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# ISOLATION AND CHARACTERISATION OF BACTERIA ASSOCIATED WITH *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE) IN HOSPITALS

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**Abstract** This study sought to determine the role of *Musca. domestica*, sampled from UK hospitals, as reservoirs of pathogenic bacteria. *M. domestica* were collected from pre-existing ultra-violet light flytraps located throughout the hospitals. External washings and macerates of *M. domestica* were prepared and inoculated onto agars and following incubation bacterial colonies identified by biochemical tests. Fourteen batches of *M. domestica* (n = 67) were sampled from 6 hospitals between March 2010 to August 2011 and 28 bacterial isolates (21 different species) were obtained. Bacterial isolates were recovered from all 14 *M. domestica* batches. There were 15 occurrences of Enterobacteriaceae (53%) (12 species), 7 Bacilli (25%) (4 species), 3 Clostridia (11%) (1 to genus level, 2 other species) 2 Staphylococci (7%) (*S. aureus*) and 1 Streptococci (4%). Bacterial species recovered multiple times were *Bacillus subtilis* Group, *Klebsiella pneumoniae* ssp *pneumoniae* and *Enterobacter cloacae*. The significance of these data is that *M. domestica* may carry pathogenic bacteria in the healthcare environment. This study highlights the potential for *M. domestica* to contribute to persistence and spread of pathogenic bacteria in hospitals and the need to consider pest control as part of infection control strategies.
**Key words** House fly, disease, pest control, infection control, healthcare.

## INTRODUCTION

The house fly *M. domestica* is a synanthropic fly and the endophilic, communicative behaviour of such flies coupled with their potential for exposure to pathogenic bacteria presents a significant threat to public health (Gracyzk et al., 2001). House flies can transmit such pathogenic bacteria by mechanical transmission (Lane and Crosskey, 1993) and bioenhanced transmission (Kobayashi et al., 1999).

*M. domestica* sampled from hospitals in Nigeria, India, and Senegal harboured pathogenic bacteria, including *Bacillus* spp, (Adeyemi and Dipelou, 1984), *Eschericia coli* (Fotedar et al., 1992), antimicrobial resistant *Klebsiella pneumoniae* ssp *pneumoniae* (Fotedar et al., 1992a), *Salmonella* sp. (Nmorsi et al., 2007), Methicillin resistant *Staphylococcus aureus* (MRSA) (Rahuma et al., 2005), and MRSA with a sensitivity profile and phenotype of resistance identical to patients (Boulesteix et al., 2005). In Europe, house flies sampled from a hospital in the Czech republic harboured antimicrobial resistant *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp, *Staphylococcus* spp and *Enterococcus* spp (Sramova et al., 1992). Laboratory models are also showing that house flies, *M. domestica* are able to transfer *Clostridium difficile*, one of the so-called 'hospital superbugs' (Davies et al., 2011).

The aim of this study was to isolate and characterise bacteria associated with *M. domestica* in hospitals, to understand the relevance of pest control as a component of infection control in healthcare facilities.

## MATERIALS AND METHODS

*M. domestica* were collected from pre-existing ultra-violet light flytraps in the form of electronic fly killers (EFK's) and professional sticky traps located throughout 6 health care facility sites from March 2010 to August 2011. The contents of the EFK's were tipped into sterile bags. The glue boards from the sticky traps were removed and covered with a sterile plastic bag. The samples were stored at 4°C in a domestic refrigerator, pending identification and microbiological analysis. External washings of *M. domestica* were serially diluted and inoculated onto CCFA+Tc, Nutrient Agar, Mannitol Salt Agar and Violet Red Bile Glucose agar (all Oxoid Ltd, Basingstoke, UK). The flies were then macerated and the above process repeated for the macerates.

Nutrient agar, Mannitol Salt agar and Violet Red Bile Glucose agar plates were incubated at 37°C for 24 hours in aerobic conditions. CCFA+Tc agar and a set of Nutrient Agar plates were incubated in anaerobic conditions at 37°C (Don Whitley Anaerobic cabinet) for 48 and 24 hours respectively. Bacterial colonies were identified by macroscopic morphology, Gram staining, microscopic examination of morphology, oxidase and catalase tests (National Standard Methods BSOP TP 26 and BSOP TP 8) API 20E test kits, API Staph test kits, rapid ID 32A API test kits (bioMérieux, Marcy l'Etoile, France) and Bacillus-ID test kits (Microgen Bioproducts Ltd, Camberley, UK).

## RESULTS

Fourteen batches of *M. domestica* (n = 67) were sampled microbiologically from 6 hospitals from March 2010 to August 2011 and 28 bacterial isolates (21 different species) were obtained (Table 1). Bacterial isolates were recovered from all *M. domestica* batches. Table 1 shows that of the bacteria isolated from *M. domestica*, there were 15 occurrences of Enterobacteriaceae (12 species), 7 isolates of *Bacillus* spp (4 species), 3 Clostridia (1 to genus level, 2 other species) 2 Staphylococci (both *S. aureus*) and 1 Streptococci. Species of bacteria recovered multiple times were *Bacillus subtilis* Group (x 4), *Klebsiella pneumoniae* ssp *pneumoniae* (x 3) and *Enterobacter cloacae* (x 2).

Figure 1 shows the proportion of isolates belonging to certain bacterial groups that were identified from *M. domestica*. Figure 1 shows that the majority of bacterial isolates taken from *M. domestica* sampled from hospitals were of the family Enterobacteriaceae (53%), followed by *Bacillus* spp (25%), Clostridia (11%), Staphylococci (7%) and Streptococci (4%). *M. domestica* carrying this variety of microorganisms were sampled from a number of locations, including hospital catering areas, ward kitchens, wards, hospital food stores and a mortuary (Table 1). To our knowledge, this study provides the first example of *B. licheniformis*, *B. pumilus*, *C. beijerinckii* / *C. butyricum*, *C. clostridioforme* and *R. terrigena* isolation from *M. domestica* (Table 1).

## DISCUSSION

The clinical significance of many of the species of bacteria isolated from *M. domestica* in this study is well known, as is the role of house flies in the dissemination of these microorganisms, much of which is discussed in the review by Graczyk et al., (2001). As a result, the focus of the discussion of this study is on the significance of the bacterial species isolated for the first time from *M. domestica*.

*B. licheniformis* was isolated from house flies sampled from a hospital mortuary (Table 1). This is of importance, because over half of bloodstream infections with *Bacillus* spp have been attributed to *B. licheniformis* where the cause was the use of non-sterilised cotton wool for skin disinfection and in one case, the patient died following infection (Ozkocaman et al., 2006). In this outbreak, *B. licheniformis*

showed some antibiotic resistance, caused pneumonia and fever and was classed as a 'new' pathogen causing serious infection in patients with neutropenia (Ozkocaman et al., 2006).

*Bacillus pumilus* was isolated from *M. domestica* collected from a hospital food store (Table 1). The significance of this is that catheter infection in children due to *B. pumilus* has been recorded in the

**Table 1.** Bacteria isolated from *M. domestica* sampled from hospitals. Key: The hospital areas sampled: catering (HC), ward kitchens (WK), wards (W), food stores (HS), mortuary (M). *Isolated from *M. domestica* for the first time, to our knowledge.

| **Bacteria isolated from *M. domestica*** | **Area** | **Significance** |
|---|---|---|
| ***Bacillus* spp** | HC | Resistant neonatal sepsis |
| *Bacillus lentus* | M | Septicaemia |
| **Bacillus licheniformis* | HS | Catheter-related bacteraemia |
| **Bacillus pumilus* | HC | Fatal brain and lung infection |
| *Bacillus subtilis* Group | WK | |
| *Bacillus subtilis* Group | HC | |
| *Bacillus subtilis* Group | W | |
| *Bacillus subtilis* Group | | |
| **Clostridia** | HC | Neonatal necrotizing enterocolitis |
| **Clostridium beijerinckii/butyrricum* | HC | Bacteraemia |
| **Clostridium clostridioforme* | HC | |
| *Clostridium* sp | | |
| **Enterobacteriaceae** | HC | Haemolytic uraemic syndrome |
| *Citrobacter freundii* | W | Wound infection |
| *Enterobacter asburiae* | HC | Resistant neonatal bacteraemia |
| *Enterobacter cloacae* | HC | |
| *Enterobacter cloacae* | HC | Haemolytic uraemic syndrome |
| *Eschericia coli* | HC | Catheter-related bacteraemia |
| *Eschericia hermannii* | HC | Haemorrhagic colitis |
| *Klebsiella oxytoca* | HC | Pneumonia |
| *Klebsiella pneumoniae* ssp *pneumoniae* | HC | |
| *Klebsiella pneumoniae* ssp *pneumoniae* | HC | |
| *Klebsiella pneumoniae* ssp *pneumoniae* | HC | Fatal neonatal septicaemia |
| *Pantoea* sp | HC | |
| *Pantoea* species 1 | HC | |
| *Pantoea* spp 3 | HC | |
| *Pantoea* spp 4 | W | Resistant neonatal sepsis |
| **Raoultella terrigena* | | |
| **Staphylococci** | W | Resistant infection of blood, skin, urine, |
| *Staphylococcus aureus* | HC | respiratory tract |
| *Staphylococcus aureus* | | |
| **Streptococci** | HC | Endocarditis |
| Streptococci | | |

literature (Bentur et al., 2007). The *B. pumilus* infection was only eradicated following catheter removal and antibiotic use (Bentur et al., 2007).

*C. beijerinckii* / *C. butyricum* was isolated from houseflies *M. domestica* sampled from a hospital catering area (Table 1). Clinically significant *C. butyricum* strains have been isolated from the faeces of newborn babies suffering from Neonatal Necrotizing Enterocolitis (NNE) and those experiencing haemorrhagic colitis and an adult with peritonitis, while *C. beijerinckii* has been detected in dairy products (Popoff and Dodin, 1985).

*C. clostridioforme* was isolated from *M. domestica* sampled from a hospital catering area (Table 1). There appear to be no records in the literature of *C. clostridioforme* isolation from insects. To our knowledge, this study reports for the first time, isolation of *C. clostridioforme* from insects, specifically *M. domestica*. *C. clostridioforme* infection has been identified in cases of bacteraemia, intra-abdominal abscess, peritonitis, wound infection and other infections (Finegold et al., 2005).

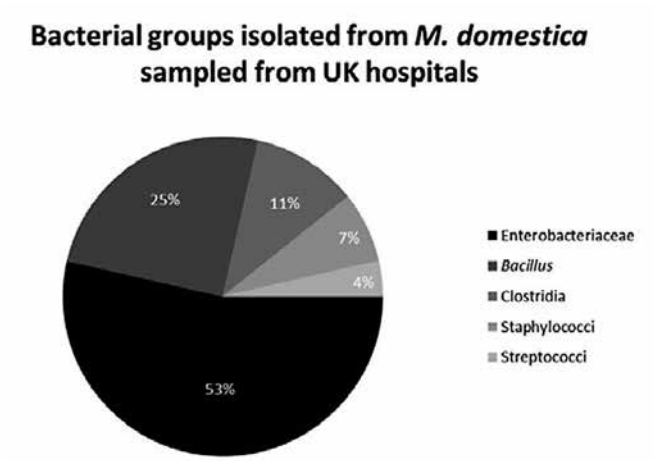


**Figure 1.** Bacterial groups isolated from M. domestica sampled from UK hospitals.

*R. terrigena* was isolated for the first time from *M. domestica*, which were sampled from a hospital ward (Table 1). Multi-drug resistant strains of *R. terrigena* have been described in over 25% of blood cultures taken from neonates, who were suffering with sepsis due to this microorganism (Elamreen, 2007). Neonatal enteral feeding tubes can be a source of bacteria and one study showed that 10% of isolates from such tubes were *R. terrigena*, representing an important risk factor for infection in neonates (Hurrell et al., 2009).

Based on 'read-across' from studies on the transmission of bacteria by *M. domestica* (Kobayashi et al., 1999), it follows that house flies in hospitals may act as a mobile reservoir and vector of clinically significant *B. licheniformis*, *B. pumilus*, *C. beijerinckii* / *C. butyricum*, *C. clostridioforme* and *R. terrigena*, which were isolated from them for the first time in this study, emphasising the importance of pest control as a component of infection control in hospitals.

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