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The role of surface adhesins in *Clostridium difficile* pathogenesis: Comparison between a non-epidemic and an epidemic strain

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Clostridium difficile is a major cause of healthcare-associated infection and inflicts a considerable financial burden on healthcare systems worldwide.Disease symptoms range from self-limiting diarrhoea to fatal pseudomembranous colitis. Whilst *C. difficile* has two major virulence factors, toxin A and B, it is generally accepted that other virulence components of the bacterium contribute to disease. *C. difficile* colonises the gut of humans and animals and hence the processes of adherence and colonisation are essential for disease onset. Previously it has been suggested that flagella might be implicated in colonisation. Here we tested this hypothesis by comparing flagellated parental strains to strains in which flagella genes were inactivated using ClosTron technology. Our focus was on a UK-outbreak, PCR-ribotype 027 (B1/NAP1) strain, R20291. We compared the flagellated wild-type to a mutant with a paralyzed flagellum and also to mutants (*fliC, fliD* and *flgE*)that no longer produce flagella *in vitro* and *in vivo*. Our results with R20291 provide the first strong evidence that by disabling the motor of the flagellum, the structural components of the flagellum rather than active motility, is needed for adherence and colonisation of the intestinal epithelium during infection. Comparison to published data on 630Δ*erm* and our own data on that strain revealed major differences between the strains: the R20291 flagellar mutants adhered less than the parental strain *in vitro*, whereas we saw the opposite in 630Δ*erm*. We also showed that flagella and motility are not needed for successful colonisation *in vivo* using strain 630Δ*erm*. Finally we demonstrated that in strain R20291, flagella do play a role in colonisation and adherence and that there are striking differences between *C. difficile* strains. The latter emphasises the overriding need to characterize more than just one strain before drawing general conclusions concerning specific mechanisms of pathogenesis.

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