

# *Clostridium difficile* ribotype 033 colitis in a patient following broad-spectrum antibiotic treatment for KPC-producing *Klebsiella pneumoniae* infection, Italy

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## SUMMARY

This report describes a case of *Clostridium difficile* ribotype 033 colitis in a patient treated with multiple antibiotics for KPC-producing *Klebsiella pneumoniae* pancreatitis. Diagnostic, clinical and therapeutic features are discussed. To the best of our knowledge, this is the first case of *C. difficile* ribotype 033 clinical infection reported from Italy.

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An 80 year-old man was admitted to dell'Angelo Hospital, Venezia-Mestre, Italy, in December 2013 for acute abdominal pain. The patient had a history of hypertension, diabetes mellitus, bilateral hip replacement in 2001 and 2003, and pulmonary embolism in 2010. Following laboratory and imaging evaluation, the patient was diagnosed with necrotic-haemorrhagic pancreatitis, and was transferred to the Intensive Care Unit. An empirical antibiotic treatment with piperacillin-tazobactam 4.5 g qid iv was started (Figure 1). At the time of admission to the ICU, a surveillance rectal swab for carbapenem-resistant enterics, performed by cultural method (chromID™ CARBA SMART bioMérieux, Marcy-l'Étoile, France), resulted negative.

At 7 days after admission, surveillance cultures revealed intestinal and respiratory tract colonization by a carbapenem-resistant *K. pneumoniae*. The strain retained susceptibility to gentamicin (MIC=2 mcg/ml) and tigecycline (MIC=1 mcg/ml) and was intermediate to imipenem (MIC=8 mcg/ml), resistant to meropenem (MIC=16 mcg/ml), ertapenem (MIC>1 mcg/ml) and colistin (MIC>8 mcg/ml) using the broth micro-dilution method (custom plates, Thermo Fisher Scientific, Oakwood Village, OH, USA).

At 30 days after admission diarrhoea was reported, and a stool sample, tested using the Xpert *Clostridium difficile* kit on the GeneXpert platform® (Cepheid, Sunnyvale CA, USA), yielded a positivity for the *C. difficile* binary toxin genes (*cdtAB*) with negativity for the *tcdB* gene (atypical

B-CDT<sup>+</sup> pattern). The diarrhoea spontaneously subsided after 24 h without administration of specific antibiotic treatment. Therefore, the patient was considered colonized by *C. difficile* and the diarrhoea was considered a spurious association. A faecal sample collected the same day was sent to Istituto Superiore di Sanità, Rome, Italy, for *C. difficile* culture and molecular typing. *C. difficile* was isolated on the chromID™ *C. difficile* medium (bioMérieux, Marcy-l'Étoile, France) after 48h of incubation at 35°C under anaerobic conditions. Typing was performed using the capillary gel electrophoresis method (Indra *et al.*, 2008), and the isolate was identified as a PCR-ribotype 033.

At 54 days after admission, a pancreatic drainage tube was positioned and a carbapenem-resistant *K. pneumoniae* phenotypically identical to the isolate previously obtained from other sites was cultured from the purulent discharge. Therefore a combination therapy with meropenem 2 g tid iv, tigecycline 50 mg bid iv (loading dose 100 mg) and colistin 4,500,000 U bid iv (loading dose 9,000,000 U) was started. The isolate belonged to the epidemic Multi Locus Sequence Type 512 and harboured the *bla*<sub>KPC-3</sub> gene.

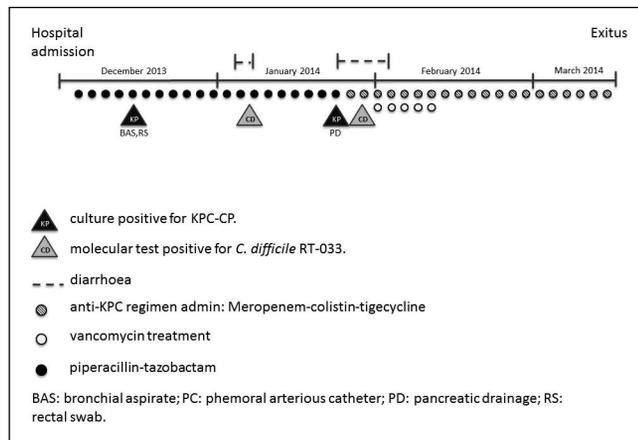
At 58 days after admission, the patient presented a relapse of diarrhoea (stool frequency of five/six stools in 24 hours). The patient was afebrile, the white blood cells count was 5.94 x 1000/mm<sup>3</sup> and the serum albumin level was 2.3 g/dl. A stool sample, processed with the Xpert *C. difficile* molecular assay was positive for *C. difficile* with a B-CDT<sup>+</sup> pattern. Other causes of diarrhoea were excluded, and vancomycin 500 mg qid was given for six days with clinical resolution of diarrhoea. *C. difficile* was again isolated from stools on selective plates and, also in this case, the isolate was identified as a PCR-ribotype 033.

At 90 days after admission a worsening in the renal function occurred, and the patient died after another four days due to multi-organ failure.

## Key words:

Antibiotic-associated colitis, Carbapenemases, Binary toxin, Antimicrobials

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**Figure 1** - Graphic representation of clinical manifestations and therapeutic decisions during the patient's hospital admission.

*C. difficile* is one of the major causes of healthcare-associated infections. In Italy, in 2013, it accounted for 12.1% of all gastrointestinal infections among inpatients

(ECDC. European Centre for Disease Prevention and Control, 2013), although these figures are probably underestimated due to existing challenges with laboratory diagnosis (Planche *et al.*, 2013; Davies *et al.*, 2014). The emergence and dissemination of strains characterized by the presence of the CDT locus, with a truncated pathogenicity locus for toxins A (*tcdA*) and B (*tcdB*), such as those of ribotypes 033 or 033-like, could also contribute to this bias. In fact, these strains yield negative results with toxin A and B enzyme immunoassays and with molecular methods targeting only the *tcdA* and *tcdB* genes (Androga *et al.*, 2014). Only molecular methods also targeting the CDT locus are able to provide A<sup>+</sup>B<sup>+</sup>CDT<sup>+</sup> results.

*Clostridium difficile* ribotype 033 (CDRT-033) was mostly found in infected and colonized animals, especially cattle and horses (Janezic *et al.*, 2014), and its role in human infections is not well established. Among the few cases of human infections by CDRT-033, four cases were described in France (Eckert *et al.*, 2015) and one in the United States (Geric *et al.*, 2003). In Italy no clinical cases have thus far been reported in the literature, although one isolate of this ribotype has recently been reported among a collection of 103 *C. difficile* isolates from Italy, published after the submission of this paper (Spigaglia *et al.*, 2015). The clinical

infection by CDRT-033 reported here in an Italian patient occurred after a prolonged hospital stay and broad spectrum antibiotic treatment. The patient did not refer any previous contact with farms or companion animals.

In conclusion, this observation confirms that CD-RT-033 can cause clinically relevant human infections in hospitalized patients treated with broad spectrum antibiotics, and emphasizes the importance of using reliable diagnostic tests for the detection of the CDT toxin genes.

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### Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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