Objective: Spreading of virulent C. difficile by ribotype 027 infection is increasing in Europe but with an unequal distribution. Up to now, only two cases by ribotype 027 strains have been reported in Spain. The aim of this study was to determine the prevalence in hospitalized and non-hospitalized patients and the epidemiology associated to C. difficile infection (CDI) in a Hospital specialized on Tropical Medicine. Methods: From October 2009 to October 2011, stool samples from patients with clinical suspicion of CDI were examined. Subjects were classified in two groups, hospitalized patients and outpatients, and a different diagnostic algorithm was performed. In the first group, detection was made by a real-time PCR which includes detection of ribotype 027 (Xpert® C. difficile). In outpatients, a two-step algorithm was used: screening with a rapid test (Techlab C. difficile Quik Chek Complete®) and positive cases were confirmed by real-time PCR. Results: A total of 690 specimens from 582 patients (279 male and 303 women) were studied. 343 corresponding to hospitalized patients, most of them natives (87.2%), and the rest immigrants (7.3%) and travellers (5.5%). Among 239 outpatients, the majority were travellers (76.6%) followed by Spaniards (19.2%) and immigrants (4.2%). A total of 40 patients were positive for CDI, 33 hospitalized patients (prevalence=9.6%) and 7 outpatients (prevalence=2.9%). Most of cases detected with CDI in hospitalized patients were >70 years old (median age, 81; interquartile range [IQR], 75.5-88.5), in contrast to outpatients (median age, 40; IQR, 33-65; p=0.005). Out of 33 CDI-positive inpatients, 31 were native and 3 immigrants. One of the immigrants was positive for 027 strain. She was a Portuguese female who had been operated in her country less than a month ago. Among 7 outpatients positive for CDI, 3 were travelers and 2 of them have developed diarrhea after antibiotic treatment for traveller’s diarrhea. The remaining outpatients were immigrants and natives who had previously taken antibiotics or proton pump inhibitors. Conclusion: A high prevalence of CDI infection in hospitalized patients with detection ribotype 027 was observed. In addition, the prevalence obtained in outpatients, notably in those with antibiotic treatment after traveller’s diarrhea, is far from negligible. These data suggest the need of diagnosis of ribotype 027 in hospitalized patients and the screening of CDI out of the traditional risk groups.