7KH UROH RI &À DVWULGLXP GLIILFLOH FHOO EL R¿OP IRUPDWL a non-epidemic and an epidemic strain

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Clostridium di cile is a major cause of healthcare-associated infection and in icts a considerable nancial burden on healthcare systems worldwide. Disease symptoms range from self-limiting diarrhoea to fatal Pseudomembranous colitis. Whilst C. di cile has two major virulence factors, toxin A and B, it is generally accepted that other virulence components of the bacterium contribute to disease. C. di cile colonises the gut of humans and animals and hence the processes of adherence ar colonisation are essential for disease onset. Bacteria within bio lms are protected from multiple stresses, including immune responses and antimicrobial agents. Increased antibiotic resistance and chronic recurrent infections have been attributed to the ability of bacterial pathogens to form bio lms. While bio lms have been well studied for several gut pathogens, little is known about bio lm formation by anaerobic gut species. We have limited understanding of how the causative bacterium C. di cile colonizes the host or how it can resist antibiotics and persist within the gut. While persistent infections have been previously linked to bio lm-formation by pathogens, bio lm development by C. di cile has not been characterized. Our work demonstrates the ability of this anaerobic pathogen to form complex bio lms, the involvement of important clostridial pathways in bio lm development and perhaps a connection between formation of spores which are believed to mediate persistence, and bio lm formation. Importantly, we show that bacterial sensitivity to antibiotics is reduced in clostridial bio lms. Bio lm formation may be a mechanism employed by C. di cile to survive in hostile environments such as the human gut. Here we tested this hypothesis by comparing agellated parental strains to strains in which agella genes were inactivated using ClosTron technology. Our focus was on a UK-outbreak, PCR-ribotype 027 (B1/NAP1) strain, R20291. We compared the agellated wild-type to a mutant with a paralyzed agellum and also to mutants (iC, iD and gE) that no longer produce agella in vitro and in vivo. Our results with R20291 provide the rst strong evidence that by disabling the motor of the agellum, the structural components of the agellum rather than active motility, is needed for adherence and colonisation of the intestinal epithelium during infection. Comparison to published data on 630 erm and our own data on that strain revealed major di erences between the strains: the R20291 agellar mutants adhered less than the parental strain in vitro, whereas w saw the opposite in 630 erm. We also showed that agella and motility are not needed for successful colonization in vivo using strain 630 erm. Finally we demonstrated that in strain R20291, agella do play a role in colonisation and adherence and that there are striking di erences between C. di cile strains. e latter emphasises the overriding need to characterize more than just one strain before drawing general conclusions concerning speci c mechanisms of pathogenesis. In addition, we also demonstrate that clinical C. di cile strains, 630 and the hypervirulent strain R20291, form structured bio lms in vitro, with R20291 accumulating substantially more bio lm. Microscopic analyses show multiple layers of bacteria encased in a proteinaceous bio lm matrix. Employing isogenic mutants, we show that virulence-associated protein, cwp84, and a putative quorum sensing regulator, luxS are all required for maximal bio lm formation by C. di cile. Interestingly, a mutant in spo0A, a transcription factor that controls spore formation, was defective for bio lm formation, indicating a possible link between sporulation and bio lm formation. Furthermore, we demonstrate that bacteria in clostridial bio lms are more resistant to high concentrations of vancomycin, a drug commonly used for treatment of CDI. Bio lm formation by C. di cile is a complex multifactorial process and may be a crucial mechanism for clostridial persistence in the host.

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