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CARRIAGE OF *CLOSTRIDIUM DIFFICILE* IN HOSPITAL PATIENTS IN SPAIN, INCLUDING MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF THE ISOLATES

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BACKGROUND

Increasing age, several co-morbidities, environmental contamination, antibiotic exposure and other intestinal perturbations appear to be the greatest risk factors for *C. difficile* infection. Therefore, hospitalized patients are considered particularly vulnerable to CDI.

OBJECTIVES

The main objective of this study was to evaluate the prevalence of *C. difficile* in a Spanish hospital and to characterise the isolates with respect to PCR-ribotype, antibiotic resistance and toxin activity

MATERIALS AND METHODS

Sampling	The study was conducted in a 1,324-bed public teaching hospital 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. *C. difficile* testing was performed on semisolid, mushy stools and watery/entire liquid feces (Bristol stool chart from levels 4 to 7).
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 ere cultured on home-made selective medium CCFAT directly and after an enrichment step of 3 days in the same liquid medium.
Molecular characterization	Identification and characterisation 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 characterisation 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.
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 manufacturing instructions. Plates were anaerobically incubated at 37°C for 48H. The resistance (r) breakpoints for metronidazole (Met r≥32 µg/mL), moxifloxacine (Mox r≥8 µg/mL) and tetracycline (r≥8 µg/mL) were those recommended by the CLSI. *Bacteroides fragilis* ATCL was included as quality control.

RESULTS

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

C. difficile detection

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

- 22 samples/week: a total of 261 samples analysed
- 32 positive samples to *C. difficile* (12.3%)
- 22 CDI cases diagnosed
- 8/32 strains were isolated from patients with acute diarrhea

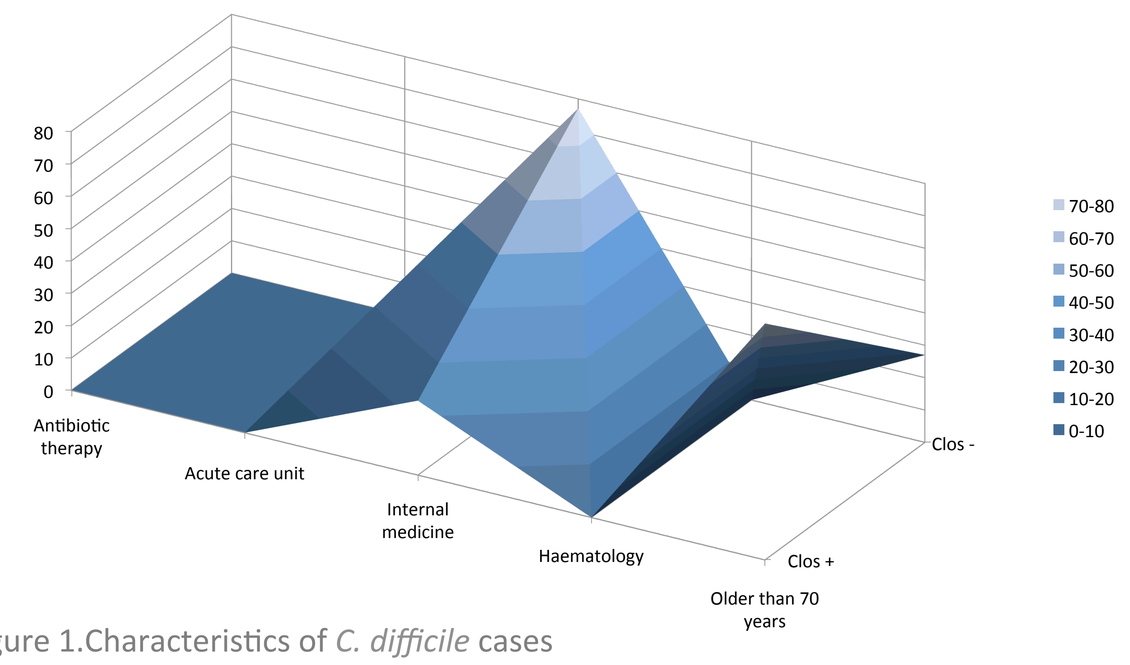

Figure 1.Characteristics of *C. difficile* cases

Significance and perspectives

This study reveals the circulation of toxigenic *C. difficile* among different types of patients in a Spanish hospital. Most of the isolates were obtained from patients without acute/bloody diarrhea. Further investigations will be performed to compare Belgian and Spanish *C. difficile* strains by whole genome sequencing analysis.