with different distribution in different geographic regions (2, 3).

RT018 has been reported as one of the most prevalent genotypes circulating in hospital settings in Italy (4, 5) and in the Far East (i.e., South Korea and Japan) (6, 7) but has been rarely described in other countries (2, 8). RT018 is highly transmissible and generally shows a multidrug-resistant phenotype. It produces both toxin A and toxin B, but is negative for binary toxin, and is associated with complicated outcomes (4, 7, 9). In this report, we announce the draft genome of *C. difficile* CD8-15 belonging to RT018, which was isolated from a patient admitted to a hospital in Florence (central Italy) in 2015 with fatal *C. difficile*-associated infection evolved as toxic megacolon.

*C. difficile* CD8-15 was grown in 5 ml of thioglycollate broth and incubated under anaerobic conditions for 48 h at 37°C. Genomic DNA was subjected to whole-genome sequencing with the MiSeq platform (Illumina Inc., San Diego, CA, USA) using a 2×300-bp paired-end approach. A total of 2,118,990 reads were generated and then *de novo* assembled using SPAdes version 3.6.1 (10) into 44 scaffolds (largest scaffold = 831,956 bp;  $N_{50}$  = 235,991 bp;  $L_{50}$  = 5; average GC = 28.63%), with an estimated genome size of 4,249,791 bp and an average coverage of 50×.

The draft genome was then subjected to the following *in silico* analyses: (i) multilocus sequence typing (MLST; <http://pubmlst.org/cdifficile/>); (ii) toxinotyping (<http://www.mf.uni-mb.si/mikro/tox/>); (iii) detection of antibiotic resistance mechanisms, using the *C. difficile* 630 strain (11). The presence of phage-related sequences and CRISPR-Cas systems were investigated using PHAST (<http://phast.wishartlab.com>) and CRISPRFinder (<http://crispr.u-psud.fr>), respectively.

MLST analysis assigned *C. difficile* CD8-15 to sequence type

(ST) 17, as previously reported for other *C. difficile* strains belonging to RT018 (12, 13). ST17 and other STs of *C. difficile* belong to clade I (12). Toxinotyping classified *C. difficile* CD8-15 as a non-variant strain (toxinotype 0) producing toxin A and toxin B but not binary toxin CDT.

*C. difficile* CD8-15 was resistant to rifampin (MIC >32 mg/L), levofloxacin (MIC >32 mg/L), and erythromycin (MIC >256 mg/L) but remained susceptible to clindamycin (MIC 2 mg/L). Sequence analysis revealed the presence of point mutations in the *rpoB* gene (R505K and I548M), which is known to be associated with high-level resistance to rifamycins (14). Mutations were detected also in the quinolone resistance-determining region of *gyrA* (T82I), which is consistent with the fluoroquinolones resistance phenotype. Resistance to erythromycin was likely attributable to the presence of a multidrug efflux system encoded by the *cme* gene (15), while *erm* genes and other genes involved in macrolides resistance (i.e., *mef*, *msr*, *cfr*) were not detected (9, 16). PHAST identified 3 intact and 5 incomplete prophage regions. CRISPRFinder identified two class I CRISPR-Cas systems.

**Accession number(s).** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [LYDP00000000](https://www.ncbi.nlm.nih.gov/nuccore/LYDP00000000).

## ACKNOWLEDGMENTS

We thank Raffaele Laureano, Christian Adamo, and Chiara Barchielli for their contribution.

## REFERENCES

- Dubberke ER, Olsen MA. 2012. Burden of *Clostridium difficile* on the healthcare system. *Clin Infect Dis* 55(Suppl 2):S88–S92. <http://dx.doi.org/10.1093/cid/cis335>.
- Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, Monnet DL, van Dissel JT, Kuijper EJ, ECDIS Study Group. 2011. *Clostridium difficile* infection in Europe: a hospital-based survey. *Lancet* 377:63–73. [http://dx.doi.org/10.1016/S0140-6736\(10\)61266-4](http://dx.doi.org/10.1016/S0140-6736(10)61266-4).
- Freeman J, Bauer MP, Baines SD, Corver J, Fawley WN, Goorhuis B,

- Kuijper EJ, Wilcox MH. 2010. The changing epidemiology of *Clostridium difficile* infections. *Clin Microbiol Rev* 23:529–549. <http://dx.doi.org/10.1128/CMR.00082-09>.
4. Baldan R, Trovato A, Bianchini V, Biancardi A, Cichero P, Mazzotti M, Nizzero P, Moro M, Ossi C, Scarpellini P, Cirillo DM. 2015. *Clostridium difficile* PCR ribotype 018, a successful epidemic genotype. *J Clin Microbiol* 53:2575–2580. <http://dx.doi.org/10.1128/JCM.00533-15>.
  5. Spigaglia P, Barbanti F, Dionisi AM, Mastrantonio P. 2010. *Clostridium difficile* isolates resistant to fluoroquinolones in Italy: emergence of PCR ribotype 018. *J Clin Microbiol* 48:2892–2896. <http://dx.doi.org/10.1128/JCM.02482-09>.
  6. Han SH, Kim H, Lee K, Jeong SJ, Park KH, Song JY, Seo YB, Choi JY, Woo JH, Kim WJ, Kim JM. 2014. Epidemiology and clinical features of toxigenic culture-confirmed hospital-onset *Clostridium difficile* infection: a multicentre prospective study in tertiary hospitals of South Korea. *J Med Microbiol* 63:1542–1551. <http://dx.doi.org/10.1099/jmm.0.070672-0>.
  7. Senoh M, Kato H, Fukuda T, Niikawa A, Hori Y, Hagiya H, Ito Y, Miiki H, Abe Y, Furuta K, Takeuchi H, Tajima H, Tominaga H, Satomura H, Kato H, Morita S, Tanada A, Hara T, Kawada M, Sato Y, Takahashi M, Higuchi A, Nakajima T, Wakamatsu Y, Toyokawa M, Ueda A, Roberts P, Miyajima F, Shibayama K. 2015. Predominance of PCR-ribotypes, 018 (smz) and 369 (trf) of *Clostridium difficile* in Japan: a potential relationship with other global circulating strains? *J Med Microbiol* 64:1226–1236. <http://dx.doi.org/10.1099/jmm.0.000149>.
  8. Martin JS, Monaghan TM, Wilcox MH. 2016. *Clostridium difficile* infection: epidemiology, diagnosis and understanding transmission. *Nat Rev Gastroenterol Hepatol* 13:206–216. <http://dx.doi.org/10.1038/nrgastro.2016.25>.
  9. Spigaglia P. 2016. Recent advances in the understanding of antibiotic resistance in *Clostridium difficile* infection. *Ther Adv Infect Dis* 3:23–42. <http://dx.doi.org/10.1177/2049936115622891>.
  10. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <http://dx.doi.org/10.1089/cmb.2012.0021>.
  11. Sebahia M, Wren BW, Mullany P, Fairweather NF, Minton N, Stabler R, Thomson NR, Roberts AP, Cerdeño-Tárraga AM, Wang H, Holden MT, Wright A, Churcher C, Quail MA, Baker S, Bason N, Brooks K, Chillingworth T, Cronin A, Davis P, Dowd L, Fraser A, Feltwell T, Hance Z, Holroyd S, Jagels K, Moule S, Mungall K, Price C, Rabinowitsch E, Sharp S, Simmonds M, Stevens K, Unwin L, Whithead S, Dupuy B, Dougan G, Barrell B, Parkhill J. 2006. The multidrug-resistant human pathogen *Clostridium difficile* has a highly mobile, mosaic genome. *Nat Genet* 38:779–786. <http://dx.doi.org/10.1038/ng1830>.
  12. Knetsch CW, Terveer EM, Lauber C, Gorbalenya AE, Harmanus C, Kuijper EJ, Corver J, van Leeuwen HC. 2012. Comparative analysis of an expanded *Clostridium difficile* reference strain collection reveals genetic diversity and evolution through six lineages. *Infect Genet Evol* 12:1577–1585. <http://dx.doi.org/10.1016/j.meegid.2012.06.003>.
  13. Dingle KE, Didelot X, Ansari MA, Eyre DW, Vaughan A, Griffiths D, Ip CL, Batty EM, Golubchik T, Bowden R, Jolley KA, Hood DW, Fawley WN, Walker AS, Peto TE, Wilcox MH, Crook DW. 2013. Recombinational switching of the *Clostridium difficile* S-layer and a novel glycosylation gene cluster revealed by large-scale whole-genome sequencing. *J Infect Dis* 207:675–686. <http://dx.doi.org/10.1093/infdis/jis734>.
  14. Curry SR, Marsh JW, Shutt KA, Muto CA, O’Leary MM, Saul MI, Pasculle AW, Harrison LH. 2009. High frequency of rifampin resistance identified in an epidemic *Clostridium difficile* clone from a large teaching hospital. *Clin Infect Dis* 48:425–429. <http://dx.doi.org/10.1086/596315>.
  15. Lebel S, Bouttier S, Lambert T. 2004. The *cme* gene of *Clostridium difficile* confers multidrug resistance in *Enterococcus faecalis*. *FEMS Microbiol Lett* 238:93–100. <http://dx.doi.org/10.1111/j.1574-6968.2004.tb09742.x>.
  16. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <http://dx.doi.org/10.1093/jac/dks261>.