Characterization of *Clostridium difficile* 027 strains from an outbreak in a Portuguese hospital

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*C. difficile* infection (CDI) is the cause of an intestinal disease mediated by two potent cytotoxins, TcdA and TcdB. Symptoms of CDI can range from asymptomatic colonization or mild diarrhea, to life-threatening inflammatory lesions such as pseudomembranous colitis, toxic megacolon or bowel perforation. In part because of the recent emergence of so-called hypervirulent strains, especially (but not exclusively) those belonging to ribotype 027, *C. difficile* is now considered a main nosocomial enteric pathogen.

Hypervirulent epidemic strains have been associated with more severe disease conditions, with higher relapse rates and increased mortality. Health care-associated CDI develops in hospitalized patients undergoing antibiotic treatment because *C. difficile* can colonize the gut if the normal intestinal microbiota is disturbed. However, *C. difficile* is also emerging as an important pathogen in the community, as well as in animal husbandry. The organism is an obligate anaerobe, and has the ability to form spores. Spores are extremely resilient and can accumulate and remain viable in the environment or in the host for long periods of time. Spores that remain latent in the gut are responsible for the recurrence of *C. difficile*-associated disease (CDAD) when antibiotic therapy is stopped. At least some of the hypervirulent epidemic strains show a greater sporulation capacity *in vitro*, as well as robust toxin production.

The first detection of *C. difficile* 027 hypervirulent epidemic strains implicated in a hospital outbreak in Portugal dates from January 2012, involving 12 patients, with a crude mortality rate of 50%. Here we report on the genetic characterization of those strains as well as the antibiotic resistance profile, toxin production, and rate and efficiency of spore formation. In parallel, *C. difficile* 027 non-outbreak strains isolated from other Portuguese health care facilities are also investigated.

**Keywords** *C. difficile* 027; nosocomial enteric pathogen; hypervirulent strains; spores; antibiotic resistance