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The housefly *Musca domestica* as a mechanical vector of *Clostridium difficile*

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SUMMARY

Background: *Clostridium difficile* is a bacterial healthcare-associated infection that may be transferred by houseflies (*Musca domestica*) due to their close ecological association with humans and cosmopolitan nature.

Aim: To determine the ability of *M. domestica* to transfer *C. difficile* both mechanically and following ingestion.

Methods: *M. domestica* were exposed to independent suspensions of vegetative cells and spores of *C. difficile*, then sampled on to selective agar plates immediately postexposure and at 1-h intervals to assess the mechanical transfer of *C. difficile*. Fly excreta was cultured and alimentary canals were dissected to determine internalization of cells and spores.

Findings: *M. domestica* exposed to vegetative cell suspensions and spore suspensions of *C. difficile* were able to transfer the bacteria mechanically for up to 4 h upon subsequent contact with surfaces. The greatest numbers of colony-forming units (CFUs) per fly were transferred immediately following exposure (mean CFUs 123.8 +/− 66.9 for vegetative cell suspension and 288.2 +/− 83.2 for spore suspension). After 1 h, this had reduced (21.2 +/− 11.4 for vegetative cell suspension and 19.9 +/− 9 for spores). Mean *C. difficile* CFUs isolated from the *M. domestica* alimentary canal was 35 +/− 6.5, and mean *C. difficile* CFUs per faecal spot was 1.04 +/− 0.58. *C. difficile* could be recovered from fly excreta for up to 96 h.

Conclusion: This study describes the potential for *M. domestica* to contribute to environmental persistence and spread of *C. difficile* in hospitals, highlighting flies as realistic vectors of this micro-organism in clinical areas.

Introduction

The housefly, *Musca domestica*, presents a significant worldwide threat to public health due to its close ecological association with humans.[1] *M. domestica* breed in faecal matter, move from filth to food indiscriminately,[2,3] and are therefore implicated in the spread of many diseases.[4–6] *M. domestica* has been sampled from hospitals previously and shown to carry potentially pathogenic bacteria in the clinical environment, including *Bacillus* spp.,[7] *Escherichia coli*,[8] *Klebsiella pneumoniae*,[9] meticillin-resistant *Staphylococcus aureus* (MRSA)[10] and *Salmonella* spp.[11]

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C. difficile infection (CDI) is the leading cause of infectious nosocomial diarrhoea worldwide.[12] It has serious implications, potentially resulting in the isolation of patients, closure of wards and hospitals, and even the death of infected individuals.[12] CDI typically affects elderly patients exposed to antimicrobials, and can cause severe disease such as pseudomembranous colitis via toxins that affect intestinal cells.[13] While it is generally thought that *C. difficile* is commonly passed from person-to-person nosocomially via the faecal–oral route, most newly ocurring cases cannot be explained by potential contact with known infected individuals. Although the main routes of transmission are unknown, transmission from unidentified symptomatic carriers and/or asymptomatic carriers is likely to be important.[14–16]

It was postulated that flying insects such as *M. domestica* could play a role in the transmission of *C. difficile* in hospitals. To the authors' knowledge, *C. difficile* has not been isolated previously from flying insects in hospitals. However, flies collected from pig farms have been found to harbour ribotype 078,[17] suggesting that there is potential for insects to be mechanical vectors of *C. difficile* in other environments where a source of contamination exists.

This study determined the ability of *M. domestica* to transfer *C. difficile* mechanically and via ingestion and excretion following exposure to vegetative cell and spore suspensions. Subsequent isolation from the alimentary canal and excreta, duration of excretion, and whether the *C. difficile* was excreted as spores or vegetative cells were also investigated.

Methods

Flies

Laboratory reared, mixed-sex adult houseflies (*M. domestica*) were provided by the Insect Supplies Unit at the Food and Environment Research Agency, York, UK.

C. difficile inocula

C. difficile NCTC11204 polymerase chain reaction ribotype 001 TOX A/B + was used in this study (Anaerobe Reference Laboratory, Cardiff, UK). A 1×10^6/mL culture of *C. difficile* vegetative cells was prepared in 15-mL Wilkins Chalgren broth (Oxoid Ltd, Basingstoke, UK) by inoculation with 10 colonies cultured previously on Wilkins Chalgren agar (Oxoid Ltd) incubated anaerobically for 48 h at 37°C. Suspensions of *C. difficile* spores (1×10^6/mL) were prepared as described by Shetty *et al.*,[18] and 1×10^6/mL quantities were chosen as realistic proxy for fly exposure in faeces given that *C. difficile* may be found at levels of 1×10^4 to 1×10^7 per g of human faeces.[19]

Mechanical transfer of C. difficile by M. domestica

Houseflies were inactivated by incubation in a sterile Petri dish in a −18°C freezer (Beko, Watford, UK) for 2 min. Inactivated houseflies were taken from the freezer, and both wings were removed by dissection with sterilized entomological spring scissors and fine entomological forceps (Watkins and Doncaster, Leominster, UK) to prevent escape by flight. *M. domestica* wings do not play an important role in the mechanical transmission of bacteria.[20] The flies were stored at 4°C in a refrigerator until required.

Pretreatment control

A pre-exposure control sample of houseflies (N=5) was macerated individually in 1 mL of sterile phosphate buffered saline (PBS) (Sigma Aldrich, Poole, UK) using the end of a sterile plate spreader. The homogenate was serially diluted to 1×10^{-3}, and 0.1 mL of each dilution was inoculated on to the surface of a CCFA plus sodium taurocholate (Tc) plate (Oxoid Ltd). The plates were incubated anaerobically for 48 h at 37°C. Colonies with typical morphology were subcultured on to Columbia blood agar (Oxoid Ltd), and identified using rapid ID 32A API test strips (bioMérieux, Marcy l'Etoile, France).

Mechanical transfer of vegetative cells

In order to confirm the flies were clear of *C. difficile* carriage prior to the experiment, a single fly was transferred from the sterile holding dish on to the surface of a CCFA plate (no spore germinant) and allowed to walk around the plate for 6 min. Subsequently, it was transferred to a CCFA plus Tc plate for a further 6 min. The same fly was transferred to a 'donor' CCFA plate that had been inoculated with 0.1 mL of the *C. difficile* vegetative cell culture immediately before the fly was introduced. After exposure to the donor plate for 6 min, the fly was transferred to a fresh CCFA plate ('recipient' plate), and then a CCFA plus Tc plate (second 'recipient' plate) (6 min on each). The 6-min contact times were chosen to reflect observations by the authors of how long adult houseflies have contact with foodstuffs and surfaces. The fly was then transferred to a sterile empty Petri dish ('resting' plate) for 1 h. The fly then underwent three further cycles of transfer to CCFA and CCFA plus Tc plates separated by 1-h periods in 'resting plates'. These experiments were replicated nine times.

Mechanical transfer of spores

This experiment used the same methodology as described above but with a 1×10^6/mL *C. difficile* spore suspension rather than a vegetative cell culture. Additionally, only CCFA plus Tc plates were used.

Isolation of C. difficile from M. domestica alimentary canal

Five houseflies were exposed to *C. difficile* for 30 min by being allowed to walk over a CCFA agar plate inoculated with 0.1 mL of the 1×10^6/mL spore suspension. Flies were then killed by incubation in a sterile Petri dish at −18°C for 5 min. Each fly was subsequently removed from frozen storage and washed five times in PBS.

The fly alimentary canal (including crop) was dissected aseptically. The fly alimentary canal was added to 1 mL of PBS in a sterile 1.5-mL universal micro test tube, macerated with the end of a sterile plate spreader, and mixed by vortexing for 30 s to release bacteria into the PBS wash. Of this PBS wash, 0.1 mL was inoculated on to the surface of a CCFA plus Tc agar plate. The PBS wash was diluted 10-fold in sterile PBS, and