C. difficile spores spread superbug

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New research suggests that antibiotic treatment could be asymptomatically inducing the transmission of the healthcare-acquired infection, *C. difficile*, contributing to the outbreaks that have recently been widely reported in hospitals and other settings. A team of scientists have successfully mirrored the infection cycle of *C. difficile* by generating a 'mouse hospital' with conditions mimicking the human environment in which *C. difficile* is transmitted.

The results have implications for infection control measures in the healthcare environment and open the door for the development of treatments and improved diagnosis of *C. difficile*.

At present, healthcare professionals manage the threat of *C. difficile* by observing stringent hygiene and isolation practices primarily by dealing with patients who exhibit the symptoms of infection - including diarrhoea and fever. But today's publication suggests that widening the targets of infection control in hospitals, to include all patients receiving antibiotic treatment - although logistically complex - is worth investigating.

"*C. difficile* is a highly resistant and highly infectious pathogen and resistant to many front line antibiotics," explains Dr Trevor Lawley, Wellcome Trust Sanger Institute researcher and lead author on the study. "Until now, animal studies have focussed on the observable, acute symptoms of *C. difficile*. But, to understand how this highly infectious pathogen spreads, investigating the entire cycle of transmission is absolutely vital. We looked at mice carrying *C. difficile* and observed

that they shed low levels of spores and, crucially, they did not infect other mice."

"But when we treated mice with antibiotics, we saw a dramatic rise in the levels of spores shed - leading to what we have described as a 'supershedder state' and transmission of *C. difficile* among mice. Importantly, transmission occurs even in the absence of clinical symptoms."

C. difficile transmission relies on the shedding of highly resistant spores in the faeces of humans. These bacterial spores are essentially dormant cells with protective outer layers making them well-adapted for survival and dispersal in a wide range of environmental conditions. When humans shed spores in their faeces, those spores are capable of surviving dormant in the environment for long periods of time, under harsh conditions and in temperatures up to 70 ºC, before reintroduction and infection in a new human host.

"We treated mice with short and longer courses of antibiotics," says Professor Gordon Dougan, Head of Pathogen Genetics at the Sanger Institute and senior author on the study. "After a short course most mice had dropped back to normal spore shedding levels around two weeks after cessation of the treatment. But after long term exposure to antibiotics some of the mice remained in their 'supershedder' state for weeks or even longer after treatment was stopped. We should consider that patients still pose a considerable transmission threat some weeks after treatment is terminated even if they have not exhibited signs of *C. difficile* disease."

The team also found that there was a considerable threat from environmental contamination. Even short-term housing of 'supershedder' mice in transfer vessels could contaminate the area, leading to infection in nad′ve - or uninfected - mice, and suggesting that even the briefest

environmental contamination is a potential infection threat.

The administration of antibiotics has been a known risk factor in the development of *C. difficile* infection for some time. The bacterium can live inside the gut of healthy human beings, existing as part of a natural, diverse and potentially beneficial ecosystem of bacteria and microorganisms -the microbiota - that we are all are host to.

When the team treated mice with antibiotics, the balance of the microbial ecosystem was disrupted. Because *C. difficile* is resistant to many antibiotics, the bacterium was able to exploit the opportunity and proliferate where other gut bacteria had succumbed to the antibiotics. This allowed the *C. difficile* to flourish and dominate the microbiota of the mouse.

Although the majority of mice exhibited no visible symptoms of *C. difficile*, the team found that one particular group of knockout mice with mutations associated with impaired immune defence began to lose weight and became moribund. The similarity with the human manifestation of the disease, where older or immune impaired patients tend to exhibit the symptoms of acute *C. difficile* infection, is clear and strengthens utility the mouse model as a valuable tool for investigation into the transmission of *C. difficile*.

Today's results are published soon after a previous publication, where the team demonstrated that *C. difficile* spores could be isolated and highly purified - essentially washed - in a way that maintains their natural characteristics and resistance to an array environmental conditions. This ability to purify spores, while preserving their characteristics, allows researchers to use them in experimental settings and to establish their structure.

"*C. difficile* is a high-profile and rapidly emerging pathogen and is

responsible for the death of a patient every hour in our hospitals - but its biology and transmission are so far poorly understood," says Brendan Wren, Professor of Microbial Pathogenesis from the London School of Hygiene & Tropical Medicine's Department of Infectious & Tropical Diseases. "At last we can monitor the transmission of this major pathogen using a tractable model system. This will be invaluable in determining the role of spore formation in transmission and how and why some *C. difficile* strains are more virulent and transmissible than others."

"It will also facilitate determining the host factors important in disease and will lead to better diagnosis and treatment strategies to reduce the burden of *C. difficile*-associated diseases."

Because the team had established how to isolate and purify spores and trigger transmission of *C. difficile* in mice, they were also in a position test disinfectants commonly used in hospitals for their success at controlling infection. The alcohol-based disinfectants tested had no success in preventing transmission. Instead, a 20-minute surface disinfection using a sporicidal agent was necessary to reduce environmental spore contamination enough to eliminate transmission. Further results detailing the success of individual hospital disinfectants will be published in the coming months.

Today's publication provides a step towards reducing the burden on healthcare systems, which have seen a ten-fold increase in the incidence of *C. difficile* in the last decade. 56,000 cases were reported in the UK in 2007, although, the first a three months of 2009 saw a decline in incidence compared to the same period in 2008. The most severe complication is pseudomembranous colitis, an infection of the colon that can be lethal in patients with weakened immune systems.

"What this research essentially provides," says Dr Fiona Cooke, working

in the Department of Medical Microbiology at Addenbrooke's Hospital, Cambridge, "is a better understanding of the interactions between *C. difficile*, the intestinal microbiota and the immune system of the host - in this case the mouse - primarily in response to antibiotic treatment but also in response to immune impaired hosts. This opens up numerous opportunities including the development of new probiotic approaches, which could restore the balance of the intestinal microbiota and promote health, are clear."

"This research has far reaching implications for treatment and infection control of a pathogen that is increasingly raising alarm among the global healthcare community."

More information:

Lawley T D et al. Antibiotic treatment of Clostridium difficile carrier mice triggers a supershedder state, transmission, and severe disease in immunocompromised hosts. *Infection and Immunity*. Published ahead of print, doi:10.1128/IAI.00558-09

Lawley T D et al. Proteomic and genomic characterization of highly infectious Clostridium difficile 630 spores. *Journal of Bacteriology*. Published online ahead of print, doi:10.1128/JB.00597-09

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