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 hypervirulent ribotypes, as R027. The first description of R027 in our hospital was in 2014. The aim of the study was to analyse the epidemiology of CDI in our community and hospital settings during the last three years.

Material/Methods: Patients with CDI (2014-2016) were recruited and their infectious processes were classified as: **i)** healthcare facility-onset, healthcare facility-associated disease **(HO-HCFA); ii)** community-onset, healthcare facility-associated disease **(CO-HCFA); iii)** community-acquired disease **(CA-CDI),** and i**v)** indeterminate, according to published guidelines. *tcdA*, *tcdB*, *tcdC*, *cdtA* and *cdtB* genes were amplified by PCR. *tcdC* deletions were detected after sequencing. Ribotyping was assessed using Capillary-Electrophoresis PCR protocol with Bidet primers and the Webribo database (https:/webribo.ages.at/). Clinical data of patients were collected from their clinical charts.

Results: Diagnostic data of CDI are included in **Table 1**. Patients' clinical and epidemiological data are included in **Table 2**. Distribution of HO-HCFA rates by medical wards is shown in **Figure 1**. Finally, a total of 292 clinical isolates of unrelated patients were molecularly typed (91 from 2014, 119 from 2015 and 82 from 2016). The most prevalent ribotypes are represented in **Figure 2**. Binary toxin was observed in 85 isolates (29.1%). Three *tcd*C deletions were identified: 18 bp (R027), 39 bp (R078/126), and 54 bp (R023), **Table 3**. All CDI caused by the hypervirulent R027 corresponded to HCFA, and the distribution was as follows: 2014 (3 patients, 3.3%), 2015 (6 patients, 5%), and 2016 (4 patients, 4.9%).

Table 1 Diagnostic data of CDI during the period of study.

	2014	2015	2016	Total
Number of CDI analysed	2613	3196	2612*	8421*
GDH positive by EIA (%)	302 (11.6)	326 (10.2)	305 (11.7)	933 (11.1)
Toxins A+B positive by EIA (%)	120 (4.6)	131 (4.1)	132 (5.1)	383 (4.6)
Toxins A+B negative by EIA/ Toxin B positive by NAAT (%)	87 (3.3)	107 (3.3)	120 (4.6)	314 (3.7)
Total Toxigenic isolates	7.9%	7.4%	9.7%	8.3%

GDH: glutamate dehydrogenase, EIA: enzyme inmmunoassay, NAAT: nucleic acid amplification test. *Data until November 2016

Table 2 Clinical and Epidemiological data of patients with CDI (2014-2016)*

Age	68±22.2			
Females	57.5%			
Recurrence	22.4%			
Surveilance definitions of CDI (%)	2014	2015	2016	Overall
HO-HCFA	55,9	53,1	48,8	52,6
CO-HCFA	21,2	20,1	18,3	19,9
CA-CDI	20,3	21,5	25,6	22,5
Indeterminate	2,5	5,3	7,3	5
HCFA-density of incidence	4.45 per 10.000 patients-days			
HCFA-accumulated incidenced	40.9 per 10.000 inpatients			
CA-CDI-accumulated incidence	7.44 per 100.000 inhabitants			
HO-HCFA-average length of stay	26.7±22.3			
HO-HCFA days from admission to CDI	14.2±15.7 (2-164)			

*Data until November 2016.

Figure 1 Distribution of HO-HCFA rates by medical wards.

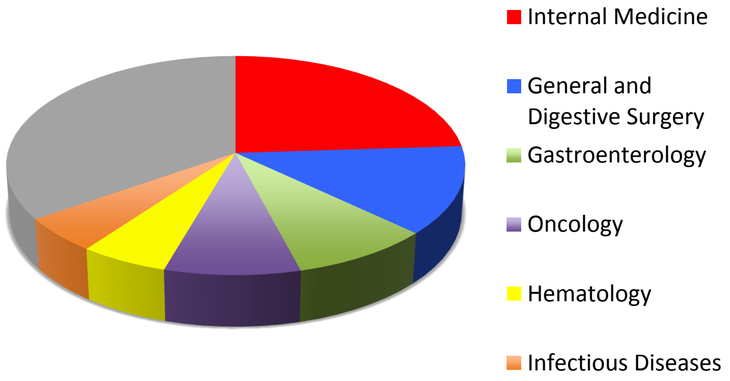
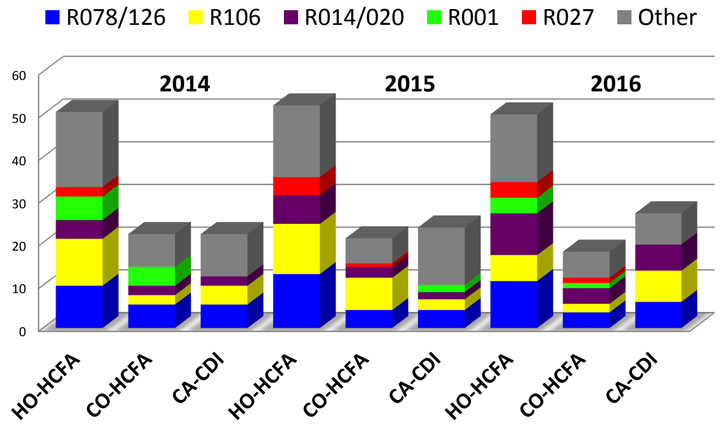


Figure 2 Distribution of the most prevalent ribotypes by year and according to the surveillance definitions*.



*Indeterminated disease is not represented

Table 3 Percentage distributions by toxins and surveillance definitions.

	2014		2015		2016	
Toxins	HCFA	CA-CDI	HCFA	CA-CDI	HCFA	CA-CDI
A+B+CDT-	54,9	17,6	49,6	17,7	45,1	19,5
A+B+CDT+	17,6	6,6	26,1	5,9	20,7	6,1
A-B+CDT-	0	0	0	0,8	1,2	0

HCFA (HO-HCFA + CO-HCFA), CDT binary toxin

Ribotypes with binary toxin: 023, 027, 078, 126, 515, 578, 593.

Conclusions: CDI epidemiology is progressively changing in our area. Ribotype distribution is variable and CA-CDI rate is increasingly. Although the R027 has been introduced, its incidence still remains low. Remarkably, a high proportion of binary toxin-producing isolates corresponding to R078/126 was observed in the absence of an epidemic scenario.