

## Introduction

Increasing age, several co-morbidities, environmental contamination, antibiotic exposure and other intestinal perturbations appear to be the greatest risk factors for *C. difficile* infection (CDI). Therefore, hospitalized patients are considered particularly vulnerable to CDI. The main objective of this study was to evaluate the prevalence of *C. difficile* in a Spanish hospital and to characterize the isolates with respect to PCR-ribotype, antibiotic resistance and toxin activity.

## Sampling

The study was conducted in a 1,324-bed Central University Hospital (HUCA) in Oviedo, Spain. During a 4-month period, from July to October 2014, all patients (hospitalised patients or outpatients in consultation) suspected to have CDI were eligible to participate. Stools were received in the routine coprology laboratory of the HUCA.

## *C. difficile* analysis

First screening for *C. difficile* was performed using a rapid membrane enzyme immunoassay for the simultaneous detection of *C. difficile* glutamate dehydrogenase antigen and toxins A and B (Cdiff QuickChek Complete® TechLab). At the same time fresh stool samples were cultured on home-made selective medium CCFAT directly and after an enrichment step of 3 days in the same liquid media.

## Molecular characterization

Identification and characterization of the colonies were done by detection of *tpi*, *tcdA*, *tcdB* and *cdtA* genes. GenomEra CDX System *C. difficile* (Abacus) was performed for the rapid identification of toxin B. Further characterization was performed by PCR-ribotyping and Genotype Cdiff test (Hain Lifescience) which detects deletions in the regulator gene *tcdC* and *gyrA* mutations associated with moxifloxacin resistance.

## PCR-ribotype analysis

Profiles were analyzed by comparison with those of reference strains from the European Brazier collection and with our own database (nomenclature beginning with UCL).

## Results

Culture was positive in 32 stools from 261 positive samples (prevalence 8.1%). Most of the positive samples were from patients older than 60 years (Figure 1). We could differentiate 18 different ribotypes. Seventeen of them were identified as toxigenic (Table 1).

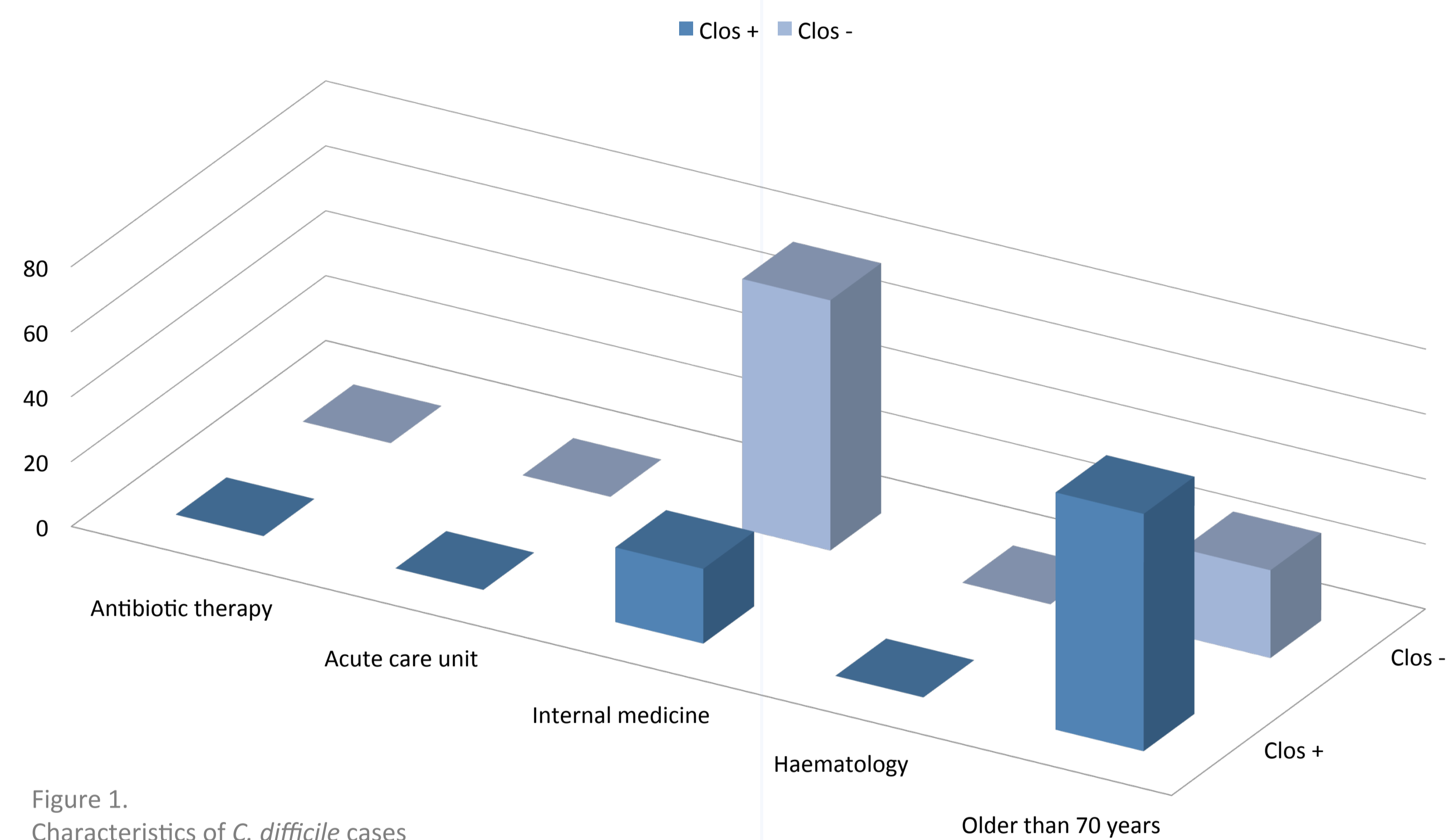


Figure 1. Characteristics of *C. difficile* cases

## Antimicrobial susceptibility

Susceptibility to metronidazole, moxifloxacin and tetracycline was determined by Etest strips (Lucron EliTech Group) on Brucella Blood Agar with hemin and vitamin K1 (Becton-Dickinson) according to the instructions of the manufacturer. Plates were anaerobically incubated at 37°C for 48H. The resistance (r) breakpoints for metronidazole (MET  $r \geq 32 \mu\text{g/ml}$ ) and tetracycline (Tet  $r \geq 8 \mu\text{g/ml}$ ) were those recommended by the Clinical and Laboratory Standard Institute (CLSI). *Bacteroides fragilis* ATCL was included as a quality control.

Table 1. *C. difficile* ribotypes in hospitalized patients and antimicrobial resistance of the isolates

PCR-ribotype	N° of strains	Toxin genes	<i>C. difficile</i> detection		
			Met	Mox	Tetra
001	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
002	3	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	+ (n=2)	-
078	2	A <sup>+</sup> B <sup>+</sup> CDT <sup>+</sup>	-	+	+
070	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
023	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
014	3	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
012	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
UCL 489	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
UCL 5a	4	A <sup>+</sup> B <sup>+</sup> CDT <sup>+</sup>	-	+	+
UCL 16b	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	+	+
UCL 9	1	A <sup>-</sup> B <sup>-</sup> CDT <sup>-</sup>	-	-	-
UCL 16o	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
UCL 55a	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
UCL 36a	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	+
UCL 16i	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
UCL 108	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
UCL 16a	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-
UCL 483	1	A <sup>+</sup> B <sup>+</sup> CDT <sup>-</sup>	-	-	-

## Conclusion

This study reveals the circulation of toxigenic *C. difficile* strains among different types of patients in a Spanish hospital. Most of the isolates were obtained from patients without acute/bloody diarrhea. The survey of the epidemiology of *C. difficile* shows a wide heterogeneity of the ribotypes, the absence of clonal spread within wards and that most frequent ribotypes mirror those found in the surveillance program in other European countries, including in Belgium.