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C.difficile (including epidemiology)

Clinical, epidemiological and microbiological characteristics of relapse and re-infection in *Clostridium difficile* infection

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ABSTRACT

Recurrent diarrhea is a common complication of *Clostridium difficile* infection (CDI). Recurrent CDI (r-CDI) may be produced by the persistence of spores (relapse) or by the acquisition of a new strain (reinfection). In this study, we analyze epidemiological, clinical, microbiological and laboratory data from patients with r-CDI, relapse, and reinfection-CDI over 5 years and compared with a control group (non r-CDI). Among 60 patients with r-CDI, 36 patients had stool samples collected from two or more episodes, which were molecularly analyzed. Based on ribotyping, 63.9% of the samples were relapse, and 36.1% reinfection. In a multivariable logistic regression analysis, previous antibiotic exposure was found to be a risk factor for r-CDI (OR: 2.23; 95% CI: 1.0–4.9; $p = 0.04$). Patients with relapse had previous antibiotic exposure more frequently than did patients with reinfection ($p = 0.03$), and patients with reinfection suffered more frequently from chronic liver disease ($p = 0.02$) than did relapse patients. Relapse patients compared with the control group had a higher percentage of previous antibiotic exposure, although the difference was statistically no significant (73.9% vs. 91.3 $p = 0.06$). No significant differences for the selected variables were observed between the reinfection and control groups, although we observed a higher percentage of patients with chronic liver disease (30.8% vs 13.3%; $p = 0.08$). All isolates were sensitive to metronidazole and vancomycin. No significant differences in antibiotic susceptibility were found between the different groups. Sporulation and germination frequency of r-CDI were higher than non r-CDI ($p = 0.02$ and $p < 0.01$, respectively). Nevertheless, there were statistically not significant differences between the relapse and reinfection groups. Both frequencies were compared between the first and second episode of CDI for the relapse and reinfection groups, but differences were not observed to be statistically significant. In conclusion, our study showed that the recurrence of CDI was associated with antibiotic use and sporulation/germination frequency, regardless of relapse or reinfection. The use of antibiotics would produce a dysbiosis and favor the persistence of the *C. difficile* spores and relapse. A possible alteration of the intestinal microbiota and the bile salts produced by chronic liver disease could favor reinfection.

1. Introduction

Recurrent *Clostridium difficile*-associated disease (CDAD) represents one of the most difficult and increasingly common challenges faced in the management of *Clostridium difficile* infection (CDI) [1]. After being properly treated with standard therapy and recovering from the first episode of *C. difficile* diarrhea, 15%–35% of patients develop a second episode between two to eight weeks after discontinuation of therapy. Up to 45% of those patients can then have a second recurrence [1–7].

Recurrent CDI (r-CDI) may be produced by the endogenous persistence of spores (relapse) or by the acquisition of a new strain (reinfection) [8]. It has been reported that the main factors involved in r-CDI are both the persistence of *C. difficile* spores in the host [9], and a non-recoverable, antibiotic-induced dysbiosis of the colonic microbiota, which is no longer able to act as a resistant barrier against *C. difficile* colonization [10]. There is interest in documenting if r-CDI is due to reinfection or relapse. The likelihood of reinfection depends on the quality of infection control procedures [11]. If r-CDI occurs by relapse, other measures, in addition to good infection control techniques, may be necessary to decrease the risk of recurrence [12].

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Many epidemiological and microbiological variables have been established as risk factors for r-CDI (old age, previous CDI, previous use of antibiotics, severe underlying disease, epidemic B1/NAP1/027) [5–7,13]. Nevertheless, the clinical characteristics and risk factors associated with relapse or re-infection have not been well established [14]. The aim of this study was to identify the clinical characteristics and risk factors associated with relapse or reinfection CDI in an area without a B1/NAP1/027 epidemic. We also analyzed antibiotic susceptibility and sporulation/germination frequency of *C. difficile* isolates to investigate the correlation between microbiological characteristics and r-CDI.

2. Methods

2.1. Study design and population

A retrospective case-control study was conducted at the Hospital Universitario 12 de Octubre in Madrid (Spain), which is a 1300-bed tertiary-care facility serving a population of 400,000. All patients older than 2 years with r-CDI from January 2010 to December 2014 were recorded for the study and compared with a randomized control group (non r-CDI patients). Patients with r-CDI and controls were paired and adjusted for age and gender in a proportion of 1 r-CDI case to 3 controls.

Demographic, epidemiological, clinical and laboratory results were extracted from the hospital's electronic medical database. This included a) Demographic data: age and gender; b) Epidemiological factors: contact with the National Health System (NHS) during the previous three months (hospitalization or visiting a healthcare facility for medical consultation or medical test), length of hospitalization, medication during the three months prior to the case (antibiotics, proton pump inhibitors (PPI), chemotherapy or corticoids); c) Clinical information: comorbidities (solid organ transplantation, chronic liver disease, diabetes mellitus, immunosuppression and death during the period of study) and clinical data recorded at admission (diarrhea caused by *C.difficile* (CDI), fever and abdominal pain); and d) Laboratory results: white blood cell count, albumin and creatinine levels.

2.2. Definitions

CDI was defined as the occurrence of diarrhea in the presence of a positive stool test for toxigenic *C. difficile* that is not attributed to other causes. R-CDI was defined as two or more episodes of CDI between 15 days and two months after the first episode was properly treated [2]. Relapse was considered when r-CDI episodes were produced by the same PCR ribotype and re-infection by different PCR Ribotype.

CDI was considered to be community-acquired if the diarrhea occurred within 72 h of admission and if the patient had no history of hospitalization within the previous 4–12 weeks [2].

2.3. Microbiological methods

Diarrheic stool samples were tested by the rapid dual device enzyme-immunoassay screening test (C. diff Quik Chek Complete; Techlab, Blacksburg, VA, USA). From January 2010 to February 2011, confirmation of toxigenic *C. difficile* in samples that were GDH positive and toxin A/B negative was performed by toxigenic culture. From February 2011 onwards, confirmation was performed by the GeneXpert®System (Cepheid, Sunnyvale, CA). Positive samples were cultured on selective cycloserine-cefoxitin-fructose agar plates (CLO-agar, BioMérieux, Marcy l'Etoile, France).

2.4. Antimicrobial susceptibility testing

Susceptibility to vancomycin, metronidazole, erythromycin, moxifloxacin, tetracycline and clindamycin was determined by the E-test method (BioMérieux, Marcy l'Etoile, France) on Brucella agar plates (in house) and incubated in an anaerobic atmosphere for 48 h. Breakpoints, as established by Barbut et al. [15], were used for erythromycin ($R > 4$), moxifloxacin ($R = 4$), tetracycline ($R > 8$) and clindamycin ($R = 4$). Breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used for metronidazole ($R > 2$) and vancomycin ($R > 2$) (http://www.eucast.org/ast_of_bacteria/previous_versions_of_documents/v.2.0).

2.5. Molecular typing

DNA was extracted (Qiacube) from a single colony as previously described [16] and PCR ribotyping was performed per the method used by Stubbs et al. [17]. PCR ribotypes 001, 027 and 078 were used as reference strains.

2.6. Sporulation and germination frequency

Were calculated as described by Oka et al. [8]. Briefly, the sporulation frequency of *C. difficile* was determined by phase contrast microscopy. A few colonies of *C. difficile* were suspended in sterile saline after 5 days of incubation onto Brucella agar plate at 37 °C under anaerobic conditions, and the sporulation frequency was calculated as (number of spores per ml)/(number of total cells per ml) ×100. For germination frequency, a suspension of *C. difficile* cells, from a pure culture, was heated at 70 °C for 10 min to kill all the vegetative cells. The heat-treated suspension was then subjected to 10-fold serial dilution with sterile saline and inoculated onto Brucella agar (in house) and incubated in an anaerobic atmosphere (37 °C) for 2 days. Germination frequency was calculated as (number of CFU per ml)/(number of spores per ml) ×100.

2.7. Statistical methods

Descriptive statistics for continuous variables were summarized as medians and interquartile ranges. Categorical variables were reported as the number and percent. For univariate analysis of categorical variables, the $\chi 2$ test was used, while the Student's t-test or Mann-Whitney tests were used for continuous variables. Odds ratios (OR) with 95% confidence interval (CI) were calculated for all significant variables. A p value < 0.05 in a two-tailed test was considered significant. Multivariate analysis between r-CDI and non r-CDI was performed with forward stepwise logistic regression. We included factors that were associated with r-CDI in the univariate analysis ($p < 0.1$). Only data corresponding to the first episode of r-CDAD were analyzed. Data were stored and analyzed by using Epi Info™ 7 for Windows software (https://wwwn.cdc.gov/epiinfo/).

3. Results

3.1. Clinical and epidemiological characteristics of patients with r-CDI

A total of 472 CDI episodes were documented during the period of study, with a recurrence rate of 12.7% ($n = 60$). Comparison of the demographic, epidemiological, clinical and laboratory data between r-CDI ($n = 60$) and control groups (non r-CDI) ($n = 180$) showed a trend between the diarrhea caused by *C. difficile* at admission (48.3% vs. 36.1%, $p = 0.09$; OR: 2.1, 95%; CI: 0.9–4.5) and antibiotic exposure prior to the first episode in patients with r-CDI (85% vs 73.9%, $p = 0.08$; OR: 2.0, 95% CI:0.9–4.4). A total of 22.9% of