

The Role of Flagella in *Clostridium difficile* Pathogenesis: Comparison between a Non-Epidemic and an Epidemic Strain

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Kim R. Hardie, Imad Kansau, Anne Collignon, Nigel P. Minton



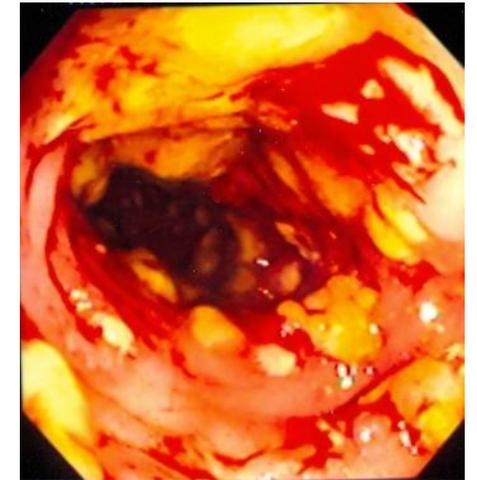
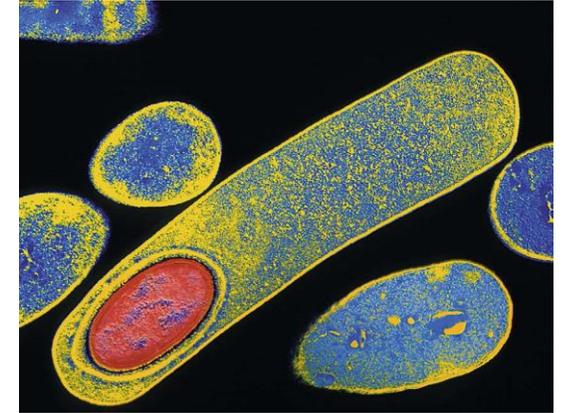
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Background- *Clostridium difficile*- the organism and the disease



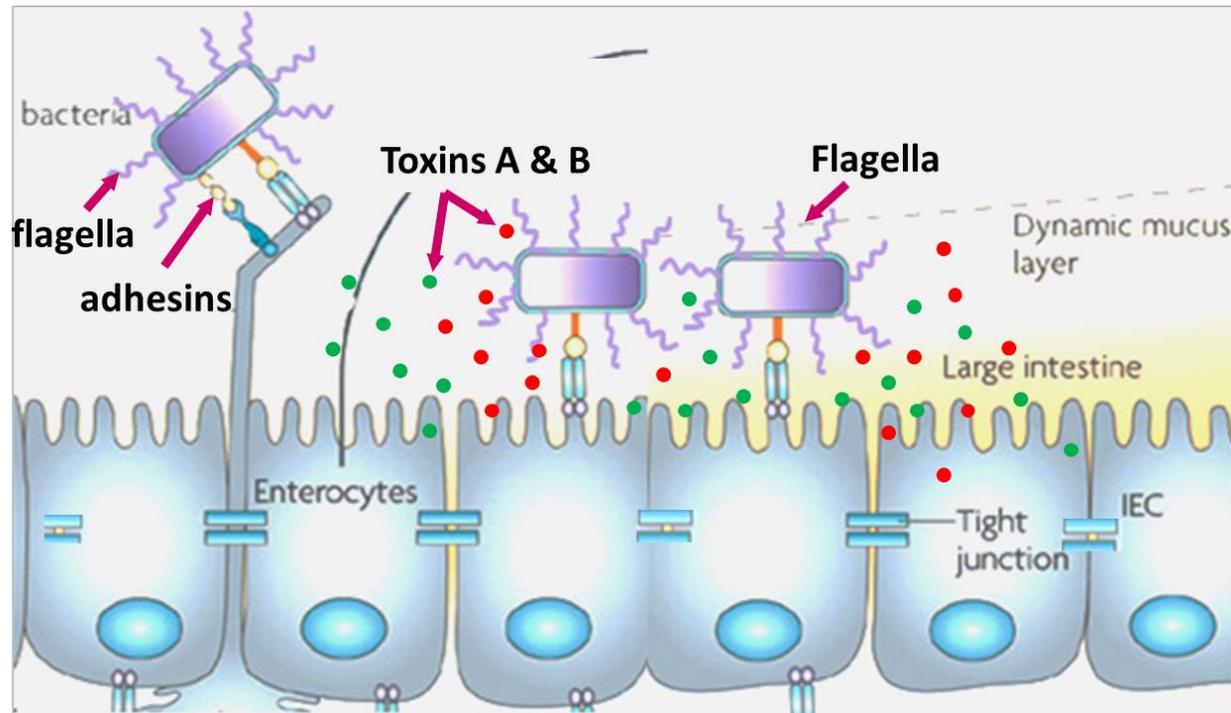
- ❑ *Clostridium difficile*- The worldwide leading cause of hospital-associated infections.
- ❑ The incidence and severity of CDI continue to increase particularly following the global emergence of epidemic hypervirulent *C. difficile* strains (027/BI/NAP1).
- ❑ The prevention and control of *C. difficile* infection in health care settings has become a global public health challenge.





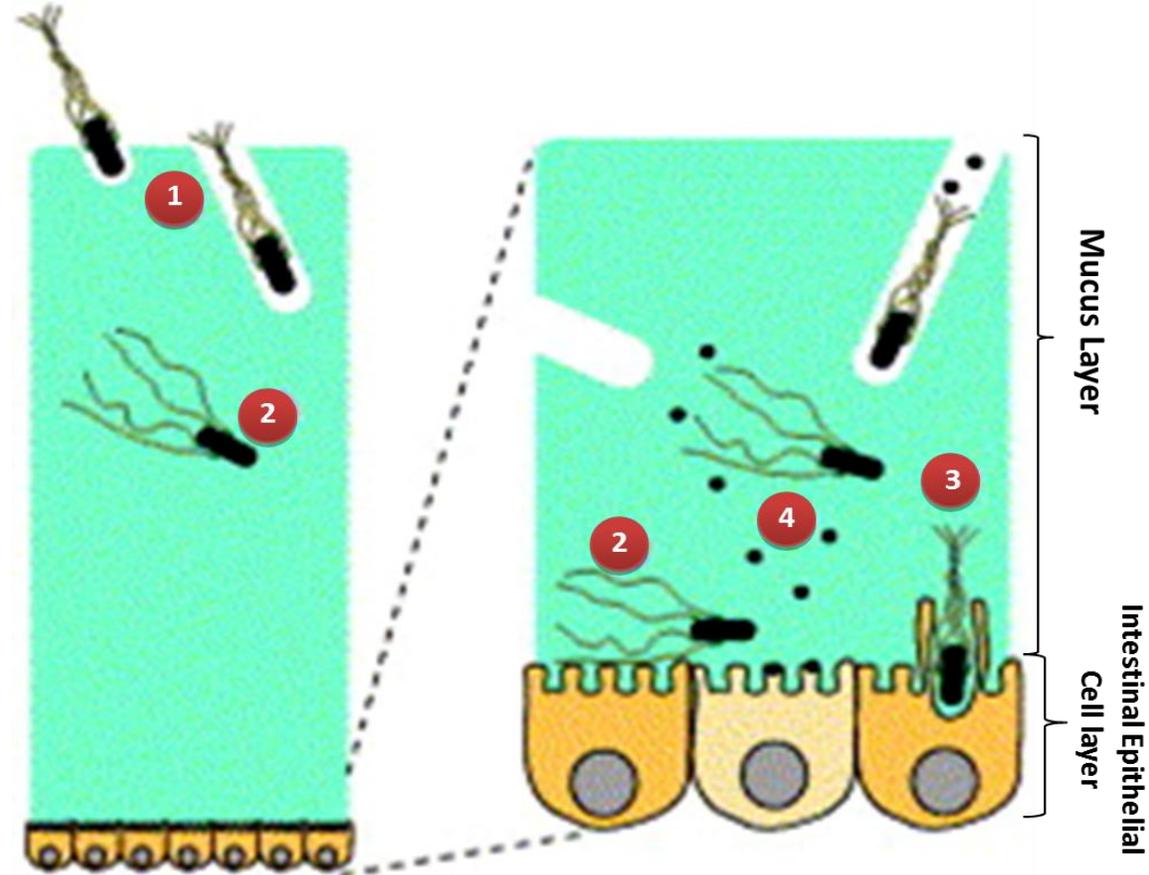
C. difficile virulence factors

- ❑ *C. difficile* produce two major toxins, toxin A and toxin B, both of which are responsible of the clinical manifestations of the disease.
- ❑ *C. difficile* expresses multiple surface adhesins, potentially functioning as colonization factors.



Role of flagella & motility in virulence

- ❑ Flagella function as an adhesin in mediating adherence of *C. difficile* bacteria to intestinal epithelial cells.
- ❑ Potential functions for flagella in virulence:
 - ❑ Motility apparatus
 - ❑ Surface adhesins and invasins
 - ❑ Secretion apparatus



Aims of this study

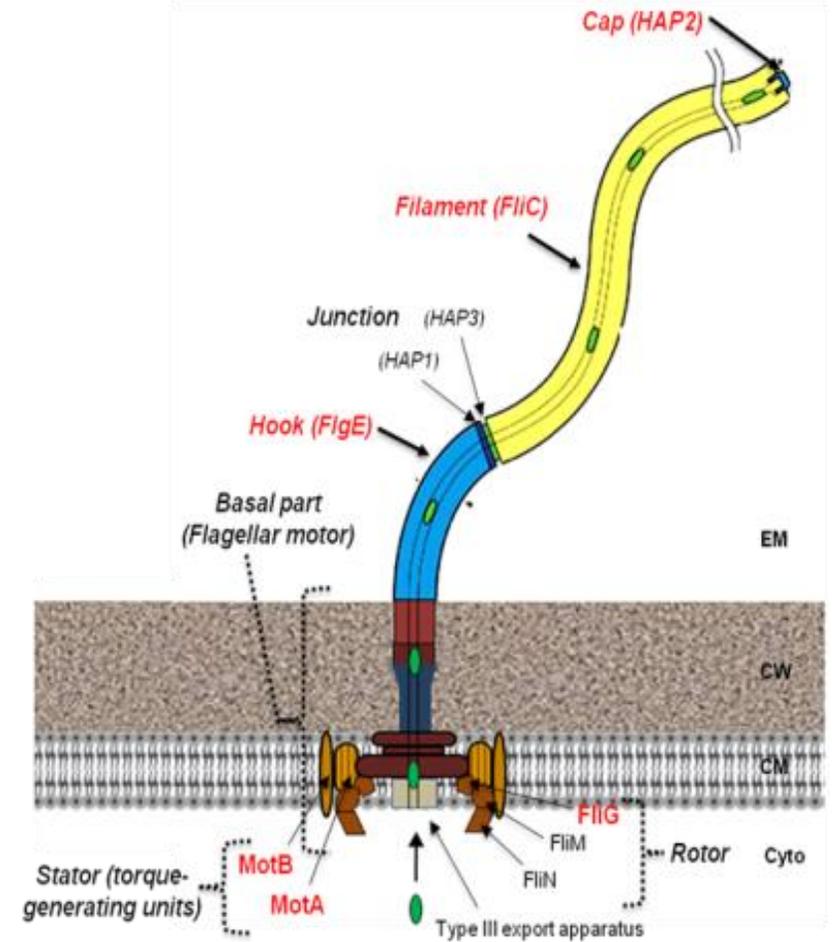
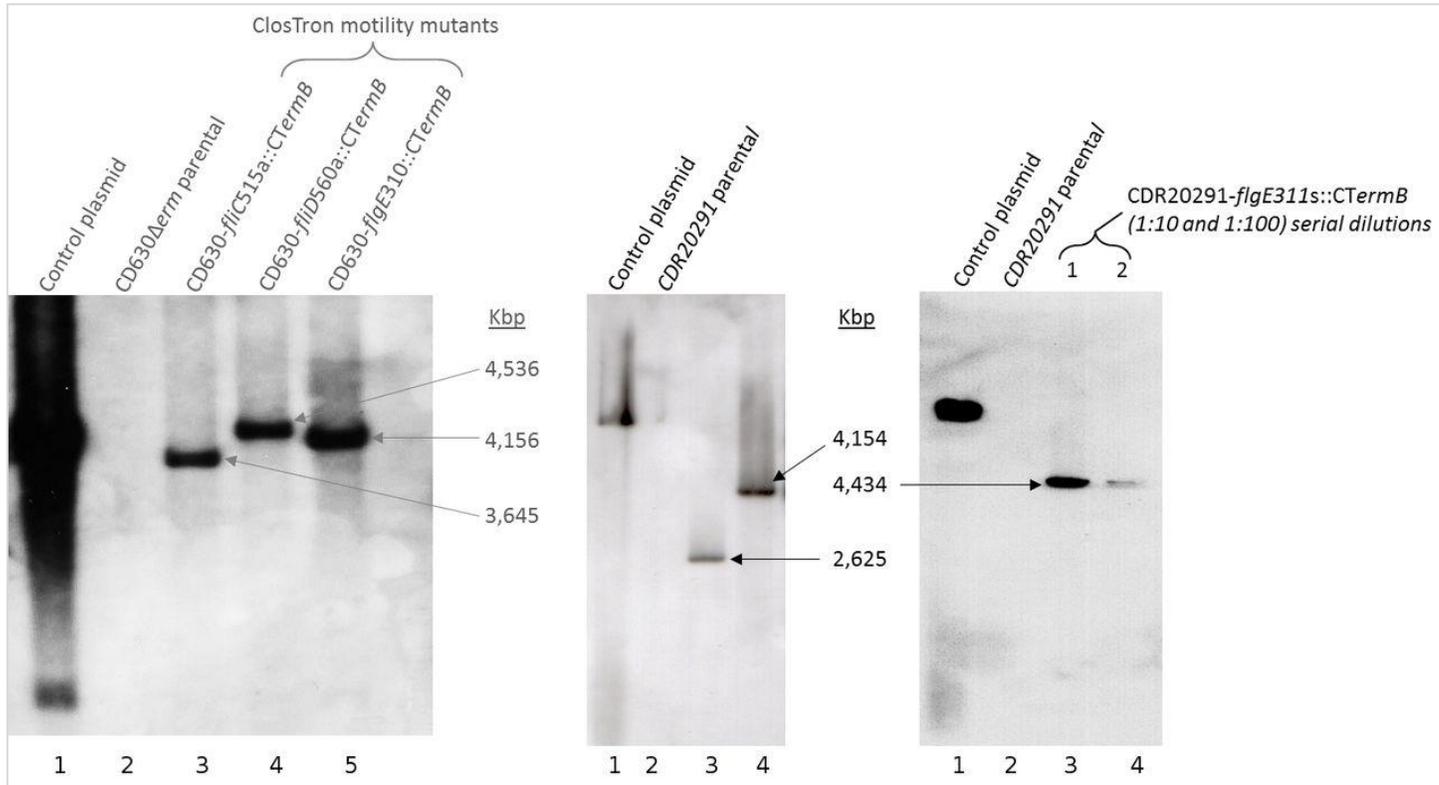


- To elucidate the mechanism by which flagella contribute to *C. difficile* adhesion to human intestinal epithelial cells
- To assess the contribution of flagellum-mediated motility in colonisation of the intestinal tract in mice
- Does some part of the polar flagellum act as an adhesin, or is it active motility that is needed for adherence and intestinal colonization?

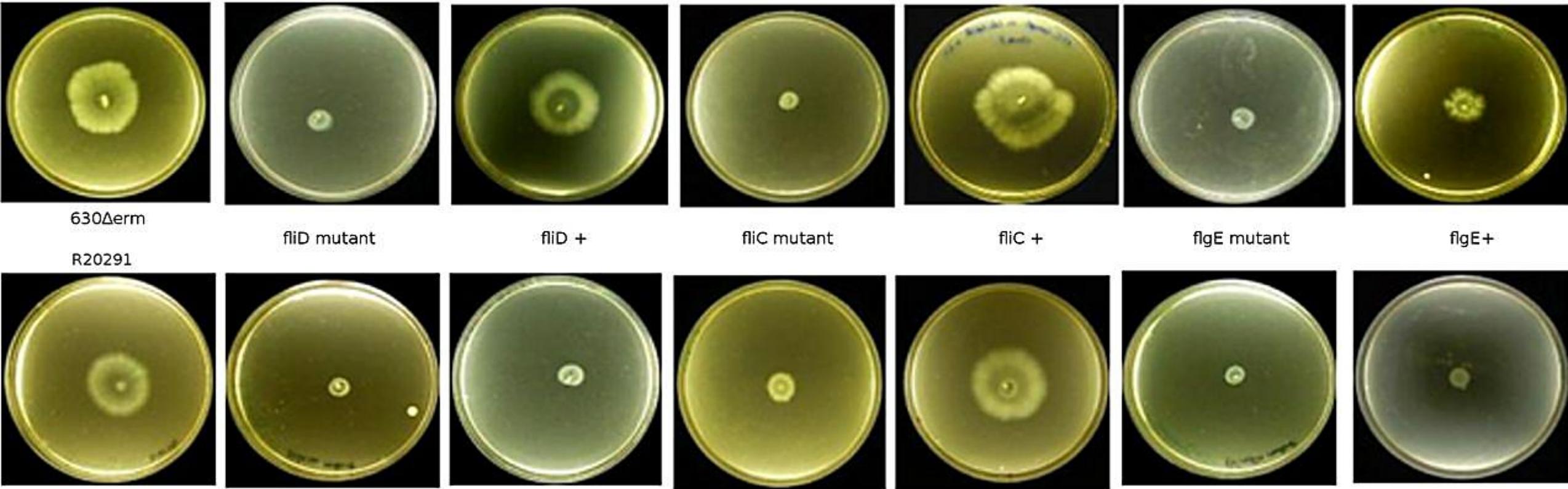
Construction and phenotypic characterization of flagella-associated mutants



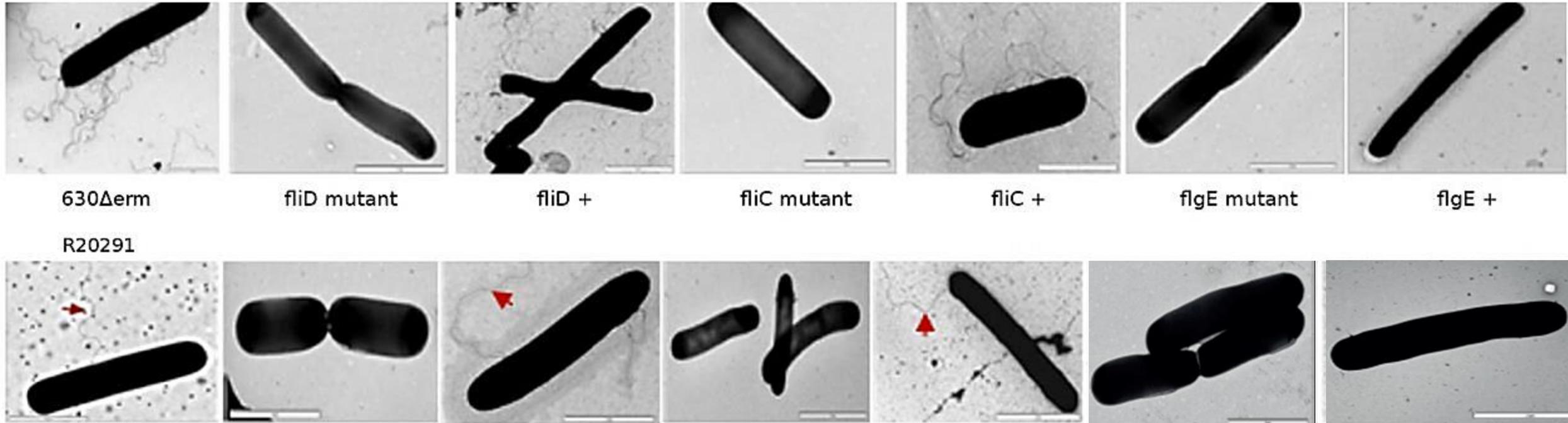
- Southern blot analysis



Phenotypic characterization of flagella-associated mutants for motility



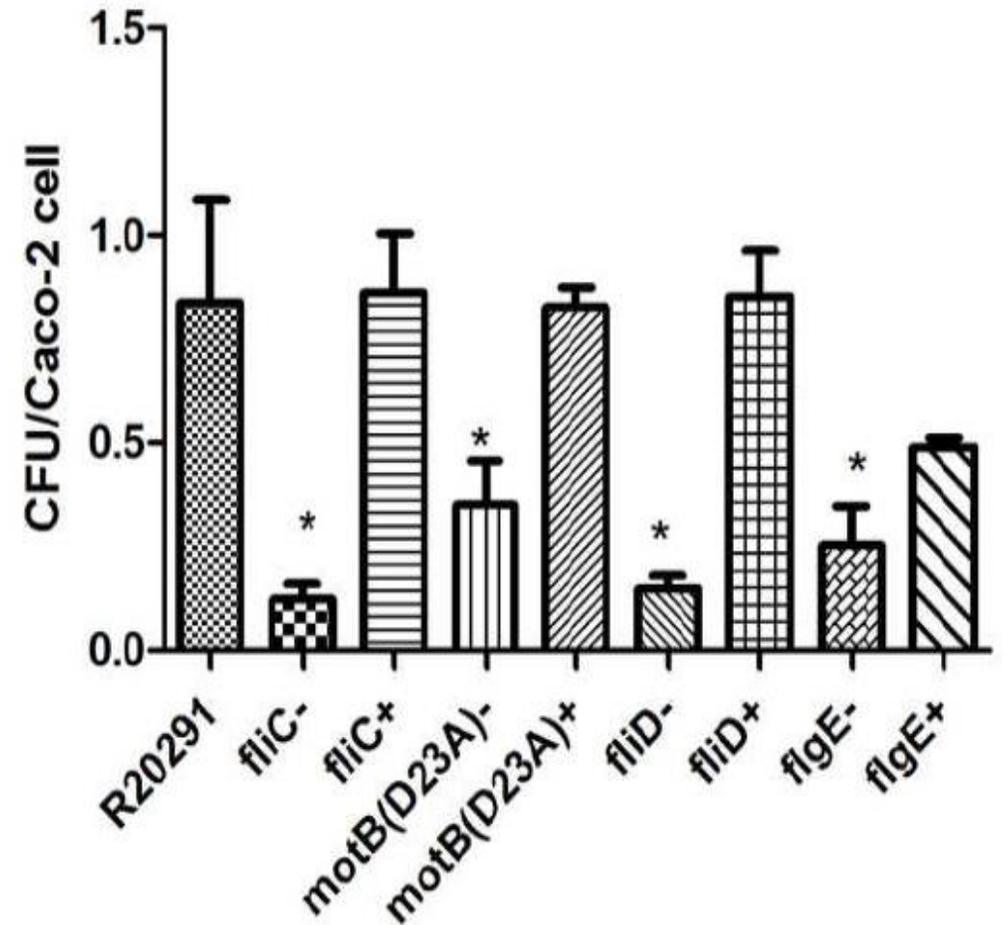
Phenotypic characterization of flagella-associated mutants for flagellation



Importance of flagella of R20291 for *in vitro* cell adherence



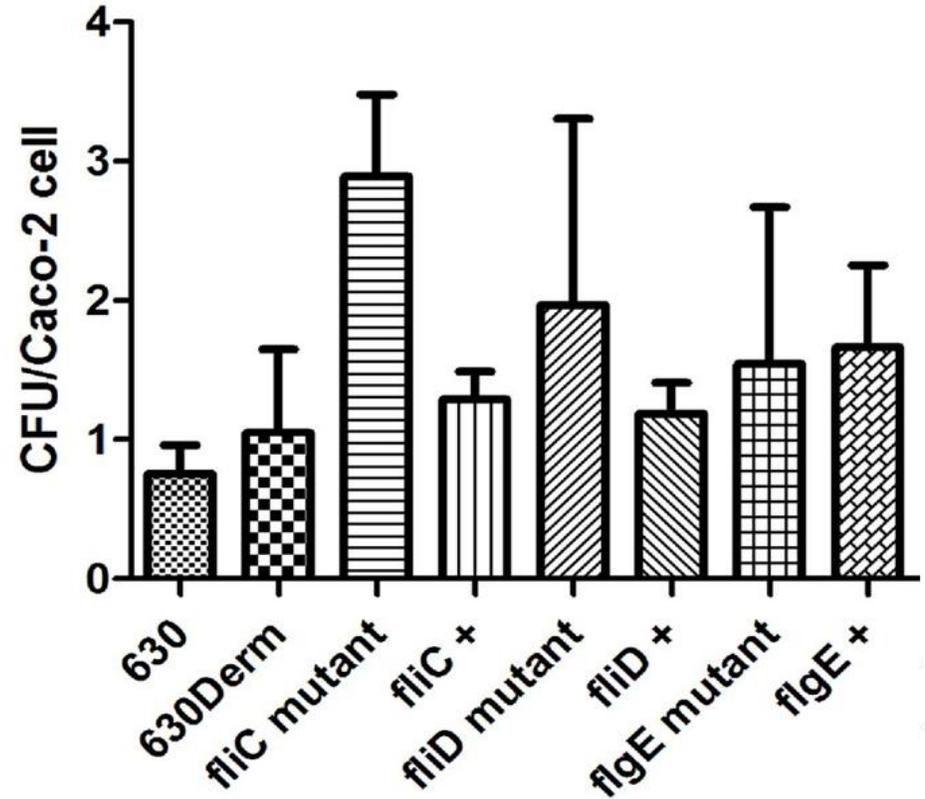
- The flagellum of R20291 is used as an adhesin to enhance bacterial association to human intestinal epithelial cells *in vitro*



Importance of flagella of *C. difficile* 630 Δ erm for *in vitro* cell adherence



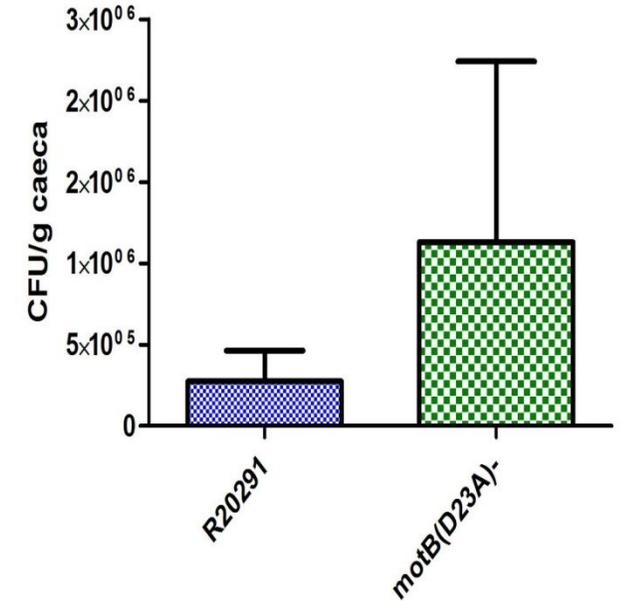
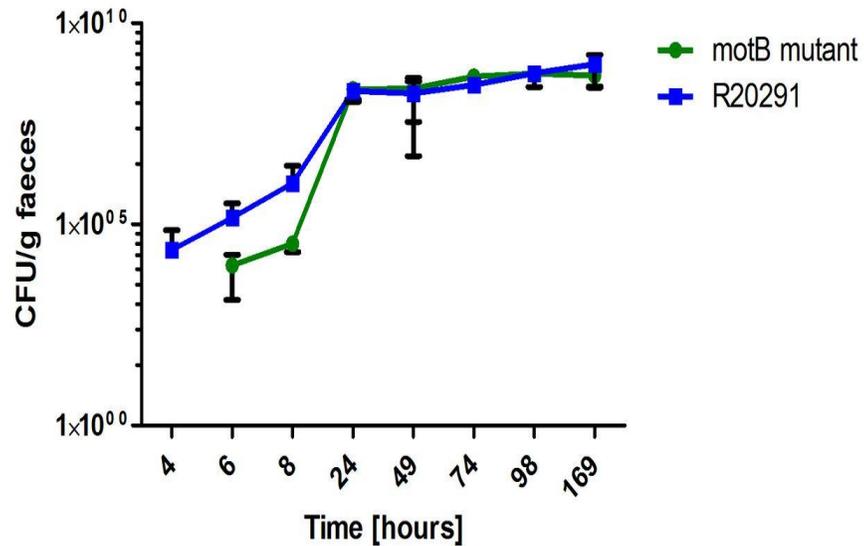
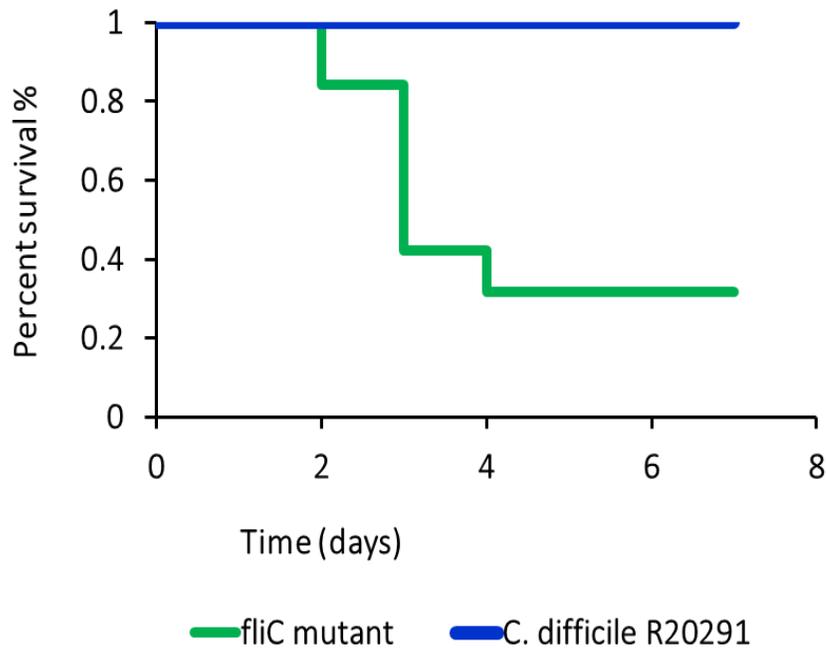
- Surprisingly, all flagellar mutants adhered significantly more strongly to human intestinal caco-2 cells than the wild-type strains.



In vivo analysis: Importance of flagellum of R20291 strain in intestinal colonization of axenic mice

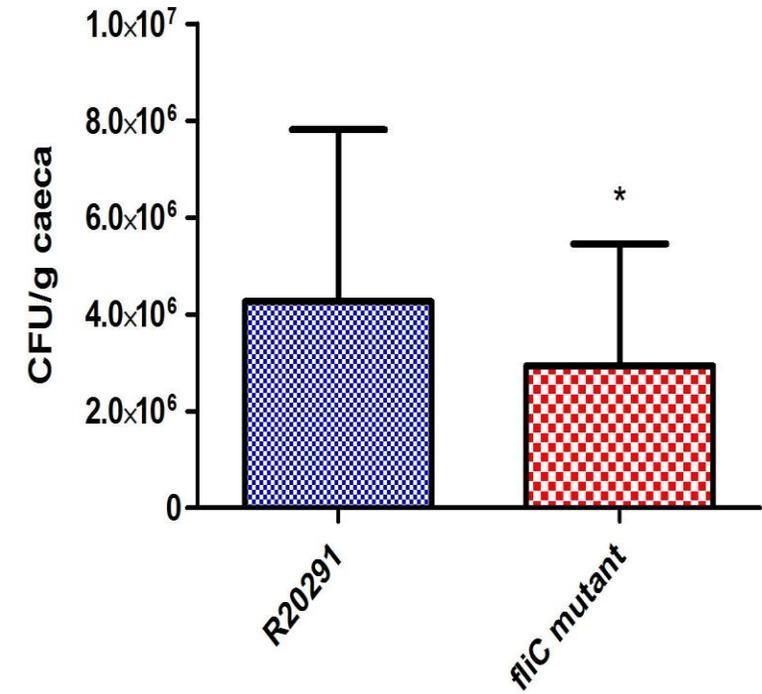
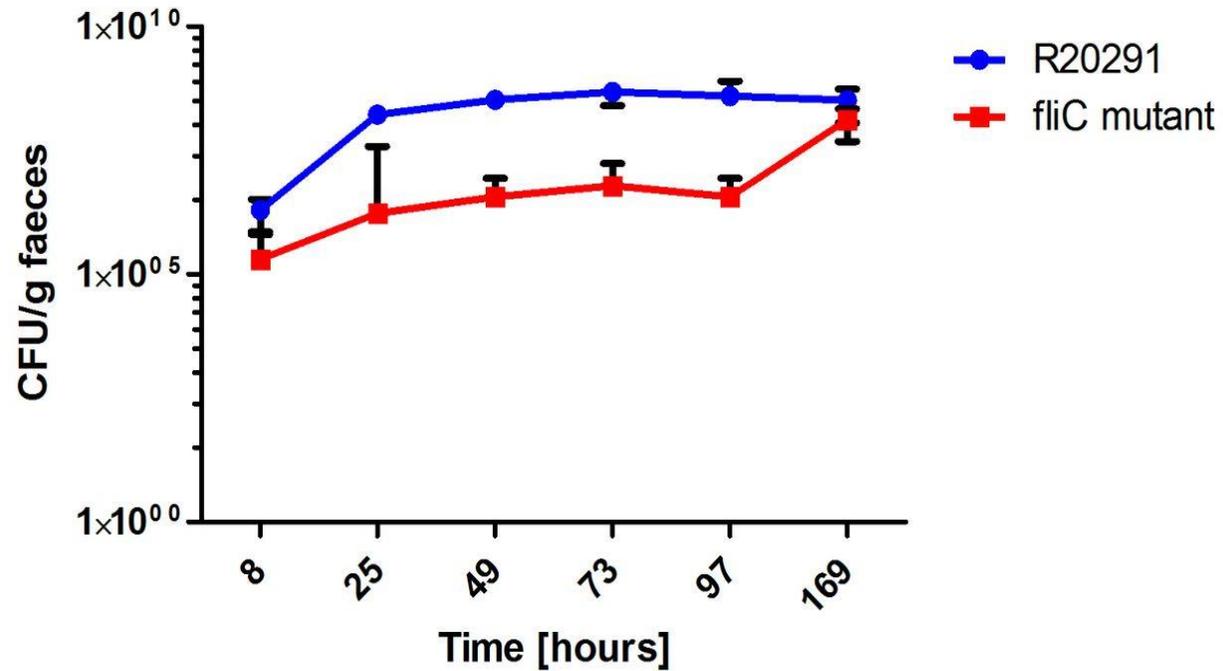


Monoxenic mouse model of colonization (Individual mouse infections)



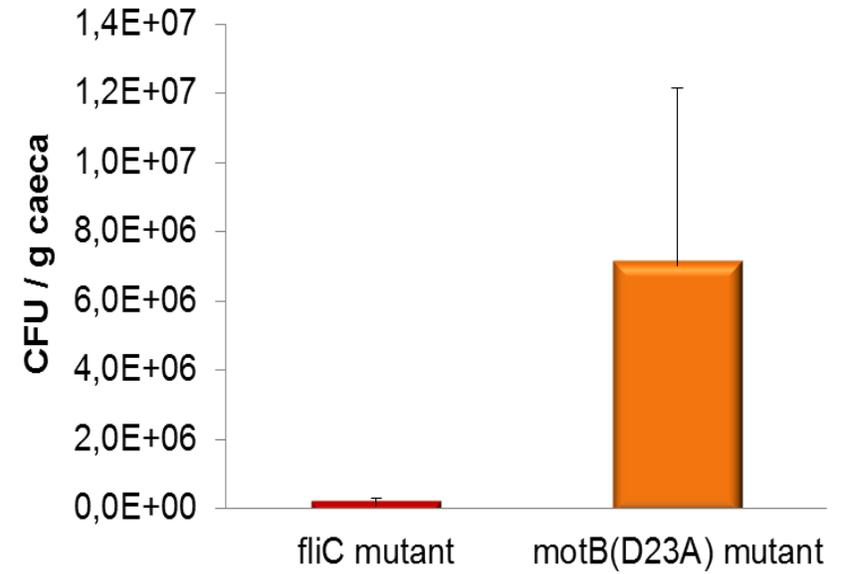
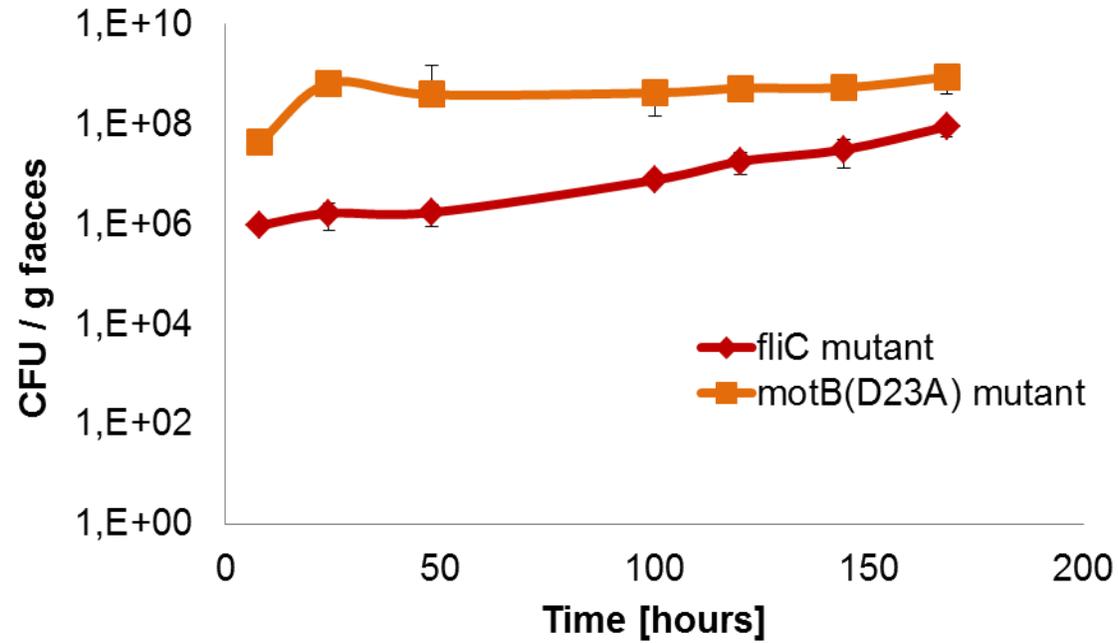


Dixenic mouse model of colonization (coinfection study)





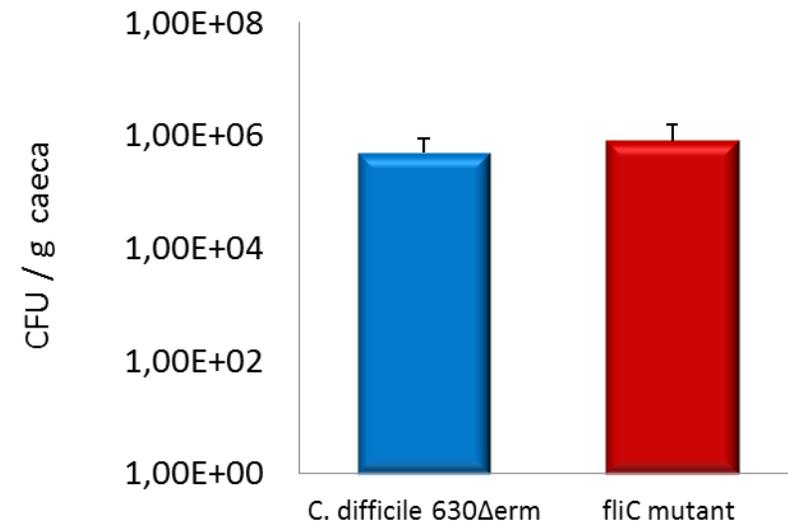
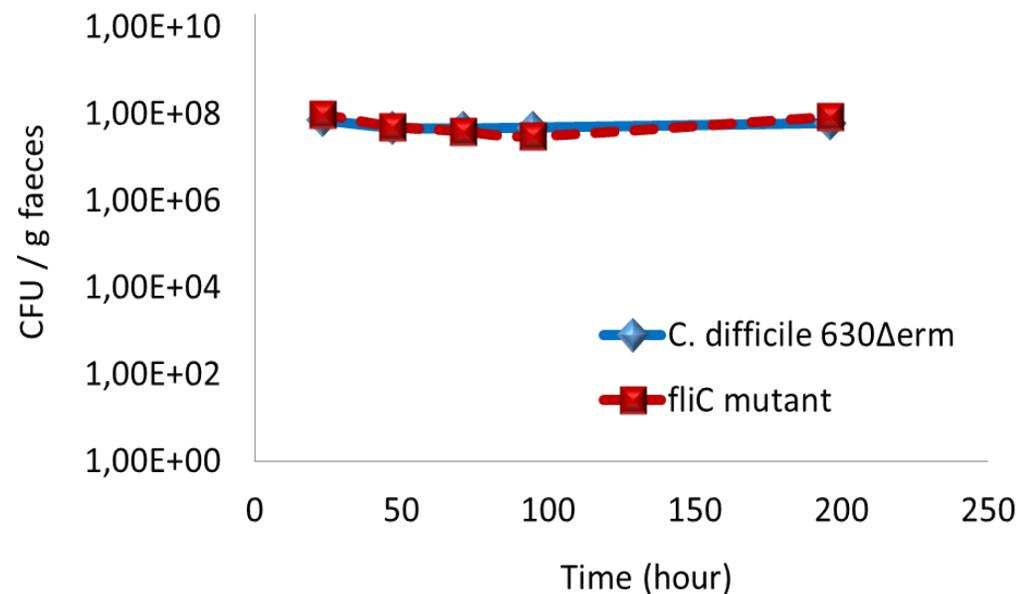
Dixenic mouse model of colonization (coinfection study)



In vivo analysis: Importance of flagellum of *C. difficile* 630 Δ erm strain in intestinal colonization of axenic mice

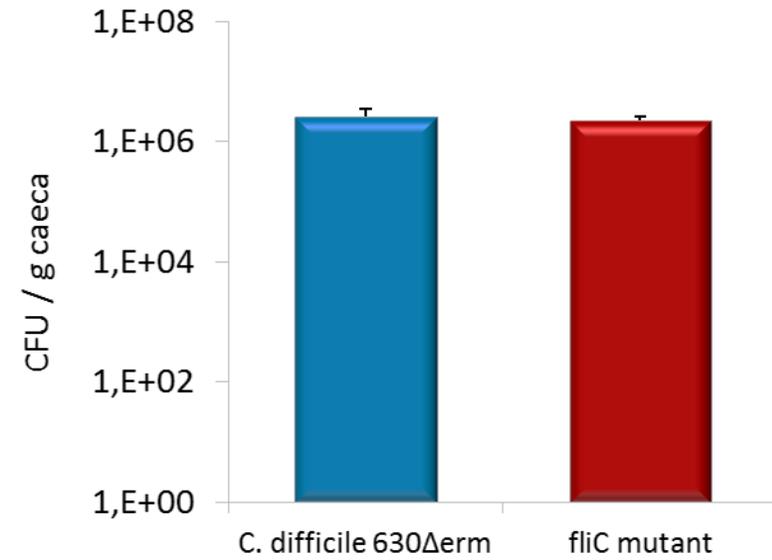
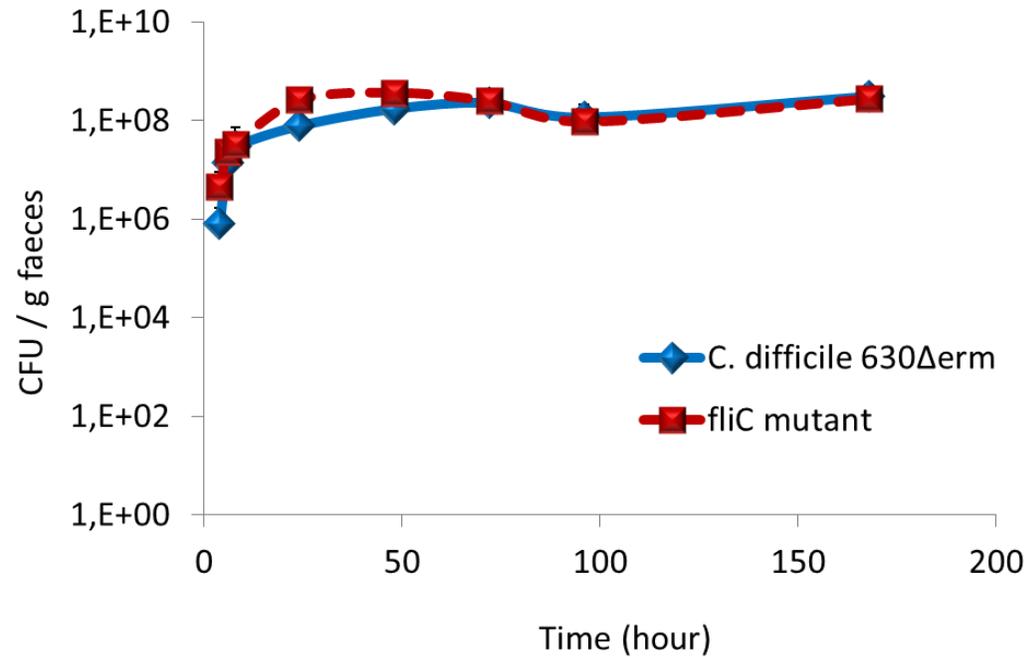


Human Microbial Flora mouse model



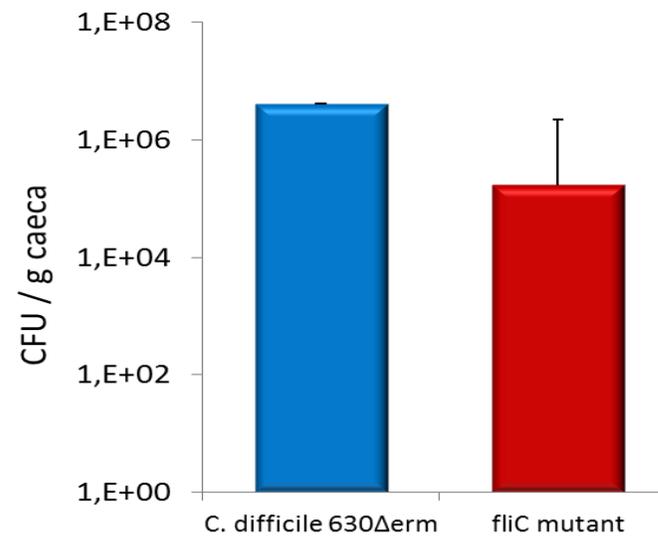
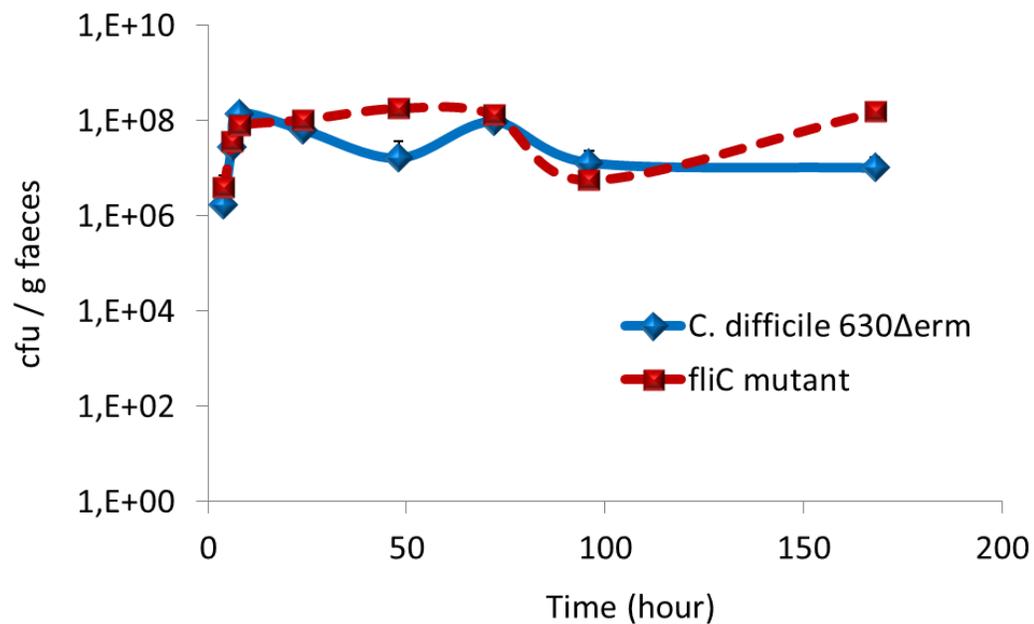


Monoxenic mouse model of colonization (Individual mouse infections)





Dixenic mouse model of colonization (coinfection study)



Conclusion



- ❑ The flagellum of R20291 functions as a surface adhesin, not as motility apparatus to enhance bacterial association to human intestinal epithelial cells and establish intestinal colonization.
- ❑ Flagella of *C. difficile* 630 Δ erm are not required for adherence and intestinal colonisation in this strain, but flagella-mediated motility might contribute to an overall fitness of the bacteria.
- ❑ The flagellum plays an important role as a virulence factor in contributing to the “hypervirulence” of the epidemic *C. difficile* R20291 which causes more severe infections than historical strain.

Acknowledgments

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