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Epidemiological data and molecular characterization of Clostridium difficile isolates causing diarrhoea between 2014-2016 in a tertiary hospital of Madrid, Spain

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Background: *Clostridium difficile* infection (CDI) is the leading cause of antibiotic-associated diarrhoea. Relevant changes in CDI epidemiology had been documented after the emergence of the epidemic hypervirulent ribotypes, as R027. The first R027 description in our hospital was in 2013. The aim of the present study was to analyse the epidemiology of CDI in our community and hospital settings during the last three years.

Material/methods: All CDI patients of our institution from January 2014 to November 2016 were recruited, and classified as having healthcare facility-onset, healthcare facility-associated disease (HO-HCFA), community-onset, healthcare facility–associated disease (CO-HCFA), indeterminate disease and community-associated disease (CA-CDI), according to 2016 ESCMID guidelines. Isolates from patients suffering from CDI for the first time (new cases) were analysed at the molecular level. *tcdA*, *tcdB*, *tcdC*, *cdtA*, and *cdtB* genes were amplified by PCR, and *tcdC* deletions were sequenced.

Ribotyping was assessed using Capillary-Electrophoresis PCR protocol with the Bidet primers, and the Webribo database (https:/webribo.ages.at/). In addition, clinical data of the patients were collected.

Results: During the studied period, a total of 8421 GDH analysis were requested to our laboratory, being 960 of them positive (11.4%), 383 (4.6%) positive for toxin B production and 314 (3.7%) positive PCR. The median age of patients was 68±22.2 years (range 1-97), being 57.5% females. CDI recurrence was documented in 22.41% of the patients. Clinical data of the patients allowed us to defined CDI as HO-HCFA (50.5%), CA-CDI (24.2%), CO-HCFA (21.5%), and indeterminate (3.8%). HCFA-density of incidence was 4.9 per 10.000 patient-days, HCFA-accumulated incidence was 36.85 per 10.000 inpatients and CA-CDI-accumulated incidence was 6.79 per 100.000 inhabitants. HO-HCFA-average length of stay was 26.7±22.3 days. HO-HCFA days from admission to CDI was 14.2±15.7 days (range 2-164). The highest HCFA rates were detected in both Gastroenterology and Internal Medicine wards.

A total of 284 clinical isolates of unrelated patients were molecularly typed (90 from 2014; 112 from 2015; and 82 from 2016). Binary toxin was observed in 84 isolates (30.3%). Three *tcdC* deletions genotypes were identified linked to ribotypes R027, R176, and RAI-33 (18 bp); R078, R126 and R620 (39 bp); and R023 (54bp). A total of 71 ribotypes were identified, being R106 and R126 the most prevalent in CA-CDI, whereas in HO-HCFA were R078, R106, R126 and R500. All CDI caused by the hypervirulent ribotype 027 corresponded to HCFA, and their distribution were as follows: 2014 (n=2, 2.2%), 2015 (n=6, 5.4%), and finally 2016 (n=3, 3.7%).

Conclusions: CDI epidemiology of our area is changing in the last three years, and although the hypervirulent R027 has been introduced, their incidence remains low. A high proportion of binary toxin producing isolates grouped into the R078 and R126 rybotypes was observed in abscense of an epidemic scenario.