ISOLATION AND CHARACTERISATION OF BACTERIA ASSOCIATED WITH FLYING INSECTS IN HOSPITALS, WITH PARTICULAR EMPHASIS ON *Clostridium difficile*

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Doctor of Philosophy

Aston University

September 2014


flies
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domestica
difficile
bacteria
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Flies of importance in UK hospitals

The housefly *Musca domestica*, life cycle. Model laboratory organism.

Right: Adults. Top Centre: Eggs.

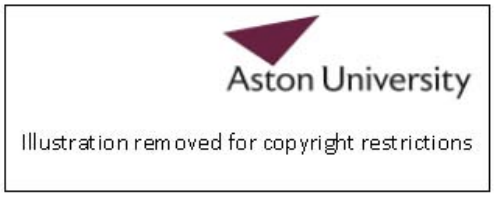
Bottom: Larvae. Top left: Pupae.

Clemson University - USDA Cooperative Extension Slide Series, Bugwood.org

Calliphora vicina, blowfly, family Calliphoridae.

The most common synanthropic fly in UK hospitals.

Gary Alpert, Harvard University, Bugwood.org



Non-biting midge, family Chironomidae.

The most common fly in UK hospitals.

Joseph Berger, Bugwood.org

Psychoda sp, family Psychodidae.

The most common ‘drain fly’ in UK hospitals.

Whitney Cranshaw, Bugwood.org

ASTON UNIVERSITY

Isolation and characterisation of bacteria associated with flying insects in hospitals, with particular emphasis on *Clostridium difficile*

A thesis submitted by Matthew Paul Davies BSc (Hons)

For the degree of Doctor of Philosophy

2014

SUMMARY

Clostridium difficile is a bacterial healthcare-associated infection, which houseflies *Musca domestica* may transfer due to their synanthropic nature. The aims of this thesis were to determine the ability of *M. domestica* to transfer *C. difficile* mechanically and to collect and identify flying insects in UK hospitals and classify any associated bacteria. *M. domestica* exposed to independent suspensions of vegetative cells and spores of *C. difficile* were able to mechanically transfer the bacteria on to agar for up to 4 hours following exposure. *C. difficile* could be recovered from fly excreta for 96hrs and was isolated from the *M. domestica* alimentary canal. Also confirmed was the carriage of *C. difficile* by *M. domestica* larvae, although it was not retained in the pupae or in the adults that subsequently developed. Flying insects were collected from ultra-violet light flytraps in hospitals. Flies (order Diptera) were the most commonly identified. Chironomidae were the most common flies, *Calliphora vicina* were the most common synanthropic fly and 'drain flies' were surprisingly numerous and represent an emerging problem in hospitals. External washings and macerates of flying insects were prepared and inoculated onto a variety of agars and following incubation bacterial colonies identified by biochemical tests. A variety of flying insects, including synanthropic flies (e.g. *M. domestica* and *C. vicina*) collected from UK hospitals harboured pathogenic bacteria of different species. Enterobacteriaceae were the group of bacteria most commonly isolated, followed by *Bacillus* spp, Staphylococci, Clostridia, Streptococci and *Micrococcus* spp. This study highlights the potential for *M. domestica* to contribute to environmental persistence and spread of *C. difficile* in hospitals. Also illustrated is the potential for flying insects to contribute to environmental persistence and spread of other pathogenic bacteria in hospitals and therefore the need to implement pest control as part of infection control strategies.

Keywords: *Musca domestica*, flies, pest control, infection control, disease.

For Granddad Ted

ACKNOWLEDGEMENTS

I thank my supervisors, Professor Anthony Hilton and Professor Moray Anderson, for their generosity of support, boundless enthusiasm and positive outlook.

I am indebted to Sufina Akram, Sarah Jewkes, Laura Searle, Nithesh Narayanan and Baljeet Baba for their excellent contributions to my work.

For the constant supply of *Musca domestica* I thank the ever efficient and reliable Insect Supplies Unit at the Food and Environment Research Agency.

To everyone in the Killgerm Technical Department, thank you for manning the fort when I have been away 'counting flies'.

A firm handshake to all the pest controllers who have generously given up so much of their time to assist with the collection of flying insects from NHS premises.

To everyone of Lab 327 over the years (Christian Lowden, Preena Lowden, Lauren Acton, Laura Lancaster and Jess Rollason to name but a few), thank you for accepting me (and my flies) into the group and always making me feel welcome, as 'fly Matt'.

Friends and family, Mum & Dad, Becky & Sophie, you are my inspiration.

To my fiancée Kayleigh, your love has kept me going.

Bradley and Kelly, thank you for keeping me smiling and laughing every single day.

Undertaking a part-time external PhD over six years while balancing a full-time job and latterly a young family has been a challenging and rewarding experience, made all the more enjoyable due to the support of everyone acknowledged here. I genuinely could not have done this without all of you. Thank you!

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ABBREVIATIONS

%	Percent
-ve	Negative
+ve	Positive
ADH	Arginine dihydrolase
A&E	Accident and emergency
ANOVA	Analysis of variance
API	Analytical profile index
ºC	Degrees centigrade
CCFA	Cycloserine cefoxitin fructose agar
CDAD	*Clostridium difficile* associated diarrhoea
CDI	*Clostridium difficile* infection
CFU	Colony forming unit
CNS	Central nervous system
CT	Computerised tomography
D	Simpson's diversity index
DHSS	Department of Health and Social Security
DoH	Department of Health
DPIL	Danish Pest Infestation Laboratory
E_D	Equitability
EFK	Electronic fly killer
EPEC	Enteropathogenic *Escherichia coli*
Etc.	*Et cetera* (and so forth)
FERA	Food and Environment Research Agency
g	Gram
h	Hour
HC	Hospital catering areas
HPA	Health Protection Agency
HS	Hospital food stores
ID	Identification
i	Number of different species
I.e.	*Id est* (that is)
IRAS	Integrated research application system
KCIIS	Killgerm Chemicals insect identification service
Ltd	Limited
M	Mortuary

mins	Minutes
ml	Millilitre
mm	Millimetre
MI	Medical illustration
MRI	Magnetic resonance imaging
MRSA	Methicillin resistant *Staphylococcus aureus*
N	Total number of individuals of all species
n	Number of individuals of a specific species
n	Number
NB	*Nota bene* (note well)
NCTC	National collection of type cultures
NHS	National Health Service
nm	Nanometre
NTCD	Non-toxigenic *Clostridium difficile*
OD	Optical density
ONS	Office of National Statistics
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCT	Primary care trust
PHE	Public Health England
PHLS	Public Health Laboratory Service
p_i	Proportion of *S* made up of $_i$th species
PMC	Pseudomembranous colitis
PPIs	Proton pump inhibitors
RAPD	Random amplification of polymorphic DNA
R&D	Research and development
REC	Research ethics committee
S	Total number of species in the community (richness)
SDW	Sterile distilled water
SE	Standard Error
SEM	Scanning electron microscope
SPI	Stored product insects
sp	Species
spp	Species (plural)
ssp	Subspecies
Tc	Sodium taurocholate
UK	United Kingdom
µL	Microlitre

µm	Micrometres
UPAS	Urban pest advisory service
URE	Urease
UV	Ultra violet
VRBG	Violet Red Bile Glucose agar
$^{v}/_{v}$	Volume per volume
$^{w}/_{v}$	Weight per volume
W	Wards
WK	Ward kitchens
WN	Neonatal and maternity wards

1 CHAPTER 1: INTRODUCTION

1.1 *Musca domestica*

The housefly, *Musca domestica*, is a synanthropic, endophilous, cosmopolitan fly. It has a propensity to breed in faecal matter and moves indiscriminately from filth to food. In addition, there are many works which show that houseflies harbour pathogenic bacteria obtained from various unsanitary sources and have been implicated in the transmission of many diseases and thus present a significant threat to public health (West, 1951, Greenberg, 1971, Greenberg, 1973, Olsen, 1998, Graczyk *et al.*, 2001, Forster *et al.*, 2009).

It is crucial that *M. domestica* is identified accurately by medical entomologists and others involved in the protection of public health. The following key features of adult housefly morphology are: a length of 6-7mm; wingspan of 13 – 15mm; grey thorax with four longitudinal stripes; the fourth vein on the wing bends sharply forward, almost reaching the third vein; the sides of the abdomen are yellowish and may be transparent and a central dark band broadens at the back to cover the final abdominal segments (Busvine, 1980, Killgerm, 2013). Identification should be confirmed in the laboratory by using a dissecting microscope and entomological references (Colyer and Hammond, 1951, Unwin, 1981, Chinery, 1993, Chinery and Falk, 2007).

The life history of *M. domestica* is typical of flies in that it is an Endopterygote undergoing holometabolous development, which is sometimes described as complete metamorphosis due to the complete change in shape from egg to larva, then pupa and finally the imago or adult (Chinery, 1993). The female housefly selects an oviposition site of moist rotting organic matter, ranging from kitchen waste to animal faeces such as those from sheep, pigs, horses, cows, poultry and humans (West, 1951), upon which she deposits 100 – 150 eggs in a day, with approximately 400 – 750 eggs produced during her lifetime (Busvine, 1980). The housefly detects the aforementioned breeding material with antennal olfactory organs and may travel up to five miles in a day in search of such media (Busvine, 1980).

The development period of *M. domestica* from egg to adult is an average of three weeks during a typical United Kingdom (UK) summer, with four to six generations and an adult lifespan of approximately one month but as insect metabolism and development is influenced by temperature, the life cycle can be prolonged significantly in cooler conditions (Busvine, 1980). It is important to note that although *M. domestica* activity peaks during summer, these flies are still found in and around

hospitals during the winter months and development can continue due to the suitable temperatures and local availability of breeding matter (personal observation).

1.2 *M. domestica* and the spread of disease

There are considered to be three possible ways in which houseflies can mechanically acquire and transmit pathogens. These mechanisms are related to the anatomy and feeding behaviour of the fly and their habit of frequently being associated with unsanitary matter such as faeces and waste (Lane and Crosskey, 1993);

1. External surfaces of the fly can become contaminated upon contact with pathogens, principally areas such as the legs particularly the tarsi and pulvilli (the 'sticky pads' on fly 'feet' where pathogens are known to adhere – see Figure 1.1 and Figure 1.2) and the mouthparts. The external surfaces of the fly are covered with many spines, hairs (setae) and microtrichia, where material can become attached and transported.
2. Regurgitation on food as a prelude to feeding.
3. Ingestion and defecation of pathogens.

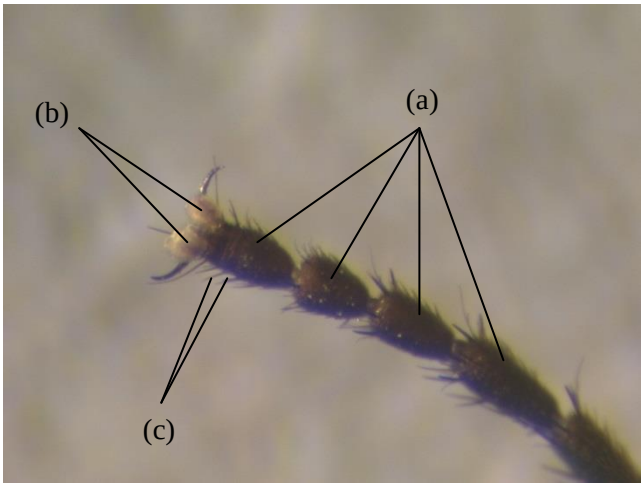

Figure 1.1 Tarsi (a), pulvilli (b) and setae (c) of *M. domestica*, which are candidate sites for adherence of pathogens in the process of mechanical transmission (Matthew Davies & Dr Kameel Sawalha, Aston University).

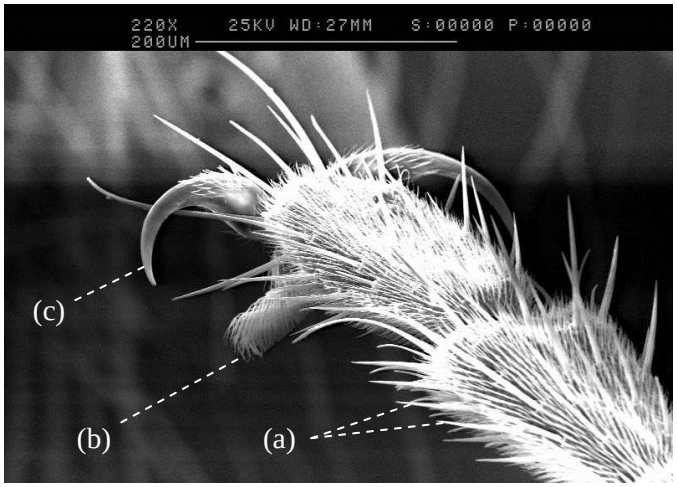

Figure 1.2 Numerous setae on tarsi (a), many setae of the pulvillus (b) and tarsal claw (c) of *M. domestica*, which are candidate sites for adherence of pathogens in the process of mechanical transmission (Matthew Davies & Dr Kameel Sawalha, Aston University).

A number of researchers have confirmed the mechanical transmission of a variety of pathogenic organisms by flies. Houseflies can mechanically transfer *Salmonella* Enteritidis from infected food to other flies, onto surfaces they contact, to mice and to uninfected food (Ostrolenk and Welch, 1942). Humans and domestic animals may also be at risk of *Salmonella* spp infection from flies via mechanical transmission. Experimental transmission of *Salmonella* Typhimurium from an infected dog to human volunteers has been carried out. In these experiments houseflies were exposed to dog faeces and subsequently to an atole drink and after consuming the drink human volunteers became infected with *S.* Typhimurium (Greenberg, 1964). Researchers described the mechanical transmission of *Salmonella enterica* serovar Enteritidis from chickens to *M. domestica* and *vice versa*, when contaminated houseflies were fed to the chickens (Holt *et al.*, 2007).

Houseflies have been incriminated as mechanical vectors of *Shigella* spp (Levine and Levine, 1991) and can transfer *Helicobacter pylori* mechanically, specifically via their excreta (Grubel *et al.*, 1997). External surfaces and the digestive tract of *M. domestica* can become contaminated with *Cryptosporidium parvum* oocysts after exposure to bovine faeces containing such oocysts, which the flies subsequently deposited onto surfaces by mechanical transmission, specifically faecal deposition (Graczyk *et al.*, 1999).

Flies other than the housefly are also able to transfer bacteria mechanically. Fruit flies, *Drosophila* sp, became contaminated with *E. coli* O157: H7 after contact with the bacteria source and these flies then successfully transferred the bacteria to uncontaminated apple wounds (Janisiewicz *et al.*, 1999). Non-biting midges, family Chironomidae, carried viable *Vibrio cholerae*, which was associated with egg masses while adult flies were able to mechanically transmit the bacteria to uncontaminated water (Broza *et al.*, 2005).

An enhanced form of mechanical transmission has been described, where enterohaemorrhagic *E. coli* O157: H7 proliferated in the pseudotrachea of the labellum of *M. domestica* mouthparts, resulting in observed persistence of the bacteria in the fly intestine and deposition in faeces for at least three days post-exposure; a process that is termed 'bioenhanced transmission', suggesting that the potential for houseflies to disseminate pathogens is greater than first thought (Kobayashi *et al.*, 1999).

The anatomy of flies provides a number of sites for bacterial contamination. The alimentary canal (inside the peritrophic membrane) and the crop (Figure 2.1) and the mouthparts of *M. domestica* have all been shown to harbour *E. coli* O157: H7 (Kobayashi *et al.*, 1999). In terms of the fly mouthparts, *E. coli* O157: H7 specifically adhered to surfaces of the labellum (Figure 1.3), actively proliferating in the pseudotrachea (Kobayashi *et al.*, 1999). Bacteria have been isolated from the internal structures of flies, specifically *Salmonella* serovar Enteritidis from *M. domestica* gut in all cases and the crop seldom (Holt *et al.*, 2007), *E. coli* O157: H7 from the crop of *M. domestica* (Sasaki *et al.*, 2000) and also *C. parvum* oocysts from *M. domestica* digestive tract (Graczyk *et al.*, 1999). Bacteria have also been isolated from the external structures of flies, specifically *C. parvum* oocysts from *M. domestica* wing bristles on the posterior wing margin and within hairs on the tibia (Graczyk *et al.*, 1999). *V. cholerae* bacteria have been located at abdominal intersegmental membranes of the exoskeleton, the tarsal pulvilli and on male external genitalia of chironomids (Broza *et al.*, 2005). Although some bacteria have been found on fly wings, *M. domestica* wings do not play an important role in the mechanical transmission of *V. cholerae* (Yap *et al.*, 2008).

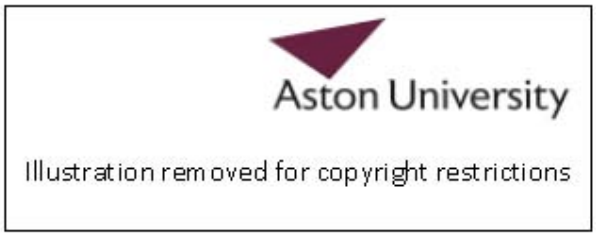

Figure 1.3 Mouthparts of the housefly *M. domestica*, showing the labellum (a), which is a known site of bacterial adherence. (West, 1951).

1.2.1 Pathogenic bacteria associated with synanthropic flies, with specific reference to *M. domestica*.

The pathogenic bacteria associated with synanthropic flies is reviewed comprehensively in the classic texts of Greenberg (1971), Greenberg (1973) and West (1951).

M. domestica is the most important fly in terms of significant pathogenic bacterial associations. Typical examples of pathogenic bacteria associated with *M. domestica* and other synanthropic flies include *Salmonella* spp, *Escherichia coli*, *Staphylococcus* spp, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Vibrio cholerae*, *Listeria* spp, *Shigella* spp, *Bacillus* spp, *Helicobacter pylori*, *Klebsiella* spp, *Serratia* spp, *Enterobacter* spp, many of which are discussed in the more recent reviews by Olsen (1998) and Graczyk *et al.* (2001), which have added to the knowledge base provided by the classic texts.

In the form of an update, a selection of work since the reviews by Olsen (1998) and Graczyk *et al.* (2001) and any significant omissions are summarized in Table 1.1.

Table 1.1 Bacterial associations of synanthropic flies e.g. *M. domestica*

Fly species	**Bacteria isolated**	**Reference**
'Flies' presumably *Musca domestica*	Haemolytic streptococci, Coagulase positive staphylococci, Coliform bacilli, *Proteus* spp	(Shooter and Waterworth, 1944)
Musca domestica	*Helicobacter pylori*	(Grubel *et al.*, 1997)
Musca domestica	*Aeromonas hydrophila*, *Citrobacter freundii*, *Enterobacter agglomerans*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Burkholderia pseudomallei*	(Sulaiman *et al.*, 2000)
Pollenia rudis	*Bacillus* spp, *Erwinia* spp (*Pantoea* spp), *Stenotrophomonas maltophilia*, *Flavibacterium odoratum*, *Staphylococcus lugunensis*, *Pseudomonas aeruginosa*	(Faulde *et al.*, 2001)
Musca domestica	*Vibrio cholerae*	(Fotedar, 2001)
Musca domestica	*Serratia marcescens*	(Cooke *et al.*, 2003)
Musca domestica	*Escherichia coli*	(Alam and Zurek,

	O157: H7	2004)
Musca domestica	Methicillin resistant *Staphylococcus aureus* (MRSA)	(Boulesteix *et al.*, 2005)
Musca domestica	*Bacillus* sp, *Coccobacillus* sp, *Staphylococcus* sp, *Micrococcus* sp, *Streptococcus* sp, *Acinetobacter* sp, *Enterobacter* sp, *Proteus* sp, *Escherichia* sp, *Klebsiella* sp	(Nazni *et al.*, 2005)
Musca domestica	*E. coli*, *Klebsiella* spp, *Aeromonas* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Streptococcus* spp	(Rahuma *et al.*, 2005)
Musca domestica and *Calliphora vomitoria*	*Bacillus atrophaeus*	(Torres, 2006)
Musca domestica	*Shigella* spp, *Salmonella* spp	(Ugbogu *et al.*, 2006)
Musca domestica	Coagulase-negative staphylococci, Non-fermentative Gram-negative bacilli, Streptococcus group D non-enterococci, *Escherichia coli*, *Klebsiella pneumoniae*, Viridans streptococci, *Morganella*	(Sukontason *et al.*, 2007)

	morganii, *Enterobacter cloacae*, *Providencia stuartii*, *Enterococcus* spp, *Providencia alcalifaciens*, *Providencia rettgeri*, *Citrobacter freundii*, *Enterobacter agglomerans*, *Bacillus* spp, *Proteus mirabilis*, Mixed Gram-negative bacilli, *Citrobacter amalonaticus*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Proteus penneri*, *Pseudomonas* spp, *Micrococcus* spp, Staphylococci spp, *Staphylococcus aureus*	
Calliphora vomitoria, *Fannia canicularis*, *Graphomya maculata*, *Helina dublicata*, *Lucilia caesar*, *Musca domestica*, *Mydaea scutellaris*, *Orthellia cornicina*,	*Acinetobacter lwoffii*, *Enterobacter aerogenes*, *Enterococcus faecium*, *Escherichia coli* (ETEC, EPEC, EAEC), *Klebsiella* spp, *Morganella*	(Forster *et al.*, 2007)

Phaonia viarum, *Polietes lardaria*, *Sarcophaga carnaria*, *Stomoxys calcitrans*	*morganii*, *Pantoea agglomerans*, *Proteus* sp, *Providencia rettgeri*, *Pseudomonas* sp, *Sphingomonas paucimobilis* and *Staphylococcus aureus* were collected from some of the sampled flies.	
Musca domestica	*Salmonella enterica*	(Holt *et al.*, 2007)
Musca domestica	*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp, *Salmonella* spp, *Shigella* spp, *Proteus vulgaris*, *Proteus* spp, *Serratia* spp, *Klebsiella* spp, *Enterobacter* spp, *Escherichia coli*	(Lamiaa *et al.*, 2007)
Musca domestica & *Drosophila* sp	*Escherichia* sp, *Proteus* sp, *Streptococcus* sp, *Klebsiella* sp, *Salmonella* sp, *Proteus* sp, *Streptococcus* sp, *Salmonella* sp	(Nmorsi *et al.*, 2007)
Telmatoscopus albipunctatus	*Nocardia* sp (probably *N. cyriacigeorgica*)	(Pelli *et al.*, 2007)

Musca domestica	*Acinetobacter baumanni*, *Bacillus cereus*, *Bacillus pumilus*, *Bacillus thuringiensis*, *Cronobacter sakazakii*, *Escherichia coli* 0157:H7, *Methylobacterium persicinum*, *Shigella dysenteriae*, *Staphylococcus saprophyticus*, *Staphylococcus sciuri*, *Staphylococcus xylosus*	(Butler *et al.*, 2010)
Musca domestica	*Enterococcus faecalis*, *E. hirae*, *E. faecium*, *E. casseliflavus*	(Ahmad *et al.*, 2011)
Musca domestica	*Achromobacter ruhlandii*, *Acinetobacter bereziniae*, *Acinetobacter haemolyticus*, *Acinetobacter radioresistens*, *Acinetobacter soli*, *Aeromonas hydrophila*, *Aeromonas veronii*, *Bacillus*	(Gupta *et al.*, 2012)

	amyloliquefaciens, *Bacillus firmus*, *Chryseobacterium haifense*, *Clostridium sordellii*, *Comamonas testosterone*, *Cronobacter sakazakii*, *Desulfovibrio senezii*, *Dysgonomonas mossii*, *Enterobacter aerogenes*, *Enterobacter cancerogenus*, *Enterococcus faecalis*, *Enterococcus sulfureus*, *Escherichia hermannii*, *Halomonas cupida*, *Holospora obtuse*, *Ignatzschineria larvae*, *Kerstersia gyiorum*, *Klebsiella pneumoniae*, *Kurthia gibsonii*, *Lactococcus garvieae*, *Lactococcus lactis*, *Morganella morganii*, *Myroides odoratimimus*,	

	Naxibacter varians, *Paludibacterium yongneupense*, *Pantoea anthophila*, *Parabacteroides distasonis*, *Paraprevotella clara*, *Phascolarctobacterium faecium*, *Photobacterium damselae*, *Plesiomonas shigelloides*, *Proteus mirabilis*, *Providencia alcalifaciens*, *Providencia rustigianii*, *Providencia stuartii*, *Pseudomonas corrugata*, *Pseudomonas fragi*, *Pseudomonas mendocina*, *Pseudomonas plecoglossicida*, *Ralstonia pickettii*, *Serratia rubidaea*, *Shewanella baltica*, *Shigella flexneri*, *Staphylococcus simiae*, *Staphylococcus warneri*, *Stenotrophomonas maltophilia*, *Vagococcus*	

	carniphilus, *Wohlfahrtiimonas chitiniclastica*,	
Musca domestica, *Fannia canicularis*, *Drosophila melanogaster*, *Psychoda alternata*	*Clostridium difficile* ribotype 078	(Burt *et al.*, 2012)
Clogmia albipunctata	*Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter haemolyticus*, *Acinetobacter junii/johnsonii*, *Acinetobacter lwoffii*, *Alcaligenes denitrificans*, *Actinomyces* spp, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Alcaligenes denitrificans*, *Alcaligenes faecalis*, *Alcaligenes* spp, *Bacillus cereus*, *Brevundimonas diminuta*, *Brevundimonas vesicularis*, *Burkholderia cepacia*, *Chryseomonas*	(Faulde and Spiesberger, 2013)

	luteola, *Citrobacter freundii*, *Citrobacter koseri*, Coagulase-negative *Staphylococcus* spp, *Comamonas acidovorans*, *Comamonas testosterone*, *Corynebacterium amycolatum*, *Corynebacterium* spp, *Enterobacter aerogenes*, *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterobacter sakazakii*, *Enterococcus casseliflavus*, *Enterococcus* spp, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* ssp *pneumoniae*, *Leuconostoc* spp, *Micrococcus* spp, *Moraxella* spp, *Myroides* spp, *Neisseria* spp, *Ochrobacterium anthropic*, Opportunistic aerobic	

	mesophilic *Bacillus* spp, *Paecilomyces lilacinus*, *Photobacterium damsel*, *Proteus* spp, *Providencia rettgeri*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas oryzihabitans*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Psychrobacter phenylpyruvicus*, *Ralstonia pickettii*, *Rhodococcus* spp, *Serratia fonticola*, *Serratia marcescens*, *Serratia rubidaea*, *Shewanella putrifaciens*, *Sphingobacterium multivorum*, *Stenotrophomonas maltophilia*, *Streptococcus* spp, *Streptomyces* spp, *Tsukamurella* spp, *Yersinia frederiksenii*	

Having outlined the clear and established relationship between flying insects and pathogenic bacteria it follows that if such insects are found in hospitals potential for dissemination of bacteria by these insects exists. Indeed, Table 1.2 lists the bacteria isolated from flying insects collected from hospitals.

Table 1.2 Bacteria isolated from flying insects collected from hospitals

Flying insect species	**Bacteria isolated**	**Location**	**Reference**
'Flies' presumably *Musca domestica*	Haemolytic streptococci, Coagulase positive staphylococci, Coliform bacilli, *Proteus* spp	Hospital wards in the United Kingdom	(Shooter and Waterworth, 1944)
Musca domestica, *Fannia canicularis*	*Bacillus* spp, *Proteus* spp, *E. coli*, *Klebsiella* spp, *Pseudomonas* spp, *Staphylococci* spp, *Serratia* spp	Hospitals in Nigeria.	(Adeyemi and Dipeolu, 1984)
Musca domestica	*Klebsiella* spp, *Pseudomonas aeruginosa*, *E. coli*, *Enterobacter* spp, *Proteus* spp, *Acinetobacter* spp, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Viridans* streptococci, *Enterococcus faecalis*, Other Streptococci, Other Micrococci, *Bacillus* spp	Surgical ward of a hospital in India.	(Fotedar *et al.*, 1992b)
Musca domestica	*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella ozanae*,	Surgical ward of a hospital in India.	(Fotedar *et al.*, 1992a)

	Klebsiella rhinoscleromatis		
Wasps *Paravespula vulgaris* **Flies** *Musca domestica*, *Fannia canicularis*, *Fannia scalaris*, Sarcophagidae, Piophilidae, Tachinidae, Lauxaniidae, *Drosophila melanogaster*, *Drosophila* sp, Chironomidae	*Enterobacter agglomerans*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Acinetobacter calcoaceticus*, (fly species unspecified): *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoninae*, *Citrobacter freundii*, *Serratia marcescens*, *Acinetobacter calcoaceticus*, *Providencia rettgeri*, *Morganella morganii*, *Staphylococcus* spp (coagulase negative), *Enterococcus* spp, *Staphylococcus* spp (coagulase negative), *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Hafnia alvei*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Acinetobacter*	Hospital in Czechoslovakia.	(Sramova *et al.*, 1992)

	calcoaceticus, Sporing bacteria – unidentified.		
Mosquitoes *Culex pipiens molestus*	*Staphylococcus* spp (coagulase negative), *Enterococcus* spp, *Enterobacter cloacae*, *Enterobacter intermedius*, *Acinetobacter calcoaceticus*		
Moths *Agrotis exclamationis*, *Nemapogon cloacellus*	*Citrobacter amalonaticus*, *Pseudomonas cepacia*, *Acinetobacter calcoaceticus*		
Pollenia rudis	*Bacillus* spp, *Erwinia* spp (*Pantoea* spp), *Stenotrophomonas maltophilia*, *Flavibacterium odoratum*, *Staphylococcus lugunensi*, *Pseudomonas aeruginosa*	Upper floors of a military hospital in Germany.	(Faulde *et al.*, 2001)
Musca domestica	Methicillin resistant *Staphylococcus* aureus (MRSA)	Intensive care facility in Dakar.	(Boulesteix *et al.*, 2005)
Musca domestica	*E. coli*, *Klebsiella* spp, *Aeromonas* spp, *Pseudomonas* spp, *Staphylococcus* spp,	Hospital in Misurata, Libya.	(Rahuma *et al.*, 2005)

	Streptococcus spp.		
Musca domestica *Drosophila* sp	*Escherichia* sp, *Proteus* sp, *Streptococcus* sp, *Klebsiella* sp, *Salmonella* sp *Proteus* sp, *Streptococcus* sp, *Salmonella* sp	Hospitals in Nigeria.	(Nmorsi *et al.*, 2007)
Telmatoscopus albipunctatus	*Nocardia* sp (probably *N. cyriacigeorgica*)	Intensive care unit in a University hospital in Brazil	(Pelli *et al.*, 2007)
Musca domestica	*Acinetobacter soli*, *Bacillus amyloliquefaciens*, *Enterobacter cancerogenus*, *Providencia alcalifaciens*, *Vagococcus carniphilus*, *Wohlfahrtiimonas chitiniclastica*	Hospital, India	(Gupta *et al.*, 2012)
Clogmia albipunctata	*Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter haemolyticus*, *Acinetobacter junii/johnsonii*, *Acinetobacter lwoffii*, *Alcaligenes denitrificans*, *Actinomyces* spp, *Aeromonas hydrophila*,	From four hospitals in Germany. Sampling sites were shower cubicles, patient wards, rest rooms, kitchens.	(Faulde and Spiesberger, 2013)

	Aeromonas salmonicida, *Alcaligenes denitrificans*, *Alcaligenes faecalis*, *Alcaligenes* spp, *Bacillus cereus*, *Brevundimonas diminuta*, *Brevundimonas vesicularis*, *Burkholderia cepacia*, *Chryseomonas luteola*, *Citrobacter freundii*, *Citrobacter koseri*, Coagulase-negative *Staphylococcus* spp, *Comamonas acidovorans*, *Comamonas testosterone*, *Corynebacterium amycolatum*, *Corynebacterium* spp, *Enterobacter aerogenes*, *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterobacter sakazakii*, *Enterococcus casseliflavus*, *Enterococcus* spp, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* ssp *pneumoniae*, *Leuconostoc* spp,		

	Micrococcus spp, *Moraxella* spp, *Myroides* spp, *Neisseria* spp, *Ochrobacterium anthropic*, Opportunistic aerobic mesophilic *Bacillus* spp, *Paecilomyces lilacinus*, *Photobacterium damsel*, *Proteus* spp, *Providencia rettgeri*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas oryzihabitans*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Psychrobacter phenylpyruvicus*, *Ralstonia pickettii*, *Rhodococcus* spp, *Serratia fonticola*, *Serratia marcescens*, *Serratia rubidaea*, *Shewanella putrifaciens*, *Sphingobacterium multivorum*, *Stenotrophomonas maltophilia*, *Streptococcus* spp, *Streptomyces* spp,		

	Tsukamurella spp, *Yersinia frederiksenii*		

1.2.2 *Clostridium* spp associated with flying insects

There are only two references relating to insects being able to carry *Clostridium difficile*, the most important cause of healthcare-associated diarrhoea worldwide. The first record of *C. difficile* in insects was isolation from a laboratory strain of termites, *Coptotermes formosanus* (Taguchi *et al.*, 1993). The second refers to detection of *C. difficile* ribotype 078 from lesser houseflies, *Fannia canicularis* (some fruit flies *Drosophila melanogaster* and houseflies *M. domestica* were included in this sample) and drain flies, *Psychoda alternata* collected from pig farms (Burt *et al.*, 2012).

Greenberg (1971) and Greenberg (1973) review other *Clostridium* spp associated with flies. Some of the associations found were *Clostridium botulinum* with *Lucilia caesar* larvae on poultry farms and from bird carcasses, also *Cochliomyia macellaria* larvae from bird carcasses. These contaminated larvae were fed to healthy birds, which subsequently became infected with *C. botulinum*. Adults and larvae of the cheese skipper, *Piophila casei*, were readily contaminated with *C. botulinum*. *Clostridium chauvoei*, the causative agent of 'blackleg' infection in poultry can contaminate *M. domestica* when the fly feeds on an animal that has died of that disease. In the same study, dead *M. domestica* were still infective by inoculation one year after infection with *C. chauvoei*. *Clostridium welchii* (now *Clostridium perfringens*) has been found associated with blowflies (Family Calliphoridae) collected from butcher shops, fish shops and foods stores in the UK. *Clostridium* spp have also been found associated with the larvae of the non-biting midge, *Chironomus plumosus* (Rouf and Rigney, 1993).

Greenberg (1971) and Greenberg (1973) list further fly - *Clostridium* sp associations, presented in Table 1.3.

Table 1.3 *Clostridium* sp – fly associations

***Clostridium* sp – fly associations recorded by Greenberg (1971) and Greenberg (1973)**	
***Clostridium* spp**	**Fly Species**
Clostridium sp	*Musca domestica vicina*, *Musca sorbens*,
Clostridium bifermentans	*Musca domestica*, *Piophila casei*, *Stomoxys calcitrans*, *Lucilia* sp, *Lucilia caesar*, *Sarcophaga* sp
Clostridium botulinum	*Eristalis* sp, *Lucilia caesar*, *Phaenicia sericata*
Clostridium chauvoei	*Musca domestica*
Clostridium parabotulinum bovis	*Musca domestica*, *Chrysoma albiceps*, *Chrysoma chloropyga*, *Chrysoma marginalis*
Clostridium perfringens (synonym *Clostridium welchii*)	*Phormia* sp, *Protophormia terraenovae*, *Lucilia* sp, *Phaenicia sericata*, *Calliphora* sp, *Sarcophaga* sp
Clostridium putrefaciens	*Musca domestica*
Clostridium tetani	*Musca domestica*

Although *C. difficile* has not previously been isolated from flying insects in hospitals, the fact that this and a number of species of the same genus have been recorded in association with insects in other settings suggests that there is potential for insects to be mechanical vectors of *C difficile* in a clinical setting.

1.2.3 *Clostridium* spp associated with insects in hospitals.

There appears to be only one reference to *Clostridium* spp associated with insects in hospitals. A UK study found *Clostridium welchii* (now *Clostridium perfringens*) associated with pharaoh ants

(*Monomorium pharaonis*) collected from a hospital kitchen and *Clostridium cochlearium* from ants of the same species collected from washrooms and toilets (Beatson, 1972). Although *C difficile* has not previously been isolated from insects in hospitals, the fact that *Clostridium* species have been isolated from *M. pharaonis* in such premises suggests that there is potential for insects to be mechanical vectors of *C. difficile* in the hospital environment. *C. difficile* probably hasn't been identified previously in insects in hospitals because this organism only really came to the 'forefront' around 1978, when it was recognised as a causative agent of human diarrhoeal disease (Cookson, 2007). Prior to this, anaerobic microbiology techniques were not particularly well-developed in terms of dealing with *C. difficile*; another reason why it may have been overlooked previously. For example, the addition of sodium taurocholate to selective media in order to enhance *C. difficile* spore recovery was only reported in 1982 (Wilson *et al.*, 1982).

1.2.4 Gram-positive spore forming bacteria, other than *Clostridium* spp, associated with flying insects.

Certain bacteria of the genus *Bacillus* share some common features with *C. difficile*, as they are Gram-positive rods and they form endospores. Evidence shows that *Bacillus anthracis* is excreted in *M. domestica* flyspots and vegetative cells have been isolated from the fly gut, where bacterial replication appears to take place (Fasanella *et al.*, 2010). As houseflies are able to harbour *Bacillus anthracis* spores, they may also be able to harbour spores of *C difficile*.

Earlier experiments have described the recovery of *Bacillus anthracis* spores from legs, tarsi and vomitus of *M. domestica* post-feeding (Graham-Smith, 1914). Vegetative cells were also recovered from the legs and tarsi of *M. domestica* after feeding experiments (Graham-Smith, 1914). The fact that spores were recovered from fly vomitus suggests ingestion and subsequent ejection of spores.

Other studies have shown that houseflies and bluebottles retained *Bacillus atrophaeus* spores after exposure to inoculated food (Torres, 2006) and that *Bacillus sp* has been found associated with vomitus and faeces of houseflies collected from food factories and restaurants (Nazni *et al.*, 2005). These findings illustrate the potential role of flies in the mechanical transmission of Gram-positive, rod-shaped, spore-forming bacteria. This suggests that such insects are potential candidates for the mechanical transmission of *C. difficile* in the hospital environment, especially as the spore-forming *Bacillus cereus* has been isolated from *M. domestica* collected from two hospitals in Nigeria (Adeyemi and Dipeolu, 1984).

1.3 *M. domestica* in the hospital environment

Previous studies investigating *M. domestica* sampled from hospitals have shown that the flies which were collected harboured pathogenic bacteria, including (as mentioned above) *Bacillus* spp from hospitals in Nigeria, (Adeyemi and Dipeolu, 1984), *Escherichia coli* (Fotedar *et al.*, 1992b) and antimicrobial resistant *Klebsiella pneumoniae* (Fotedar *et al.*, 1992a) from a hospital in New Delhi, India, Methicillin resistant *Staphylococcus aureus* (MRSA) from a hospital in Libya (Rahuma *et al.*, 2005), MRSA with a sensitivity profile and phenotype of resistance identical to patients, from a hospital in Senegal (Boulesteix *et al.*, 2005) and *Salmonella* sp from a hospital in Nigeria (Nmorsi *et al.*, 2007). More recent studies have highlighted even more bacterial species associated with *M. domestica* in a hospital in India (Gupta *et al.*, 2012) and the moth fly *Clogmia albipunctata* from four German hospitals (Faulde and Spiesberger, 2013), the details of which are listed in Table 1.2. It is possible that the above locations are at a greater risk of experiencing fly ingress compared to UK hospitals. These locations will experience higher temperatures and therefore a longer outdoor breeding season than those in the UK, allowing faster development of houseflies and a greater number of generations per annum to occur.

In the study by Adeyemi and Dipeolu (1984), the location of the hospitals appeared to have an important impact on the bacterial carriage of *M. domestica*. A University Teaching Hospital with high standards of hygiene and a State Hospital located in the slums where unsanitary conditions prevailed were both sampled for flies. Consistently greater quantities of bacteria were isolated from houseflies sampled from the State Hospital compared to the University Teaching Hospital, suggesting that bacterial carriage by the housefly is indicative of the fly's habitat. Similarly, the bacterial species carried by *C. albipunctata* sampled from four German hospitals were representative of the bacteria isolated from the fly breeding sites, which were shower cubicles, patient wards, rest rooms and hospital kitchens (Faulde and Spiesberger, 2013).

1.3.1 Clinical significance of bacteria associated with flying insects

Not all bacteria found associated with flying insects in hospitals are of clinical significance. The bacteria considered to be human pathogens are described below and their significance and drug resistance status are discussed.

Flies may be able to transmit or become contaminated with pathogenic medically-important bacteria from the hospital environment, for example, Group A Beta-haemolytic streptococci (*Streptococcus pyogenes*) cultured from flies were of the same type (type 4), as those found in a nurse and patients wound and throat infections, (Shooter and Waterworth, 1944). A study in a hospital in India

confirmed that most of the bacteria isolated from houseflies are medically important (Fotedar *et al.*, 1992b). It was hypothesized that *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from the houseflies were probably obtained from accessing infected wounds of patients or associated dressings. *Klebsiella* spp isolated from hospital houseflies showed multiple drug resistance at a significant level, when compared to control houseflies sampled from a residential area 5 km away from the hospital (Fotedar *et al.*, 1992b). It was suggested that houseflies acquired antimicrobial resistant strains of *Klebsiella* spp associated with patients.

Enterobacter spp., *Klebsiella* spp., *Citrobacter* spp, *Staphylococcus* spp and *Enterococcus* spp. associated with houseflies in hospitals also exhibited multiple antimicrobial resistance (Sramova *et al.*, 1992). Enterobacteriaceae isolated from hospital houseflies were shown to be significantly resistant to some common antibiotics and isolates of *Staphylococcus aureus* were resistant to Methicillin (Rahuma *et al.*, 2005). Methicillin-Resistant *Staphylococcus aureus* (MRSA) with a sensitivity profile and phenotype of resistance identical to patients was isolated from houseflies collected from a hospital in Senegal (Boulesteix *et al.*, 2005). Multidrug resistant bacteria been have isolated from hospital houseflies *M. domestica*, including *E. coli*, *Streptococcus* spp, *Serratia* spp, *Proteus* spp and *Klebsiella* spp (Nmorsi *et al.*, 2007). In summary and quoting an important review paper, 'houseflies in hospital environments are vectors of multiple antibiotic-resistant strains of pathogenic bacteria' (Graczyk *et al.*, 2001).

Apart from work on houseflies, little research has been done on the bacteria associated with other fly species that are found in hospitals. Fruit flies, *Drosophila* sp sampled from a hospital in Nigeria were found to harbour *Proteus* sp, *Streptococcus* sp and *Salmonella* sp (Nmorsi *et al.*, 2007), all of which may cause infection in compromised patients. Cluster flies, *Pollenia rudis* sampled from a hospital in Germany were found to harbour opportunistic pathogens such as *Pseudomonas aeruginosa* and *Erwinia* spp which are also known as *Pantoea* spp (Faulde *et al.*, 2001). Cluster flies form aggregations of thousands of individuals and their presence in hospitals in such great numbers may present a risk to health when considering the opportunistic pathogens that these flies carry. In contrast with houseflies, other species of flies collected from hospitals are not as well-known for the carriage of multi-drug resistant bacteria - *C. albipunctata* was positive for many species of Enterobacteriaceae but none of these were multi-drug resistant and only *Stenotrophomonas maltophilia* exhibited resistance (Faulde and Spiesberger, 2013).

Regarding the acquisition of antibiotic resistant bacteria, there is evidence that flies obtain such microorganisms from animal faeces and transmit these to new substrates, even showing horizontal transfer of resistance genes between different species of bacteria carried within the fly gut (Zurek and Ghosh, 2014).

1.4 Fly control and disease - intervention studies

While there is a lack of direct evidence of the role of flies in disease transmission to humans, there is a wealth of indirect evidence in the form of intervention studies. The lack of direct evidence is understandable – the final experiment of infecting a human volunteer via contaminated flies is unlikely to be viable on ethical grounds, although a study by Greenberg (1964), referred to previously, did show experimental transmission of *Salmonella* Typhimurium from an infected dog to human volunteers via flies.

A number of studies have shown that reducing fly numbers by undertaking fly control measures reduces disease incidence. Levine and Levine (1991) showed that a fly control programme which reduced fly density also cut the *Shigella* incidence and subsequent human mortality in treatment areas. The effect of housefly control on diarrhoeal diseases has been evaluated and it was discovered that the incidence of diarrhoea and shigellosis decreased as fly counts declined (Cohen *et al.*, 1991). Likewise, after application of insecticides had effectively eliminated fly populations in treatment villages the incidence of diarrhoea fell (Chavasse *et al.*, 1999). In another similar study it was shown that a decrease in muscid fly numbers as a result of effective control led to fewer Trachoma *Chlamydia trachomatis* cases in the fly control areas (Emerson *et al.*, 1999). In contrast to these studies, (Allen *et al.*, 2004) showed that fly control may not always result in a major effect on disease incidence when they showed that there was no significant difference between control and treatment (insecticide use) groups in terms of human infection with *Helicobacter pylori*, in the Gambia.

1.4.1 Fly control and disease - Electronic Fly Killers

Electronic Fly Killers (EFKs) and professional sticky traps are used as a component of integrated flying insect control in UK hospitals. Research suggests EFKs and the flying insects captured by them are a potential source of bacterial contamination of the local environment. The spread of bacteria and a bacterial virus during electrocution of houseflies has been quantified (Urban and Broce, 2000). Flies were loaded with *Serratia marcescens* or with the *Escherichia coli* phage FX174. Flies sprayed with inoculum released one of every 10,000 of the added bacteria or viruses and fed flies released one of every 1,000,000 of the consumed bacteria or viruses. Results of the study suggested EFKs could play a role in the spread of infectious disease microorganisms but the potential was influenced by the insect's route of contamination. More microorganisms were released from surface-contaminated flies than from fed flies. Most microorganisms were detected in the Petri dishes immediately under the EFKs. Many bacteria and phages survived in or on the corpses of electrocuted flies. Each dead fly was potentially almost as contaminated as it was when it was alive.

The survival of *Serratia marcescens* (as a representative of the enteric bacteria) within houseflies following their electrocution by an EFK has been determined (Cooke *et al.*, 2003). *S. marcescens* was successfully ingested by houseflies and survived on and within the corpses after electrocution for up to five weeks. The highest levels of bacteria were recovered 24 hours post-electrocution. It was concluded that fly corpses provided a favourable environment for bacterial multiplication.

In summary, EFKs, while being an effective component of integrated flying insect control, can represent a dissemination risk for bacteria carried by flies. It is for this reason that EFKs designed to reduce the dissemination risk, such as those that minimise insect 'shatter' upon electrocution should be selected for pest control, while professional sticky traps which retain insect fragments (due to the glueboard) should be used in food preparation / production / storage areas (Killgerm, 2013). Regular maintenance of EKFs and professional sticky traps should be undertaken in terms of insects being removed from the units and surfaces wiped down with an appropriate disinfectant, in order to minimise the risk of dissemination of bacteria (Killgerm, 2013).

1.4.2 Flies in 'risk factor' studies

Further evidence for the significance of flies in disease transmission is provided by 'risk factor' studies. Knight *et al.* (1992) identified flies (houseflies and blowflies) as a risk factor for acute diarrhoea in Malaysian children. Households that did not use fly covers to protect stored food were twice as likely to be 'case' households. The risk factor attributed to flies was almost equivalent to that of the children's carers not washing their hands. Sengupta *et al.* (1995) recovered *Vibrio cholerae* O139 from flies (houseflies and blowflies) found associated with families of patients hospitalised due to cholera infection. The level of recovery of *Vibrio cholerae* from flies was comparable to the level of recovery from the washings of the hands of contacts of the index cases.

It is accepted that thorough hand-washing is an essential component of infection management (Coia *et al.*, 2006, HPA, 2009) and the risk factor studies by Knight *et al.* (1992) and Sengupta *et al.* (1995) suggest that the failure to control flies had a risk comparable to that associated with a lack of hand-washing.

1.4.3 Pest control in hospitals

Baker (1982) reviewed the issue of pests in hospitals, concentrating on cockroaches, Pharaoh ants, feral cats and birds. Also discussed were the conditions present within hospitals which were conducive to pest activity. In 1985, Jonathan Peck, Managing Director of Killgerm, reviewed pest control problems in NHS hospitals, discussing the public health risks of pathogenic bacteria

associated with pests of hospitals. Peck (1985) listed bacteria isolated from cockroaches from five London hospitals; *Citrobacter* spp. *Enterobacter* spp, *E. coli*, *Klebsiella pneumoniae*, *Proteus* spp, *Pseudomonas aeruginosa* and *Serratia marcescens*. Small birds, especially sparrows were implicated in a case of *Salmonella* Typhimurium phage type 160 in a patient in a mental hospital, with the organism being isolated from both the patient and the birds. Pharaoh ants infesting hospitals have been shown to be carrying *Salmonella* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Streptococcus* spp, *Klebsiella* spp and *Clostridium* spp. Robinson (1988) discussed the factors resulting in what at the time was an unsatisfactory situation regarding pest control in UK hospitals. Cockroach, rodent and Pharaoh ant problems were a concern and these problems were attributed to poor management of pest control in hospitals.

The Department of Health and Social Security (DHSS) issued advice notes on 'pest control contract management' and 'an introduction to pest control in hospitals', which attempt to address some of the issues raised in the papers above.

Murphy and Oldbury (1996) describe the role of Environmental Health departments in pest control in hospitals, particularly the enforcement of legislation facilitating control of pests, servicing of pest control contracts, survey results of pest control in hospitals and subsequent recommendations. The removal of Crown Immunity to allow Local Authority Environmental Health departments to enforce legislation, which facilitates the control of pests in hospitals, has been attributed as a major contribution to protecting public health in hospitals (Murphy and Oldbury, 1996). More recent documents exist to facilitate pest control in National Health Service (NHS) hospitals - the 'NHS conditions of contract for pest control' specify terms for the riddance of pests from hospitals (NHS, 2007).

Pest control in hospitals is of importance in other countries and (Gliniewicz *et al.*, 2006) assessed the levels of pest infestations of hospitals in Poland. The most prevalent pests were cockroaches and Pharaoh ants. Pest control measures were less reliant on the more traditional insecticides such as pyrethroids and carbamates, with a shift towards the use of alternative methods such as baits, traps and gel baits containing hydramethylnon or imidacloprid. The same study noted an increase in flies in hospitals, from 6.8% of hospitals reporting flies from a 1990 to 1995 survey, to 35.2% of hospitals reporting flies in a 2003/04 survey.

1.5 *Clostridium difficile*

This research focuses on the highly-infectious healthcare-associated pathogen *C. difficile*, because it is the leading cause of nosocomial diarrhoea worldwide, with serious implications in that it can result in

the isolation of patients, closure of wards and hospitals and even the death of infected individuals (Dawson *et al.*, 2009). *C. difficile* infection (CDI) typically affects elderly patients on antibiotics (e.g. Beta-Lactams, clindamycin), causing severe disease such as pseudomembranous colitis (PMC) via toxins that affect intestinal cells (Schroeder, 2005), with infections contributing to deaths in England and Wales that have peaked at over 8,000 per annum (ONS, 2013).

C. difficile is a slender Gram-positive, anaerobic, spore-forming, rod-shaped, motile, pathogenic bacterium present in the hospital environment (Dawson *et al.*, 2009) belonging to the family Clostridaceae, genus *Clostridium* (Cowan *et al.*, 2003) and potentially could be mechanically transferred by *M. domestica. C. difficile* vegetative cells measure 0.5 to 1.9 by 3.0 to 16.9μm, forming oval subterminal spores (Hatheway, 1990). The first isolation of *C. difficile* was in 1935 (it was originally named *Bacillus difficilis*) from infant stools and was only recognised as a causal agent of diarrhoeal disease in humans by 1978, with the name referring to the difficulties of culturing it (Cookson, 2007). When cultured successfully, by incubation for 48 hours at 37°C in anaerobic conditions, *C. difficile* produces a distinctive 'farmyard' smell, due to it producing the metabolic products iso-valeric acid, iso-caproic acid and p-cresol (Levett, 1984), with colonies that are glossy, grey, circular, with a rough edge and are usually non-haemolytic (HPA, 2011c).

There are more than 100 species of *Clostridia*, 13 of which show pathogenicity to humans (Dupuy *et al.*, 2006). The pathogenicity of *Clostridia* is linked to toxin production and bacteria of this genus produce the greatest number of toxins compared to any other genus and it is these toxins that are the key root of their pathology (Johnson, 1999). In order for *C. difficile* to act as a pathogen, vegetative cells must germinate from ingested spores that have survived the acidic conditions in the human gut (Giannasca and Warny, 2004) and been stimulated to undergo germination by bile salts (Jump *et al.*, 2007). Vegetative *C. difficile* cells, which can survive on moist surfaces for a number of hours, are passed out in the faeces of infected individuals and subsequently die or form spores on exposure to air (Jump *et al.*, 2007). The spores are the main transmissible form of the bacteria and can persist in the environment for a long period of time (Dawson *et al.*, 2009). The spores are resistant to most disinfectants and alcohol hand gels (HPA, 2009), so sporicidal agents such as bleach are required to eliminate them from the environment (Wheeldon *et al.*, 2008b).

1.5.1 Cases of *C. difficile* infections and related deaths

Cases of *C. difficile* infections reported to the Health Protection Agency from 1990 to 2005 rose from fewer than 5,000 per year to over 45,000 per year (HPA, 2009). Since 2005 there has been a decrease in the number of infections, with increases in CDI since 2003 reported to be due to the more virulent 027 strain (HPA, 2009). Although recent decreases in CDI have been reported (most likely due to

improved cleaning and disinfection measures), incidences are unlikely to decline further, as broad-spectrum antibiotics continue to be used and the number of immunocompromised and elderly patients is rising (Dawson *et al.*, 2009).

C. difficile is a notifiable disease in the UK and from 1999 to 2012 in England and Wales there were 42,475 death certificates with *C. difficile* mentioned and 20,660 where the disease was identified as the underlying cause of death (ONS, 2013).

These figures are plotted in Figure 1.4 (ONS, 2013).

Figure 1.4 *C. difficile* related deaths in England and Wales from 1999 – 2012 (ONS, 2013)

1.5.2 Transmission of *C. difficile*

Infection is passed from person-to-person nosocomially via the faecal-oral route (Fordtran, 2006). *C. difficile* may be spread by the hands of hospital workers, patients and via faecal deposits of patients with *C. difficile* associated diarrhoea (CDAD). Environmental contamination of sites such as carpets, clothing, blood pressure cuffs, thermometers, telephones and commodes can occur (Fordtran, 2006). Hospital surfaces contaminated with *C. difficile* present an infection risk to patients, as do the hands of hospital staff, which are just as likely to become contaminated from contacting these surfaces as they are by directly touching infected patients (Rutala and Weber, 2013). In proximity to symptomatic

patients, there are other surfaces that can be contaminated with *C. difficile,* including beds, floor, tables, sinks, ward storeroom handles and bins (Best *et al.*, 2010). All of these surfaces are areas where flies could alight. In the same study, *C. difficile* was sampled from the air, suggesting airborne dispersal and it was concluded that 'recognition of the risk of airborne dissemination provides an opportunity to reduce transmission' (Best *et al.*, 2010).

If flies transfer *C. difficile*, appropriate terminology would be 'facilitated airborne dissemination'. While it is generally thought that *C. difficile* is commonly passed from person-to-person nosocomially via the faecal-oral route, other research shows that most new cases cannot be explained by contact with infected individuals and the main routes of transmission are unknown (Walker *et al.*, 2012). Flying insects such as *M. domestica* may be one of the 'unknown' routes of transmission.
Cases of community acquired *C. difficile* infection have occurred and this is defined as when the patient has not been in hospital before becoming infected or has become infected 12 weeks after leaving hospital (HPA, 2009). Wilcox *et al.* (2008) reported that approximately a third of cases of community acquired *C. difficile* infection were not associated with key risk factors, such as antibiotic use and hospitalisation, suggesting that other risk factors should be explored. Flies could be one of the 'unknown' risk factors for community acquired *C. difficile* infection.

1.5.3 Prevention of *C. difficile*

It is accepted that thorough hand-washing by hospital staff is an essential component of *C. difficile* infection management, as well as the use of disposable gloves and aprons when caring for patients (HPA, 2009).

Cleaning and disinfection measures are a recommended technique for the prevention and management of *C. difficile* infection (HPA, 2009) and as spores are resistant to most disinfectants, sporicidal agents such as bleach are required to eliminate them from the environment (Wheeldon *et al.*, 2008b). Specifically, the Department of Health (DoH) recommend that daily application of a chlorine-based disinfectant (minimum 1,000ppm) is required to disinfect hard surfaces of rooms that hospitalised *C. difficile* patients have resided in (HPA, 2009). Once the *C. difficile* patient has left the room permanently, the mattress, bed linen and curtains should be replaced (HPA, 2009). Apart from disinfection, the use of copper surfaces has shown potential as a preventative measure and *C. difficile* spores can be killed on such surfaces when exposed to a germinant solution under aerobic conditions (Wheeldon *et al.*, 2008c).

An important preventative measure regarding *C. difficile* infection is the restriction of antibiotic prescription, with judicious use of antibiotics such as clindamycin having resulted in a decrease in the

number *C. difficile* infections (Climo *et al.*, 1998). Furthermore, restriction not just of the use of antibiotics but of the type used has also had an impact, as a reduction in broad spectrum antibiotic use compared to the use of more specific narrow-spectrum antibiotics has reduced *C. difficile* infections (McNulty *et al.*, 1997).

1.5.4 *Clostridium difficile* infection (CDI)

C. difficile is one of the main causes of nosocomial diarrhoea in hospitals and is sometimes the most common pathogen isolated from stools (Barbut and Petit, 2001). However, interpretation of figures regarding the detection of *C. difficile* must take into account the fact that it is carried asymptomatically in less than 3% of healthy adults and up to 70% of healthy neonates in some cases (Barbut and Petit, 2001).

When *C. difficile* causes human infection, typically when patients have been in hospital and received antibiotic therapy, a number of conditions can result. For example, the most virulent *C. difficile* strain 027, produces A and B toxins, causing diarrhoea and pseudomembranous colitis (Dawson *et al.*, 2009). *Clostridium difficile infection* (CDI) occurs when antibiotic use has disrupted the gut flora, which allows the growth of *C. difficile*, resulting in diarrhoea and associated symptoms such as nausea, abdominal pain and fever (Fordtran, 2006).

Pseudomembranous colitis (PMC) is a complication of CDI in 90% of cases (Surawicz and McFarland, 1999). PMC symptoms are similar to CDI but more severe and include severe abdominal pain, diarrhoea that is profuse and watery, sometimes including fever, as well as a tender and swollen abdomen (Kelly *et al.*, 1994). PMC is also characterised by the occurrence of yellow pseudomembranous plaques in the colon, which are made up of necrotic tissue, fibrin and inflammatory cells (Kelly *et al.*, 1994).

Another condition that occurs in CDI (1-3% of cases) is fulminant colitis (Kelly *et al.*, 1994), which is where patients experience severe illness with distension of the abdomen, abdominal pain and fever and are at risk of death without surgical intervention due to the high mortality rate of this condition (Fordtran, 2006, Schroeder, 2005). Toxic megacolon can also occur, which is a severe life-threatening illness and is characterised by dilation of the colon, with associated distension of the abdomen, tenderness of the abdomen and a risk of colonic perforation or peritonitis that can be fatal if not treated quickly (Kelly *et al.*, 1994).

Extraintestinal infections with *C. difficile* are uncommon, with infection sites including areas close to the colon (suggesting faecal contamination), such as abdominal abscesses, abdominal wounds and

peritonitis (Garcia-Lechuz *et al.*, 2001). Some cases of extraintestinal *C. difficile* infection are found away from the colon, including brain abscesses, bacteraemia and foot infections (Garcia-Lechuz *et al.*, 2001). In most cases of extraintestinal *C. difficile* infection, other bacteria were found as part of the infection (polymicrobial infection), there was no antimicrobial therapy before infection, diarrhoea was not noted and strains were non-toxigenic (Garcia-Lechuz *et al.*, 2001).

1.5.5 Risk factors for CDI

Clostridium difficile infection typically infects the elderly or individuals whose gut flora has been disturbed as a result of antibiotic use (Schroeder, 2005), with the infection flourishing under the selective pressure of antibiotics (Fordtran, 2006). In fact, prior treatment with antibiotics, particularly broad-spectrum antibiotics, is considered to be the most important risk factor for CDI (over 90% of CDI cases are associated with antibiotic use) and many antibiotics have been implicated, including clindamycin, third-generation cephalosporins and flouroquinolones (Bartlett, 2006). As described in section 1.5.3, restriction of antibiotic use has resulted in fewer cases of CDI (Climo *et al.*, 1998). Antibiotic use is such an important risk-factor because antibiotics disturb the normal gut flora by eliminating many of the commensal gut bacteria, reducing colonisation resistance and permitting establishment and proliferation by opportunistic *C. difficile* in the colon (Barbut and Petit, 2001).

Although conflicting results exist, some authors report that proton pump inhibitors (PPIs) are a risk factor for CDI infection (Jump *et al.*, 2007). This is because PPIs cause reduced gastric acid production, which while not likely to impact on acid-resistant *C. difficile* spores, may allow greater survival of vegetative cells which could have been caused to germinate by the presence of bile salts in the stomach (Jump *et al.*, 2007).

Age is a risk factor for CDI, with those under 2 years of age (Al-Jumaili *et al.*, 1984) and those over 65 years of age being at the greatest risk (Barbut and Petit, 2001), although this factor should be considered in combination with other risk factors, such as antibiotic use and being admitted to hospital, as this age group typically shows weakened immune responses, has underlying illness and is more likely to be prescribed antibiotics and also to receive treatment in hospital (Fordtran, 2006). Regarding the risk factor of hospital admission, CDI or *C. difficile* colonisation is more likely in patients staying in hospital (Barbut and Petit, 2001) and the length of stay also has an influence, with isolation rates being greater the longer the hospital stay (Kuijper *et al.*, 2006).

Clostridium difficile infection is diagnosed by the presentation of clinical symptoms described in section 1.5.4, toxin testing of stool samples, endoscopy with evidence of PMC, white cell counts, serum creatinine levels and abdominal CT (computerised tomography) scanning if required (HPA, 2009). Culture of *C. difficile* from stool samples using selective media (e.g. CCFA, similar to that

described in section 2.3) is also used as a method of laboratory diagnosis, preferably in combination with toxin testing to improve detection (Delmee *et al.*, 2005). Molecular techniques such as PCR (polymerase chain reaction) ribotyping and optimized RAPD (random amplification of polymorphic DNA) protocols are used to identify different genetic types of clinical isolates of *C. difficile* (Green *et al.*, 2011).

1.5.6 Treatment of CDI

Treatment options have recently been reviewed and Public Health England (PHE) have issued updated guidelines (PHE, 2013). These guidelines recommend supportive care and attention to levels of hydration, electrolytes and nutrition, avoiding the use of antiperistaltic agents, stopping the use of the causative antibiotic if possible and replacing with a more suitable substitute (PHE, 2013). PHE also recommend that the use of PPIs should be stopped or their need reviewed where possible (PHE, 2013).

Whereas recent first-line treatment options for CDI were limited to metronizadole and vancomycin, due to resistance to commonly used antibiotics (Dawson *et al.*, 2009), current advice adds the use of fidaxomicin, which was approved for the treatment of CDI in Europe in 2012 (PHE, 2013). Generally, metronizadole is recommended for mild CDI and vancomycin or fidaxomicin for severe disease (PHE, 2013). Surgical procedures, such as colectomy, are required for patients with toxic megacolon, perforated colon or septic shock (PHE, 2013).

Treatment agents other than the recommended antibiotics were discussed in the PHE guidance (PHE, 2013). Studies on probiotics (*Saccharomyces boulardii* is an example) have failed to show statistical significance in their efficacy results for treatment or prevention of CDI (PHE, 2013). Use of intravenous immunoglobin and anion exchange resin are not recommended for CDI treatment, as insufficient evidence exists regarding efficacy (PHE, 2013). The use of non-toxigenic *C. difficile* (NTCD) is at the clinical trials stage (PHE, 2013) and evidence exists that an oral suspension of NTCD may prevent primary or recurring CDI (Villano *et al.*, 2012). A relatively new treatment is faecal transplant, a technique that has resolved 92% of recurring CDI cases (PHE, 2013). Fusidic acid has been compared to metronidazole but resistance problems mean that use will be limited (PHE, 2013). Rifampicin and rifamixin are not currently recommended for CDI treatment (PHE, 2013).

1.5.7 Potential for transmission of *C. difficile* by *M. domestica*

C. difficile can be excreted by a human patient at levels of 1×10^4 to 1×10^7 per gram of faeces (Mulligan *et al.*, 1979) and adult *M. domestica* are attracted to, land on, feed on and oviposit on

human faeces, upon which the resulting larvae feed and develop (West, 1951). It is well known that *M. domestica* visit faeces then become contaminated with bacteria, which they disseminate (Greenberg, 1964) and this process is likely to occur with *C. difficile* and result in mechanical transmission of this pathogen. Indeed, *C. difficile* has been isolated from fly species, which were collected on pig farms (Burt *et al.*, 2012) and this supports the assertion that *M. domestica* could become contaminated with *C. difficile* by interacting with 'infected' faecal matter and that *M. domestica* is an, as yet, unconsidered factor in the spread of *C. difficile* in the hospital setting.

It is accepted that thorough hand-washing is an essential component of *C. difficile* infection management (HPA, 2009). Risk factor studies on acute diarrhoea (Knight *et al.*, 1992) and *Vibrio cholerae* O139 (Sengupta *et al.*, 1995) suggest that failure to control flies has a risk comparable to that associated with a lack of hand-washing. The level of recovery of *V. cholerae* from flies can be as high as the level of recovery from the hands of people that have been in contact with cholera sufferers.

Although *C. difficile* has not previously been isolated from *M. domestica* or other flying insects in hospitals, there could be potential for flies to act as vectors in the hospital environment. The biology of *C. difficile* may present opportunity for mechanical transmission by *M. domestica*, which frequents faecal deposits and alights on surfaces, all of which are potential sources of *C. difficile* in hospitals. The current study examined the mechanical transfer, ingestion and faecal deposition of *C. difficile* by *M. domestica* in laboratory conditions.

1.6 Insect defences against bacterial infection

Insect defence mechanisms against bacterial infection, particularly any immune system defences in *M. domestica*, are important as they may affect carriage of *C. difficile*. The insect immune system, including that of *M. domestica*, may provide protection from infection with *C. difficile*, which may affect the ability of insects to transfer this bacterium mechanically.

Although the insect immune system does not have specific immunoglobulin-based memory, there are a number of defences against bacterial infection, the main mechanisms being the physical barrier provided by the cuticle and an immune system in the form of innate immune effector systems (Siva-Jothy *et al.*, 2005). Insect cuticle is not just a simple physical barrier however, as it also provides a biochemical barrier, showing antimicrobial activity in response to abrasion and bacterial challenge (Siva-Jothy *et al.*, 2005). The digestive system of insects has defences such as protective peritrophic membrane surrounding the otherwise relatively unprotected midgut, a cuticle lining of the foregut that can be shed when bacteria bind to it and a host of defence peptides, such as antimicrobial peptides and lysozymes (Siva-Jothy *et al.*, 2005). Insects also utilise the cytotoxic activity of nitric oxide (Schmid-

Hempel, 2005) and 'reactive oxygen species' to protect against bacterial infection (Whitten and Ratcliffe, 1999), as well as exhibiting other immune responses to bacterial challenge, such as phagocytosis (Ratcliffe and Rowley, 1974).

The antimicrobial peptides produced by insects are effective against bacteria, including Gram-positive bacteria (Siva-Jothy *et al.*, 2005). The Gram-positive *C. difficile* may be susceptible to destruction by insect defences because Gram-positive bacteria trigger the 'Spaetzle-Toll' pathway, resulting in the production of antimicrobial peptides, while lysozymes can digest cell walls of Gram-positive bacteria (Schmid-Hempel, 2005).

Antimicrobial peptides showing action against Gram-positive bacteria have been isolated from adult *M. domestica* (Wang *et al.*, 2006, Liang *et al.*, 2006). Although activity of fly (Order Diptera) antimicrobial peptides against *C. difficile* is unknown, *Clostridium perfringens* was shown to be resistant to cecropin (Moore *et al.*, 1996). Other antimicrobial peptides may offer protection against infection of flies by *C. difficile* as the growth of *Clostridium perfringens*, *Clostridium ramosum* and *Clostridium paraputrificum* is inhibited by the antimicrobial peptide sarcotoxin IA, which has been isolated from the flesh fly *Sarcophaga peregrina* and exists as a homologue in other insects (Mitsuhara, 2001). Although antimicrobial peptides with activity against *C. difficile* have not yet been observed in *M. domestica*, one called coprisin is present in the Korean dung beetle, *Copris tripartitus* and its antimicrobial activity is thought to take place by disrupting the membrane of *C. difficile* (Kang *et al.*, 2011). As antimicrobial peptides are widely conserved it insects, it is possible that peptides exist in *M. domestica* which are similar to coprisin and have an antimicrobial effect against *C. difficile* but have yet to be discovered, although it is expected that the highly resistant spores of *C. difficile* are likely to resist to degradation by such peptides.

1.7 The significance of bacteria (and some protozoa) in the immature stages of flies

The majority of studies relating to flies in hospitals refer to bacteria isolated from adult flies (Graczyk *et al.*, 2001). If flies are found to be breeding within the hospital environment then eggs, larvae and pupae of flies will be present as well as adults. Larvae are an active, feeding, mobile stage of the fly life cycle and could therefore present infection risks if they are acquiring bacteria from breeding sites that may be present in hospitals, such as excrement, rotting organic matter associated with drains, animal carcasses and food spillage. For example, housefly larvae have been shown to harbour *Staphylococcus aureus*, *Pseudomonas aeruginosa* and the Gram-positive spore-forming *Bacillus cereus* in their gut and on their external surfaces (Banjo *et al.*, 2005). Indeed, bacteria such as *E. coli* are required for the proper development of fly larvae, particularly aiding successful pupation and eclosion (Watson *et al.*, 1993).

Some evidence shows that the vast majority of larval gut microorganisms are destroyed during metamorphosis and that at the point of emergence, approximately 20% of adult houseflies are sterile (Greenberg, 1973) and although *Cryptosporidium parvum* oocysts have been detected on housefly larvae, pupae and adults, it is considered unlikely that oocysts would be transmitted from larvae to adults (Graczyk *et al.*, 1999). In contrast, a number of authors have shown that housefly larvae retain a considerable number of bacteria acquired at this stage, through to the pupal stage and finally adulthood (Glaser, 1923) and it is considered that retention of *E. coli* from larval to adult houseflies could play a role in the transmission and spread of *E. coli* (Rochon *et al.*, 2005).

1.7.1 Bacteria isolated from field-sampled *M. domestica* larvae

Some field studies have taken place, which have focused on isolating bacteria from housefly larvae collected from varied environments.

Providencia rettgeri has been isolated from the gut of housefly larvae collected from turkey bedding and corn silage (Zurek *et al.*, 2000). In the same study, two mammalian pathogens, *Yersinia pseudotuberculosis* and *Ochrobactrum anthropi*, were isolated from the gut of the housefly larvae. The full list of bacteria species isolated in the study is as follows; *Bacillus coagulans*, *Bacillus* sp, *Clavibacter michiganensis*, *Corynebacterium aquaticum*, *Corynebacterium seminale*, *Gordona amarae*, *Lactococcus garviae*, *Microbacterium barkeri*, *Microbacterium esteraromaticum*, *Microbacterium lacticum*, *Microbacterium liquefaciens*, *Morganella morganii*, *Ochrobactrum anthropi*, *Providencia rettgeri*, *Providencia stuartii*, *Serratia marcescens*, *Sphingobacterium spiritivorum*, *Sphingomonas capsulate*, *Staphylococcus epidermidis*, *Staphylococcus lentus*, *Streptococcus sanguis*, *Xanthobacter flavus* and *Yersinia pseudotuberculosis*. From this work, it is clear that housefly larvae can harbour many species of bacteria, some of which are human and animal pathogens.

There appears to be no evidence in the literature of *C. difficile* being isolated from housefly larvae (or from larvae of any flies) that have been collected from the field. However, other *Clostridium* species have been recovered from fly larvae. For example, *Clostridium* spp have been found on external surfaces and in the gut of non-biting midge larvae, *Chironomus plumosus*, which were sampled from mud dredged from Lake Winnebago (Rouf and Rigney, 1993). Greenberg (1971) and Greenberg (1973) review other *Clostridium* spp associated with flies. Some of the associations found were; *Clostridium botulinum* with *Lucilia caesar* larvae on poultry farms and from bird carcasses, also *Cochliomyia macellaria* larvae from bird carcasses. These contaminated larvae were fed to healthy birds, which subsequently became infected with *C. botulinum*.

1.7.2 Bacteria (and some protozoa) used to artificially contaminate *M. domestica* in controlled laboratory experiments

There appears to be no evidence in the literature of *C. difficile* being isolated from housefly larvae (or from larvae of any flies) in controlled laboratory experiments. Experimental work does exist, however, showing that fly larvae can become contaminated with other bacteria, following artificial feeding experiments. In particular, populations of *E. coli* can be experimentally introduced into housefly larvae via ingestion (Rochon *et al.*, 2004, Rochon *et al.*, 2005).

While there appears to be no research regarding *C. difficile* and fly larvae, experimental observations have been made showing that housefly larvae can become contaminated with other Gram-positive, spore-forming bacteria such as *Bacillus anthracis* (Graham-Smith, 1914).

Some protozoan parasites of humans have been isolated from housefly larvae in controlled laboratory experiments. Although Protozoa are very different to bacteria, protozoan oocysts and bacterial spores share some common features, as they can survive for extended periods of time in unfavourable conditions and have a tough outer wall or 'cortex'. *Cryptosporidium parvum* oocysts have been detected in the housefly larval gut and on external surfaces of larvae, after experimental exposure (Graczyk *et al.*, 1999). Approximately 150 oocysts per larva were recovered from the external surfaces in the same study. Also, *Toxoplasma gondii* oocysts have been isolated from housefly larvae and blowfly (*Chrysoma megacephala*) larvae that were reared in experimentally infected cat faeces (Wallace, 1971).

1.7.3 Persistence of bacteria (and some protozoa) in housefly larvae

As the fact that housefly larvae (and other fly larvae) can harbour pathogenic bacteria is clearly established, persistence of bacteria in the immature stages of flies becomes important, especially when considering whether these pathogens can survive through the full cycle of fly development and retain their infectivity.

It has been shown that *E. coli* can persist for a number of hours in fly larvae - the level of *E. coli* in housefly larvae declined up to 48 hours after ingestion but remained constant in stable fly larvae *Stomoxys calcitrans* during this period (Rochon *et al.*, 2004). The amount of *E. coli* in stable fly larvae increased regardless of the different concentrations used in the feeding inocula. In housefly larvae, however, the abundance of *E. coli* only increased when the larvae were exposed to a feeding inoculum with a low concentration of bacteria. When a higher concentration of bacteria was used in the feeding inoculum, the level of *E. coli* in housefly larvae decreased.

Cryptosporidium parvum oocysts can persist unchanged in housefly larvae and thus retain infectivity. This is known because the recovered oocysts had cellular morphology similar to the infectious oocysts in the bovine faeces inoculum (Graczyk *et al.*, 1999).

1.7.4 Requirement for bacteria in the larval diet

With the presence and persistence of bacteria in housefly larvae confirmed, the survival of 'infected' fly larvae is important if bacteria are to survive through the fly life cycle. Research has shown that fly larvae do actually require some bacteria in the larval diet. These bacteria improve the survival rate of the flies. On average, 62% of housefly larvae and 25% of stable fly larvae were able to survive when fed on pure cultures of *E. coli*, which may suggest that houseflies digest *E. coli* more readily and utilise it as a food source (Rochon *et al.*, 2004).

Housefly larvae survive and develop through pupation and subsequent eclosion more successfully on growth media that has been inoculated with *Escherichia coli*, than without bacteria (Watson *et al.*, 1993). Larval survival was only 4% without *E. coli* incorporated into growth media. Housefly pupation and eclosion were significantly higher (72% and 63% respectively) on egg yolk media that had been inoculated with *E. coli*, when compared to other larval growth media that lacked this bacterial supplement.

1.7.5 Bacteria (and some protozoa) in the pupal stage

When *E. coli* is fed experimentally to housefly and stable fly larvae, the bacteria persist through the pupal stage (Rochon *et al.*, 2005). The *E. coli* population increased in the early stages of pupal development, before declining prior to emergence of adult houseflies. In stable flies, the *E. coli* population increased and remained at a high level during pupal development. The fly pupal cases (puparia) were also examined for the presence of *E. coli* and the shed puparia of the stable fly usually contained more of the bacteria than that of the housefly. All housefly puparia were positive for *E. coli*.

Housefly pupae that developed from larvae reared in a bovine faeces and *Cryptosporidium parvum* medium were externally contaminated with up to 320 oocysts per pupa. Pupae that were externally washed to remove *C. parvum* oocysts were then crushed and 70% of them found to harbour oocysts, with figures ranging from 10 to 94 per pupa. As the *C. parvum* oocysts were found in crushed pupae that had previously been washed externally, it was suggested that the fly larvae had ingested the oocysts.

T. gondii oocysts have also been isolated from housefly and blowfly (*Chrysoma megacephala*) pupae that were reared from larvae in experimentally infected cat faeces (Wallace, 1971).

1.7.6 Retention of bacteria (and some protozoa) into the imago

Few studies have examined the retention of bacteria acquired by the larval housefly, through the pupal stage and finally into the imago (adult). Most work has focused on bacteria acquired by adult flies. This may be because work by early researchers appeared to show that levels of bacteria decline through metamorphosis. The review by Greenberg and Klowden (1972) describes much of this early research. From the mature larval stage to the prepupa stage of houseflies, there is a greater than 90% reduction in numbers of bacteria, mainly due to feeding ceasing and evacuation of bacteria from the larval gut continuing. A further reduction in the number of bacteria occurs when the larval foregut and hindgut are shed during pupation and become deposited in the puparium upon adult fly emergence. It is also thought that destruction and synthesis of structures and general reorganisation of tissues in metamorphosis probably reduces the amount of bacteria present. These factors all contribute to 17% of houseflies being sterile upon emergence.

Competition with normal housefly gut flora appears to be a main reason why experimentally introduced bacteria are not retained during metamorphosis. Work by Greenberg, referred to in the aforementioned review by Greenberg and Klowden (1972), confirms the studies of earlier researchers that bacteria such as *Salmonella* and *Shigella*, when introduced to larvae with normal gut flora, are unable to survive pupation and were not isolated from any adults that emerged. In the same experiment, the introduced bacteria could not even be isolated from the majority of the fly larvae. Only by using aseptic rearing techniques and gnotobiotic flies can experimentally introduced bacteria at the larval stage be recovered from pupae and adults. Even then, although the bacteria survived metamorphosis, there was still a reduction in numbers. The conclusion of the review by Greenberg and Klowden (1972) was ‘the adult fly has the most potential for disease transmission, as the maggot has limited motility and possesses autosterilization mechanisms which limit its capacity to carry pathogens over into the adult stage’.

Of the 'autosterilization mechanisms' referred to by Greenberg and Klowden (1972), secretions of fly larvae and specifically antibacterial peptides from *M. domestica* and *Lucilia sericata* have been shown to be active against bacteria (Wang *et al.*, 2006, Liang *et al.*, 2006, Ratcliffe *et al.*, 2011). Larval secretions of the blowfly *L. sericata* have a bactericidal property against *Staphylococcus aureus*, Haemolytic streptococci and *Clostridium perfringens* (Simmons, 1935). Cecropin, an antibacterial peptide, present in larvae and pupae of the blowfly / ‘bluebottle’ *Calliphora vicina*, was at its highest levels in individuals that had just pupated and they showed the greatest immune response

to *E. coli* challenge at this life stage (Crowley and Houck, 2002). An antibacterial peptide, seraticin, has been extracted from larvae of *L. sericata* and shows activity against MRSA and *C. difficile* as well as a range of Gram-positive (e.g. *Bacillus cereus* and Gram-negative (e.g. *E. coli*) bacteria (Bexfield *et al.*, 2008). It is possible that the presence of antibacterial peptides in *M. domestica* larvae could be influential in the lack of retention of *C. difficile* through metamorphosis and although yet to be discovered in *M. domestica*, some insects (the Korean dung beetle, *Copris tripartitus*) do possess antimicrobial peptides (coprisin) with activity against *C. difficile* (Kang *et al.*, 2011) and a hybrid of the insect antimicrobial peptides cecropin and melittin showed inhibitory action against *C. difficile* (Edlund *et al.*, 1998).

More recent work provides a different view that *E. coli* experimentally introduced to housefly larvae survives metamorphosis and can be isolated from the external surfaces of 72% of emerged adult houseflies and the internal structures of 66% (Rochon *et al.*, 2005). *E. coli* also persisted through the adult stage of stable flies, with 29% of the flies being positive for the bacteria on their external surfaces and 27% within their internal structures. With the presentation of this newer evidence, it is possible that the spread and transmission of *E. coli* by flies could be influenced by acquisition in the larval stage and subsequent retention of the bacteria through metamorphosis into the pupal and adult stages.

Vibrio cholerae is another species of bacteria that has recently been shown to be capable of surviving through fly metamorphosis. Larvae of the non-biting midge, family Chironomidae, were experimentally exposed to *V. cholerae* while in flasks of water and the flying adults that emerged were caught and found to be positive for the bacteria (Broza *et al.*, 2005).

Human protozoan parasites are not retained into the fly adult after metamorphosis. This may seem surprising, as oocysts are hardy, thick-walled structures that are capable of surviving in the environment for long periods of time. Although *T. gondii* oocysts have been isolated from housefly and blowfly *Chrysoma megacephala* larvae and pupae that were reared in experimentally infected cat faeces, none were recovered from newly emerged adult houseflies (Wallace, 1971). It is also deemed unlikely that *Cryptosporidium parvum* oocysts are passed from fly larvae through to the adult stage (Graczyk *et al.*, 1999).

Like oocysts, bacterial spores are hardy and able to survive in the environment for long periods of time, so they may be more likely candidates for successful survival through fly metamorphosis, compared to vegetative cells. Indeed, research shows that the ability or inability of bacteria to form spores has a bearing on their survival through the stages of fly metamorphosis. Non-spore-forming bacteria, such as *Bacillus typhosus, B. enteritidis* and *B. dysenteriae* are not found in association with adult flies derived from larvae experimentally exposed to these species but the spore-forming *Bacillus*

anthracis does survive (Graham-Smith, 1914). As research shows that Gram-positive spore-forming rod-shaped bacteria such as *Bacillus anthracis* can be acquired by fly larvae, retained through metamorphosis and isolated from adult flies, it is hypothesised that the same may also hold true for the Gram-positive spore-forming rod-shaped *C. difficile*.

1.8 Veterinary significance of *M. domestica* and *C. difficile* interaction

As *M. domestica* is often the most common fly in human occupied premises (Mallis, 1964) and can disperse for a number of miles (Greenberg, 1973, Busvine, 1980), it may also have the potential to be involved in community-associated *C. difficile* cases.

C. difficile infection can be of veterinary concern and has been reported in horses, ostriches, companion animals, calves, pigs (Songer, 2004) and poultry (Zidaric *et al.*, 2008). There is also an overlap between *C. difficile* types present in animals and humans, with identical isolates of type 078 increasingly encountered in pigs and humans (Debast *et al.*, 2009). It is possible that because of their synanthropy and the fact that they can develop in human, calf, horse, pig, poultry and other animal faeces (Busvine, 1980), *M. domestica* may have a role to play in the potential interspecies transmission of *C. difficile*. This potential has been shown in other flies, such as lesser houseflies, *Fannia canicularis* (some *Drosophila melanogaster* and *M. domestica* were included in this sample) and drain flies *Psychoda alternata*, which have been collected from pig farms and been shown to be positive for *C. difficile* type 078 (Burt *et al.*, 2012). It is possible therefore, that flies may act as mechanical vectors of *C. difficile* and transfer it into and even out of hospitals.

1.9 Other arthropods associated with disease in hospitals

Although this thesis concentrates on bacteria associated with flying insects in UK hospitals, other arthropods, which harbour bacteria, are found in hospitals. For example, Pharaoh ants (*Monomorium pharaonis*) in UK hospitals were found to harbour *Salmonella* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Streptococcus* spp, *Klebsiella* spp and *Clostridium* spp (Beatson, 1972).

Sramova *et al.* (1992) collected spiders (Class Arachnida, Order Araneae), mealworm beetles (*Tenebrio molitor*), German cockroaches (*Blattella germanica*), a hemipteran bug, a ladybird (*Coccinella septempunctata*), aphids (Aphidoidea), lacewings (*Chyrsopa vulgaris*), a pollen beetle (*Meligethes* sp), a sap-sucking beetle (Nitidulidae), garden ants (*Lasius niger* and *Lasius emarginatus*) and a wasp (*Paravespula vulgaris*) from a hospital premises in Prague. The bacteria found associated with the sampled insects included *Staphylococcus* spp (coagulase negative), *Enterococcus* spp, *Enterobacter* spp, *Klebsiella* spp, *Citrobacter* spp, *Serratia* sp, *Providencia* sp, *Morganella* sp,

Pseudomonas sp, *Acinetobacter* sp, *Flavobacter* spp, *Corynebacterium* sp and unspecified sporing bacteria, with cockroaches being the major carriers of bacteria.

The role and bacterial associations of cockroaches in UK hospitals have been examined by a number of authors (Burgess and Chetwyn, 1979, Baker, 1982, Peck, 1985). The Cockroach species recorded in hospitals were *Blatta orientalis, Blattella germanica, Periplaneta americana* and *Supella longipalpa*. These cockroaches harboured *Citrobacter* spp. *Enterobacter* spp, *E. coli, Klebsiella pneumoniae, Proteus* spp, *Pseudomonas aeruginosa* and *Serratia marcescens*.

There exists extremely convincing evidence of the role played by German cockroaches (*Blattella germanica*) in an outbreak of a bacterial infection caused by *Klebsiella pneumoniae* in a neonatal unit (Cotton *et al.,* 2000). The study showed that the 'strain' isolated from the cockroaches was indistinguishable from that colonizing and causing invasive disease in new-born infants.

1.10 Flies and climate change

If climate change influences fly populations, there may be an impact on fly populations in hospitals, which requires consideration. Models have been produced which predict that housefly populations could increase substantially under the likely scenarios of climate change. These models anticipate increases of up to 244% by 2080 when compared with current levels, with the greatest increases occurring in the summer months (Goulson, 2005). If this prediction holds true, it is possible that increases in the incidence of fly-borne diseases may also occur, which may be of significance in terms of an increased reservoir of flies available to enter hospitals. Some fly populations may not experience increases, for example, although populations of stable flies (*Stomoxys calcitrans*) are unlikely to worsen in response to climate change, a shift in the activity period could occur, which is still of importance (Gilles *et al.*, 2008).

1.11 Aims & Objectives

The aim of this study was to investigate the role of flying insects in the spread of hospital-associated infections with particular emphasis on *M. domestica* as a reservoir and vector of *C. difficile*.

Specific objectives were:

- To determine the ability of *M. domestica* to transfer *C. difficile* mechanically following exposure to vegetative cell and spore suspensions.
- To collect and identify the flying insects associated with a number of UK hospitals and classify any bacteria associated with them.
- To analyse a pre-existing database containing data on insects identified in UK hospitals, in order to classify and enumerate the reports of insects and establish their seasonality and location in such premises.
- To establish whether *C. difficile* is ingested by *M. domestica* and can subsequently be isolated from the alimentary canal.
- To verify the isolation of *C. difficile* from the excreta of *M. domestica*.
- To establish the duration of excretion of *C. difficile* in the excreta of *M. domestica*.
- To determine the physiological state of *C. difficile* as excreted by *M. domestica*; spores or vegetative cells.
- To explore the retention of *C. difficile* through the life stages of *M. domestica*, from larvae to pupae to adults.
- To determine the initial and long-term survival of *C. difficile* associated with flies that have been exposed to vegetative cell and spore suspensions and subsequently electrocuted in an Electronic Fly Killer (EFK).

2 CHAPTER 2: MECHANICAL TRANSFER OF *CLOSTRIDIUM DIFFICILE* BY *MUSCA DOMESTICA* ADULTS

2.1 INTRODUCTION

The housefly, *Musca domestica*, presents a significant worldwide threat to public health due to its synanthropic and endophilous behaviour (West, 1951). Part of the behaviour that results in its threat to public health is a propensity to breed in faecal matter and move from filth to food indiscriminately (Greenberg, 1971, Greenberg, 1973). It is these unsanitary sources from which houseflies obtain pathogenic bacteria, thus being implicated in the spread of many diseases (Olsen, 1998, Graczyk *et al.*, 2001, Forster *et al.*, 2009).

M. domestica has been sampled from hospitals before and was shown to carry pathogenic bacteria in the clinical environment, including *Bacillus* spp (Adeyemi and Dipeolu, 1984), *Escherichia coli* (Fotedar *et al.*, 1992b), *Klebsiella pneumoniae* (Fotedar *et al.*, 1992a), Methicillin resistant *Staphylococcus aureus* (MRSA) (Rahuma *et al.*, 2005) and *Salmonella* sp (Nmorsi *et al.*, 2007).

Regarding pathogens in hospitals, the highly-infectious healthcare-associated pathogen *C. difficile* is one of the most important, in that it is the leading cause of nosocomial diarrhoea worldwide (Dawson *et al.*, 2009). Infection with *C. difficile* has serious implications in that it can result in the isolation of patients, closure of wards and hospitals and even the death of infected individuals (Dawson *et al.*, 2009). It is an important infection in England and Wales, with infections contributing to deaths having peaked at over 8,000 per annum (ONS, 2013). *C. difficile* infection (CDI) typically affects elderly patients on antibiotics, causing severe disease such as pseudomembranous colitis (PMC) via toxins that affect intestinal cells (Schroeder, 2005). While it is generally thought that *C. difficile* is commonly passed from person-to-person nosocomially via the faecal-oral route, other research shows that most new cases cannot be explained by contact with infected individuals and the main routes of transmission are unknown (Walker *et al.*, 2012). Flying insects such as *M. domestica* may be one of the 'unknown' routes of transmission of *C. difficile*.

Insects can carry *C. difficile*, although there are only two references in the literature and the insects were not sampled from hospitals. A laboratory strain of *Coptotermes formosanus* termites were the first example of insects carrying *C. difficile* (Taguchi *et al.*, 1993). A second reference describes flies collected from pig farms as being positive for *C. difficile* ribotype 078 (Burt *et al.*, 2012). The flies carrying *C. difficile* were lesser houseflies *Fannia canicularis* (some fruit flies *Drosophila*

melanogaster and houseflies *M. domestica* were included in this sample) and drain flies *Psychoda alternata* (Burt *et al.*, 2012).

Clostridium spp apart from *C. difficile* have been isolated from insects in UK hospitals, specifically *Clostridium welchii* (now *Clostridium perfringens*) and *Clostridium cochlearium* carried by Pharaoh ants *Monomorium pharaonis* (Beatson, 1972). Although *C. difficile* has not previously been isolated from flying insects in hospitals, the fact that this and species of the same genus have been recorded in association with insects suggests that there is potential for insects to be mechanical vectors of *C difficile* in a clinical setting.

In this chapter, the ability of *M. domestica* to transfer *C. difficile* mechanically following exposure to vegetative cell and spore suspensions was determined. Specific aims within this were to examine whether *C. difficile* is ingested by *M. domestica* and can subsequently be isolated from the alimentary canal, verify the isolation of *C. difficile* from the excreta of *M. domestica*, establish the duration of excretion of *C. difficile* in the excreta of *M. domestica* and determine the physiological state of *C. difficile* as excreted by *M. domestica*; spores or vegetative cells.

2.2 MATERIALS AND METHODS

2.2.1 Flies

Laboratory reared, mixed-sex adult houseflies, *M. domestica*, were provided by the Insect Supplies Unit at the Food and Environment Research Agency (FERA, York, UK). Flies were stored up to a maximum duration of two weeks in a refrigerator at 4°C and fed on 5% $^{w}/_{v}$ sterile sucrose solution (sucrose obtained from Sigma Aldrich, Poole, UK) as required. Where the word 'sterile' is used in descriptions of methodology, it should be taken to mean that media and equipment was sterilised in an autoclave (Prestige Medical, Coventry, UK) if it was not supplied as sterile. Exceptions to this technique of sterilisation are noted within the text.

2.2.2 *C. difficile* inocula

C. difficile NCTC11204 PCR ribotype 001 TOX A/B + (Anaerobe Reference Laboratory, Cardiff, UK) were stored on Microbank® beads (Pro-Lab Diagnostics, Cheshire, UK) and maintained at -70°C until required. A 1 x 10^6/ml culture of *C. difficile* vegetative cells was prepared in 15ml Wilkins Chalgren broth (Oxoid Ltd, Basingstoke, UK) by inoculation with 10 colonies previously cultured on Wilkins Chalgren Agar (Oxoid Ltd, Basingstoke, UK) incubated for 48 hours at 37°C in anaerobic conditions (anaerobic cabinet, Don Whitley scientific, Shipley, UK). The culture concentration was determined by comparison of the suspension with uninoculated Wilkins Chalgren Broth in a Pharmacia LKB visible spectrophotometer, Novaspec II (Pharmacia, Freiburg, Germany). The spectrophotometer was used to measure the optical density (OD) of the culture at 600nm, giving a reading which was then compared to a calibration curve (showing the relationship between optical density and CFU/ml of *C. difficile*) for verification / standardisation of a 1 x 10^6/ml concentration.

A 1 x 10^6/ml suspension of *C. difficile* spores was prepared as described by (Shetty *et al.*, 1999), with the presence of spores being verified by examination under a haemocytometer.

2.2.3 Media

Cycloserine Cefoxitin Fructose Agar (CCFA) was prepared according to manufacturer's instructions (Oxoid Ltd, Basingstoke, UK). The bile salt Sodium Taurocholate (Tc) (Sigma Aldrich, Poole, UK) was added to the agar at 0.1% $^{w}/_{v}$ as a germinant (Wheeldon *et al.*, 2008a) along with the *C. difficile* selective supplement 'selectavial' (Oxoid Ltd, Basingstoke, UK) and 7% $^{v}/_{v}$ defibrinated horse blood

(Southern Group Laboratories, Corby, UK). These agar plates were stored at 4°C in a refrigerator until required.

2.2.4 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 two minutes. Inactivated houseflies were removed from the freezer and both wings removed by dissection with entomological spring scissors and fine entomological forceps (Watkins and Doncaster, Kent, UK). Removal of fly wings was a risk control measure, to prevent escape by flight. The flies were stored at 4°C in a refrigerator until required. Prior to any manipulations of the flies, the entomological spring scissors and fine entomological forceps were sterilised as per the process described for entomological tweezers in section 2.2.6.

2.2.5 Pre-treatment control

A pre-exposure control sample of houseflies (n=5) were macerated individually in 1ml of sterile Phosphate Buffered Saline (PBS) (Sigma Aldrich, Poole, UK), using the end of a sterile plate spreader. The homogenate was serially diluted down to 1 x 10^{-3} and 0.1ml of each dilution was inoculated onto the surface of a CCFA plate plus Tc, selective supplement and 7% $^{v}/_{v}$ defibrinated horse blood. When considering any reference to CCFA from here onwards it should be assumed that the agar includes selective supplement and horse blood as described. The plates were incubated for 48 hours at 37°C in anaerobic conditions, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

2.2.6 Mechanical transfer of vegetative cells

The following experiment was replicated nine times and the same individual fly was allowed to explore each agar plate for six minutes and kept in each 'resting' plate for one hour.

To confirm the fly was clear of *C. difficile* carriage prior to the experiment, a single housefly was transferred with sterile entomological tweezers (Watkins & Doncaster, Kent, UK) from the sterile holding dish on to the surface of a CCFA plate (no spore germinant) and allowed to walk around the plate for six minutes after which, it was transferred to a CCFA plus Tc plate for another six minutes. Entomological tweezers were sterilised by submerging in ethanol (70% $^{v}/_{v}$) and passing through the flame of a Bunsen burner. Flies were picked up with the tweezers by grasping the femur of one of the legs.

The same housefly was then transferred to a 'donor' CCFA plate that had been inoculated with 0.1ml of the *C. difficile* vegetative cell culture (prepared as per section 2.2.2) immediately before the fly was introduced. After exposure to the 'donor' / inoculum plate for six minutes, the fly was transferred to a first fresh CCFA plate ('recipient' plate) for a further six minutes, after which it was transferred to a first plate of CCFA plus Tc, again for a further six minutes. The housefly was subsequently transferred to a first sterile empty Petri dish or 'resting' plate for one hour. The housefly was then transferred to a second fresh CCFA plate for six minutes, after which it was transferred to a second fresh CCFA plus Tc plate for six minutes followed by return to a second fresh sterile empty Petri dish for one hour. This process was repeated until the housefly had finished contact with the fourth fresh agar plates after which, it was transferred to a final 'resting' plate (a final fresh sterile empty Petri dish) where it was kept at room temperature overnight for further analysis i.e. attempted isolation of *C. difficile* from external and internal structures (see sections 2.2.8 and 2.2.9) the following day. The six minute time period was chosen in order to reflect realistic conditions because the author has made personal observations that adult houseflies contact and explore foodstuffs and surfaces for a number of minutes at a time in the field.

2.2.7 Mechanical transfer of spores

This experiment used the same methodology as section 2.2.6 but with the 1 x 10^6/ml *C. difficile* spore suspension rather than the vegetative cell culture, CCFA plus Tc plates rather than CCFA to expose the flies to the inoculum and without the use of CCFA plates thereafter.

2.2.8 Isolation of *C. difficile* from external structures of *M. domestica*

Houseflies used in the previous mechanical transfer experiments (see sections 2.2.6 and 2.2.7), were washed individually in 1ml of sterile PBS in a sterile 1.5ml universal micro test tube (Eppendorf, Stevenage, UK) and mixed by vortexing for 30 seconds. The resulting PBS wash was serially diluted down to 10^{-3} in sterile PBS and 0.1ml of each dilution used to inoculate the surface of a CCFA plus Tc agar plate. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

2.2.9 Isolation of *C. difficile* from internal structures of *M. domestica*

External vs. internal control; the houseflies that had been washed to remove external bacteria (as described in section 2.2.8) were then washed a further four times in PBS. The final set of PBS

washings was retained and serially diluted down to 10^{-3} in sterile PBS and 0.1ml of each dilution used to inoculate the surface of a CCFA plus Tc agar plate. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

This process of repeated washing was undertaken to remove *C. difficile* from external surfaces, to avoid issues of contamination when attempting to isolate *C. difficile* from internal structures.

After washing four times, houseflies were macerated individually in 1ml of sterile PBS in a sterile 1.5ml universal micro test tube, using the end of a sterile plate spreader. The homogenate was serially diluted down to 10^{-3} in sterile PBS and 0.1ml of each dilution used to inoculate the surface of a CCFA plus Tc agar plate. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

2.2.10 Isolation of *C. difficile* from *M. domestica* alimentary canal

Houseflies (n=5) were exposed to *C. difficile* for 30 minutes, by being allowed to walk over a CCFA agar plate that had been inoculated with 0.1ml of the 1 x 10^6/ml suspension spore suspension. Flies were then killed by incubation in a sterile Petri dish at -18°C for five minutes in a freezer (manufacturer etc.). Each fly was subsequently removed from frozen storage and washed as per sections 2.2.8 and 2.2.9.

A few drops of molten wax (from tea light candles, Morrisons, Wakefield, UK) were added to the base of a Petri dish and each washed fly was placed ventral-side down into the molten wax, to secure it for dissection. The fly was then fully immersed in sufficient quantity of PBS (approximately 25ml, depending on the layer of wax deposited) in the Petri dish to allow the internal structures to float freely while dissection was taking place. Ethanol (70% $^{v}/_{v}$) was added drop-wise to the PBS to 'colour' and therefore enhance visibility of the internal structures of the fly to aid dissection. A dissection microscope (Stereo Zoom Model GXM XTL 3101, GX Optical, Haverhill, UK), iridectomy scissors (Surgins Surgical Ltd, Birmingham, UK) and fine entomological forceps were used. The scissors and forceps were sterilised before use as per section 2.2.6. The fly alimentary canal and crop (Figure 2.1) were then dissected aseptically as follows.

Figure 2.1 *M. domestica* alimentary canal (Hewitt, 1914). The alimentary canal as it is seen on dissection from the dorsal side. The malpighian tubes have been omitted and also the distal portion of the lingual salivary gland (*sl.g*.) of the right side. The duct of the crop (*Cr*.) is shown by the dotted line beneath the proventriculus (*Pv*.) and ventriculus (*Ven*.). *p.int*: Proximal intestine. *d.int*: Distal intestine. *rect*: Rectum.

The thoracic cavity was breached using the iridectomy scissors by an incision being made at the tip of the thoracic scutellum. The thoracic cuticle was then broken along the dorsal side and the whole thoracic cuticle was removed by an incision being made along the top of the prescutum and upper thoracic segment and waist area, which enabled the sides to be cut. The two dorsal segments were then removed, revealing the longitudinal flight muscles which were removed in segments. The foregut (proventriculus and stomach) and salivary glands (located either side of the stomach) were observed. The salivary glands were cut free at the distal and proximal ends of the thoracic cavity and the stomach and proventriculus was then removed. The crop was extracted last from the thoracic segment and was pulled through from the abdominal cavity. Abdominal dissection began with an incision being made centrally along the dorsal side which allowed the cuticle abdomen to be removed. The reproductive organs, now visible were removed. The midgut (proximal) and hindgut (distal intestine and malpighian tubules) were extracted using a pulling motion which disentangled and separated out the intestines.

The fly alimentary canal was then added to 1ml PBS in a sterile 1.5ml universal micro test tube, macerated with the end of a sterile plate spreader and mixed by vortexing for 30 seconds to release

bacteria into the PBS wash. Of this PBS wash, 0.1ml was then inoculated onto the surface of a CCFA plus Tc agar plate. The PBS wash was diluted 10x in sterile PBS and 0.1ml of this 10^{-1} dilution inoculated onto a further CCFA plus Tc agar plate. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

2.2.11 Initial isolation of *C. difficile* from *M. domestica* excreta

Houseflies (n=5) were exposed to *C. difficile* for 30 minutes, by being allowed to walk over an agar plate that had been inoculated with 0.1ml of the 1 x 10^6/ml suspension spore suspension.

The flies were inactivated by incubation in a sterile Petri dish in a -18°C freezer for two minutes and subsequently transferred to 1ml of PBS in a sterile 1.5ml universal micro test tube. Each fly was subsequently washed as per sections 2.2.8 and 2.2.9. Each fly was then introduced onto its own Petri dish, with 1ml of 5% $^{w}/_{v}$ sterile sucrose solution, to encourage feeding and throughput of any potential spores in the gut, to be deposited as flyspots. Flyspots on the surface of the Petri dishes were sampled immediately as they were produced, by removal with a sterile swab, which was used to directly inoculate CCFA plus Tc agar plates. After the flyspot was removed, the sampled fly was transferred to a new Petri dish. The flyspots were sampled in this way for a period of three hours.

2.2.12 Isolation of *C. difficile* from *M. domestica* excreta over time

Houseflies (n=25) were exposed to *C. difficile* spores for 30 minutes by being allowed to walk over filter paper that had been inoculated with 0.6ml of the 1 x 10^6 CFU/ml spore suspension. The flies were inactivated by incubation in a sterile Petri dish in a -18°C freezer for two minutes and transferred to 1ml of PBS in a sterile 1.5ml universal micro test tube. Each fly was washed as per sections 2.2.8 and 2.2.9. Each fly was then individually introduced onto a Petri dish containing 0.1ml of 5% $^{w}/_{v}$ sterile sucrose solution to encourage feeding and throughput of any potential spores in the gut, to be deposited as excreta. Excreta (vomitus and faeces a.k.a. 'flyspots') on the surface of the Petri dishes were sampled immediately as they were produced for 4 hours and then every 24 hours for 4 days by swabbing with a sterile cotton swab and transferred to CCFA plus Tc and CCFA plates. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

2.2.13 Isolation of bacteria from laboratory *M. domestica*

The laboratory reared, mixed-sex adult houseflies, *M. domestica*, used as the model organism in aspects of this study, were examined microbiologically. Three separate batches were analysed, received from the supplier a number of months apart, to check for any consistency of organisms isolated from different batches. The laboratory flies (n=10) were pooled into 10ml of PBS in a 30ml universal container and washed/mixed by vortexing for 30 seconds. These external washings were then serially diluted down to 1 x 10^{-3} and 0.1ml of each dilution was inoculated onto CCFA plus Tc, Nutrient Agar, Mannitol Salt Agar and Violet Red Bile Glucose agar (VRBG) (all Oxoid Ltd, Basingstoke, UK) that were all prepared according to manufacturers' instructions. Note that VRBG is sterilised by boiling in a microwave and not by autoclave. Remaining PBS was disposed of from the 30ml universal container, 10 ml of fresh PBS was added and the flies were then macerated with the end of a sterile plate spreader and the above process of vortexing, dilution and inoculation repeated for the macerates. This experiment was replicated three times in total.

2.2.14 Identification of bacteria from laboratory *M. domestica*

Nutrient agar, Mannitol Salt agar and VRBG agar plates were incubated at 37°C for 24 hours in aerobic conditions. CCFA plus Tc agar and a set of Nutrient Agar plates were incubated in anaerobic conditions at 37°C for 48 and 24 hours respectively. Bacterial colonies were identified by macroscopic morphology, Gram staining (HPA, 2011f) with a Gram stain kit (Oxoid, Basingstoke, UK), microscopic examination of morphology (Zeiss Axio Scope microscope fitted with a Zeiss AxioCam HRc camera and AxioVision software version 3.1 (Carl Zeiss Ltd, Hertfordshire, U.K.)), oxidase tests (HPA, 2011e) with oxidase disks (Sigma Aldrich, Poole, UK), catalase tests (HPA, 2011a) with hydrogen peroxide solution (Sigma Aldrich, Poole, UK), Analytical Profile Index (API) 20E test kits (bioMérieux, Marcy l'Etoile, France) and Bacillus-ID test kits (Microgen Bioproducts Ltd, Camberley, UK). Isolates of *Staphylococcus aureus* were cultured on Mannitol Salt Agar with Oxacillin (Oxoid Ltd, Basingstoke, UK) for presumptive identification of Methicillin Resistant *Staphylococcus aureus* (MRSA).

2.2.15 Identification of *C. difficile* colonies

Colony counts were made after the CCFA and CCFA plus Tc plates had been incubated anaerobically for 48 hours at 37°C. Colonies were sub-cultured onto Columbia blood agar (Oxoid Ltd, Basingstoke, UK) and were subsequently identified by macroscopic morphology, Gram staining, microscopic examination of morphology (HPA, 2011c), characteristic 'farmyard' smell (Levett, 1984) and rapid ID 32A API tests that provided a result of 0000022000 (bioMérieux, Marcy l'Etoile, France).

2.3 RESULTS

2.3.1 Pre-treatment control

No colonies were present on the pre-treatment control plates, confirming that the houseflies were not contaminated with *C. difficile* prior to being exposed to the bacterial suspensions.

2.3.2 Mechanical transfer of vegetative cells

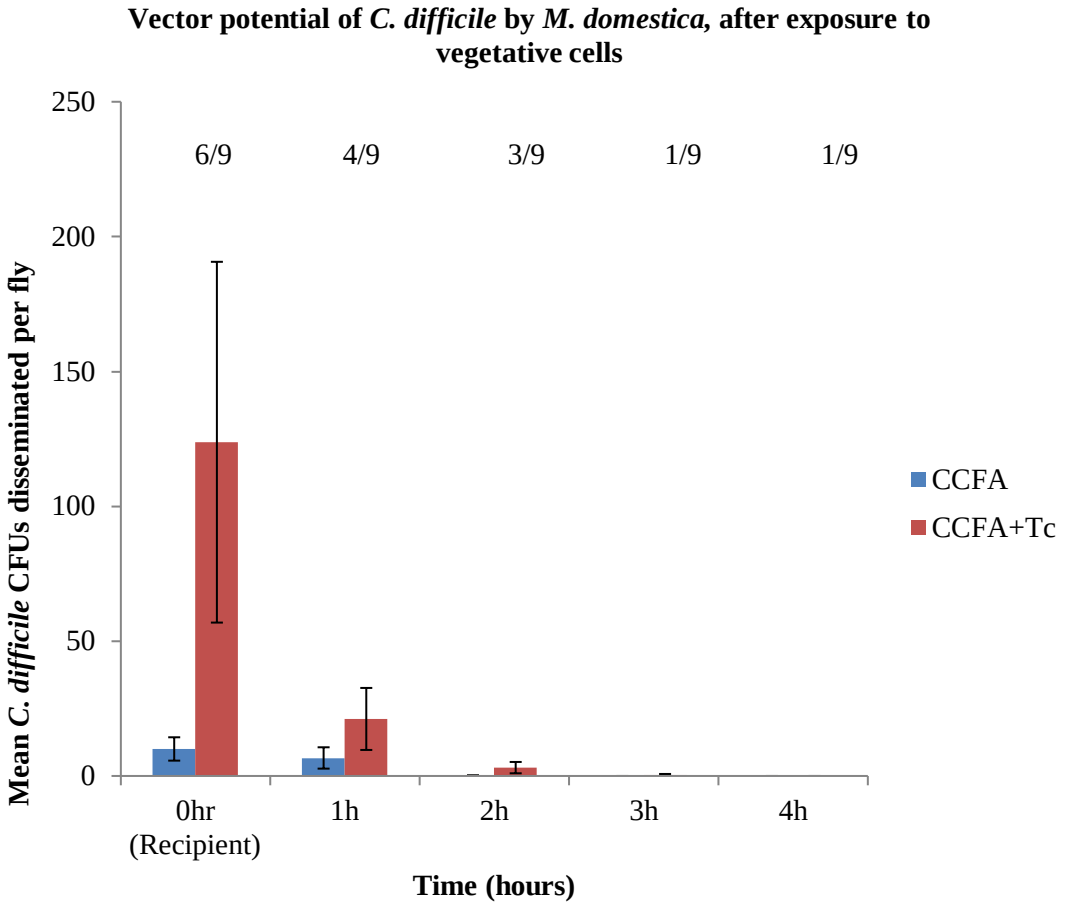

Figure 2.2 Vector potential of *C. difficile* by *M. domestica*, after exposure to vegetative cells. Mean number (± Standard Error (SE)) of *C. difficile* cells disseminated per fly (n=9), over time, after exposure to a 1 x 10^5 suspension of vegetative cells. Numbers above the columns are numbers of positive flies / number of flies tested. 'CCFA' is the recovery of *C. difficile* from Cycloserine Cefoxitin Fructose Agar without a germinant, which is likely to represent vegetative cell transfer by *M. domestica*. 'CCFA+Tc' is the recovery of *C. difficile* from Cycloserine Cefoxitin Fructose Agar with the germinant sodium taurocholate, which is likely to represent combined spore and vegetative cell transfer by *M. domestica*.

The most colony forming units (CFUs) per fly were transferred immediately and 1 hour following exposure to the vegetative cell suspension and this transfer continued, albeit with low numbers of CFUs transferred, up to four hours following exposure (Figure 2.2).

The mean number of CFUs of *C. difficile* transferred immediately by *M. domestica* (n=9) to the recipient agar plates without a germinant (CCFA) and therefore likely to represent vegetative cell transfer, was 10.2 +/- 4.3 and after 1 hour this had reduced to 6.7 +/- 3.9 (Figure 2.2). The reduction

in vegetative cell transfer may be influenced by a loss of viability of these cells in the aerobic conditions of the experiment.

The mean number of CFUs of *C. difficile* transferred immediately by *M. domestica* (n=9) to the recipient agar plates incorporating the germinant Sodium Taurocholate (CCFA+Tc) and therefore likely to represent combined spore and vegetative cell transfer, was 123.8 +/- 66.9 and after 1 hour this had reduced to 21.2 +/- 11.4 (Figure 2.2).

2.3.3 Mechanical transfer of spores

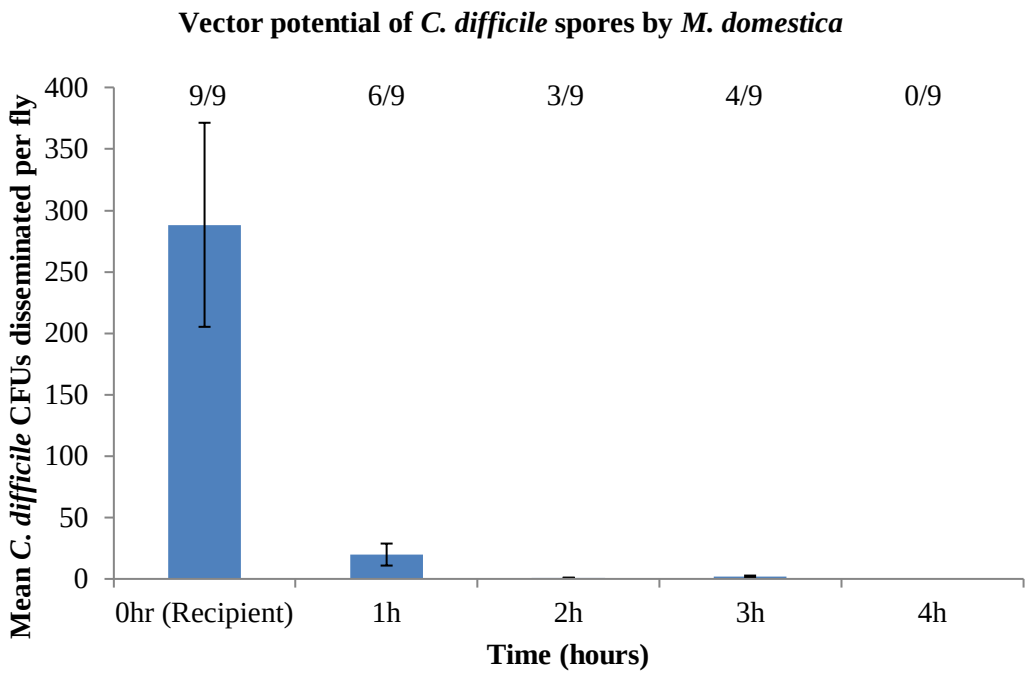

Figure 2.3 Vector potential of *C. difficile* spores by *M. domestica*. Mean number (± SE) of *C. difficile* CFUs disseminated per fly (n=9), over time, after exposure to a 1 x 10^5 suspension of spores. Numbers above the columns are numbers of positive flies / number of flies tested.

The most CFUs per fly were transferred immediately and 1 hour following exposure to the spore suspension, with minimal transfer after 2 hours, 3 hours and no transfer apparent after 4 hours (Figure 2.3).

The mean number of CFUs of *C. difficile* transferred immediately by *M. domestica* (n=9) to the recipient CCFA+Tc plates was 288.2 +/- 83.2 and after 1 hour this had reduced to 19.9 +/- 9 (Figure 2.3).

It appears that *M. domestica* pick up a higher number of spores versus vegetative cells, which may be explained by the greater hydrophobicity of *Clostridium* spp spores compared to vegetative cells (Wiencek *et al.*, 1990).

2.3.4 Isolation of *C. difficile* from external and internal structures of *M. domestica*

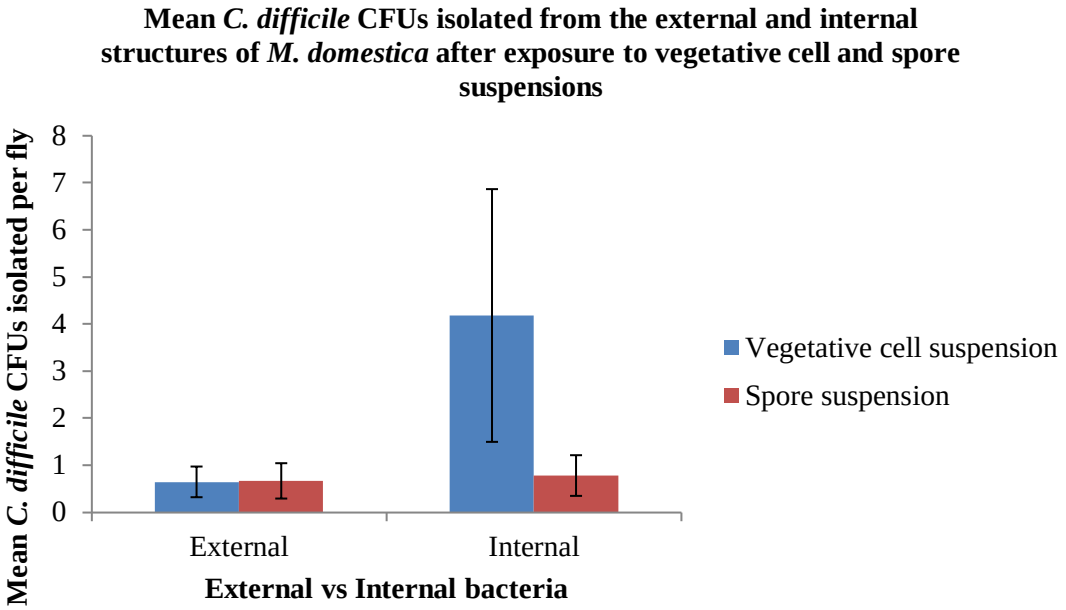

Figure 2.4 Isolation of *C. difficile* from external and internal structures of *M. domestica*. Mean number (±SE) of *C. difficile* CFUs isolated from external and internal structures of flies (n=9) exposed to a 1 x 10^5 suspension of vegetative cells, including flies (n=9) exposed to a 1 x 10^5 spore suspension, with both sets of flies left overnight before analysis.

C. difficile was isolated from the external and internal structures of the same *M. domestica* (n=18) used in the previous mechanical transfer experiments, the experimental flies being retained in Petri dishes overnight prior to analysis.

M. domestica (n=9) exposed to the vegetative cell suspension harboured 0.64 +/- 0.33 mean *C. difficile* CFUs externally and 4.18 +/- 2.69 internally. *M. domestica* (n=9) exposed to the spore suspension harboured 0.67 +/- 0.37 mean *C. difficile* CFUs externally and 0.78 +/- 0.43 internally (Figure 2.4).

External vs internal control: No *C. difficile* was recovered, indicating that the washing method was sufficient to remove external bacteria prior to maceration.

2.3.5 Isolation of *C. difficile* from *M. domestica* alimentary canal

The mean number of *C. difficile* CFUs isolated from *M. domestica* alimentary canals (n=20) was 35 +/- 6.5.

2.3.6 Initial isolation of *C. difficile* from *M. domestica* excreta

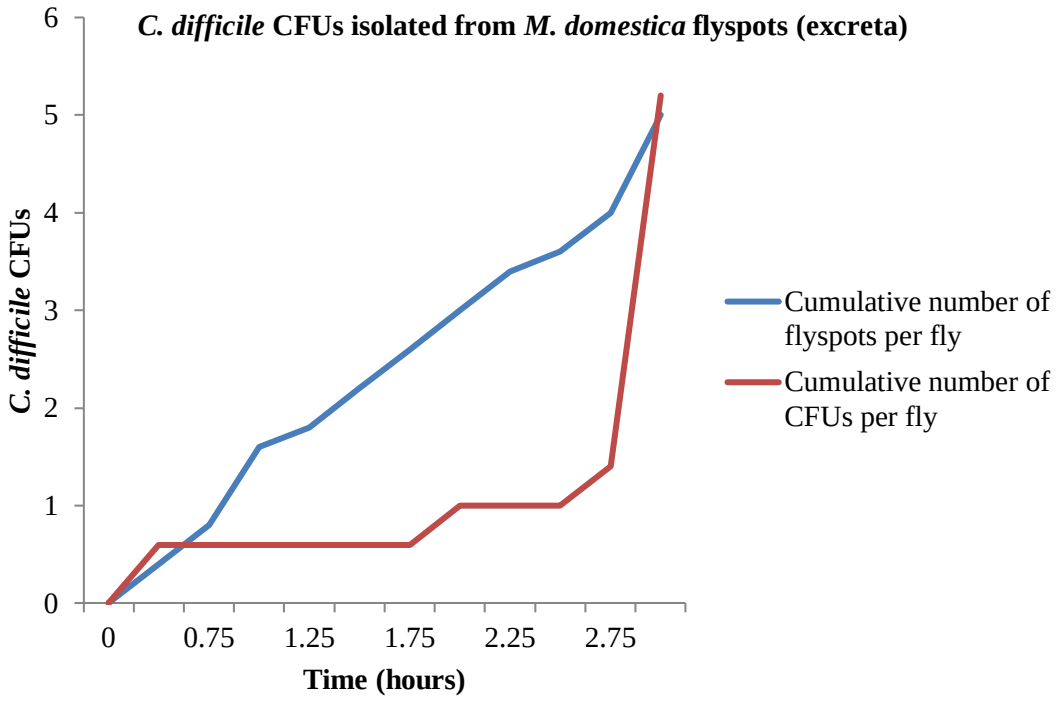

Figure 2.5 *C. difficile* CFUs isolated from *M. domestica* flyspots (excreta). The cumulative number of faecal spots produced per fly (n=5) over a 3hr period and *C. difficile* CFUs isolated from the faecal spots, after flies were exposed to a 1 x 10^5 suspension of spores.

The mean number of *C. difficile* CFUs isolated per *M. domestica* faecal spot was 1.04 +/-0.58, over a 3 hour period (Figure 2.5).

2.3.7 Isolation of *C. difficile* from *M. domestica* excreta over time

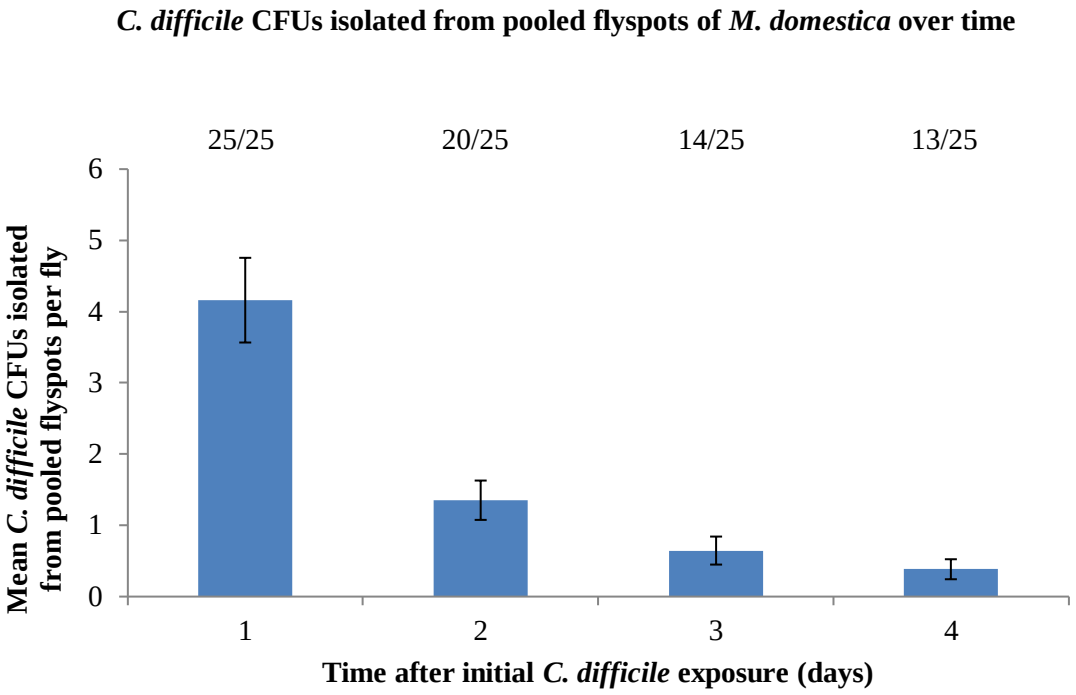

Figure 2.6 Isolation of *C. difficile* from *M. domestica* excreta over time. Mean number (±SE) of *C. difficile* CFUs isolated per *M. domestica* (n=25) from pooled flyspots sampled over time, after flies were exposed to a 1 x 10^5 suspension of spores. Numbers above the columns are numbers of positive flies / number of flies tested.

C. difficile spores could be recovered from *M. domestica* excreta for 96hrs (Figure 2.6). *C. difficile* was isolated from *M. domestica* excreta, with means of 4.16 +/- 0.59 CFUs per fly at day 1, decreasing to 1.35 +/- 0.27 after 2 days, decreasing further still to 0.64 +/- 0.19 after 3 days and 0.38 +/- 0.14 at 4 days (Figure 2.6). No growth was observed on CCFA plates (no germinant), which suggests that *C. difficile* vegetative cells were not excreted by *M. domestica*, suggesting that germination does not take place in the fly. A lack of *C. difficile* spore germination in *M. domestica* is possibly due to absence of bile salts in the fly digestive system.

2.3.8 Isolation and identification of bacteria from laboratory *M. domestica*

Bacteria isolated from laboratory reared / insectary-supplied adult *M. domestica* used as the model organism in this study are listed in Table 2.1.

Table 2.1 A checklist of bacteria isolated from laboratory stock of adult *M. domestica*

Bacteria isolated	**ID kit code**	**Estimated CFUs per fly per ml (mean)**	**Batch (month & year)**
***Bacillus* spp**			
**Bacillus circulans*	77270521	380,000	04 2013
**Bacillus circulans*	77272121	355,667	12 2013
Bacillus subtilis Group	66376427	1,490,000	01 2014
Enterobacteriaceae			
Providencia rettgeri	0274301	11,167	04 2013
Providencia rettgeri	0274301	851,433	12 2013
Serratia marcescens (non-pigmented strain)	4306721	1,434,932	04 2013
Serratia marcescens (non-pigmented strain)	4317721	532,700	12 2013
Enterobacter cloacae	2305573	730,000	01 2014
Enterobacter cloacae	3305573	730,000	01 2014
Klebsiella oxytoca	1255773	730,000	01 2014
Serratia marcescens (non-pigmented strain)	4307721	730,000	01 2014
Staphylococci			
Staphylococcus aureus		1,023,333	04 2013
Staphylococcus aureus		506,667	12 2013
Staphylococcus aureus		3,570,000	01 2014

*Isolated from *M. domestica* for the first time, to the knowledge of the author.

2.4 DISCUSSION

Results show that adult houseflies, via direct contact with their external surfaces, are able to mechanically transfer *C. difficile* for up to 4 hours, after initial exposure to vegetative cells and / or spores, thus potentially presenting an infection risk to patients via 'facilitated airborne dispersal' of this pathogen.

The route of *C. difficile* infection in patients is often unknown (Walker *et al.*, 2012) and transmission via flies could be one of the 'unknown' routes. Although the infectious dose in humans is not currently known, it is expected to be low and ingestion of tens of spores may be sufficient to cause infection in compromised individuals. As an example of infective dose in mammals, ingestion of only one or two spores may be enough to result in colonisation and *C. difficile* associated disease of hamsters that have been treated with antibiotics (Larson and Borriello, 1990). The levels of *C. difficile* mechanically transferred per adult *M. domestica* and isolated from the alimentary canal and excreta were low but this could still be of great significance in terms of infecting compromised human hosts, whose infective dose is low as described. So, the potential role of adult *M. domestica* in the transfer of *C. difficile* should not be underestimated, especially when considering that hundreds of these flies can be present in hospitals (Fotedar *et al.*, 1992a, Fotedar *et al.*, 1992b), numbers which could further enhance infection risk. It is also important to note that the results presented in this study are in the form of CFUs and one CFU often represents more than one bacterium, so the results of this study could actually be an underestimation of the carriage of *C. difficile* by *M. domestica*.

Although mechanical transfer via adult *M. domestica* external surfaces only occurred for up to 4 hours, *C. difficile* can still be isolated from the external and internal structures of houseflies after a longer period. Candidate areas of the fly external anatomy involved in initial mechanical transfer include tarsi and pulvilli. As the flies continued to harbour *C. difficile* even though mechanical transfer by direct contact ceased shortly after initial exposure to a source, the remaining bacteria could be located on or in areas of the fly anatomy where deposition onto surfaces via the normal processes of direct contact is not detectable, such as areas other than tarsi and pulvilli.

C. difficile was isolated specifically from the alimentary canal of adult *M. domestica*, showing that ingestion of the bacteria occurs. Excretion of *C. difficile* is also possible, as the bacteria were isolated from excreta 96 hours after exposure to the bacterial suspensions.

There appears to be a 'timeline of transfer'; initial transfer of *C. difficile* via direct contact of external surfaces of the fly is highest, decreasing over time as bacteria are deposited although some remain

associated with parts of the fly anatomy where transfer may not be detectable, with ingestion and subsequent excretion of bacteria in excreta potentially being responsible for continuing the transfer.

There was an observed absence or minimal amount of mechanical transfer by contact with surfaces after 4 hours and a low recovery of *C. difficile* from adult *M. domestica* alimentary canals and excreta, the reasons for which are unclear. Although not experimentally considered in this study, perhaps the action of the fly immune system had an influence. Antimicrobial peptides showing action against Gram-positive bacteria have been isolated from adult *M. domestica* (Wang *et al.*, 2006, Liang *et al.*, 2006). Although activity of fly (Order Diptera) antimicrobial peptides against *C. difficile* is unknown, *Clostridium perfringens* was shown to be resistant to cecropin (Moore *et al.*, 1996). Other antimicrobial peptides may offer protection against infection of flies by *C. difficile* as the growth of *Clostridium perfringens*, *Clostridium ramosum* and *Clostridium paraputrificum* is inhibited by the antimicrobial peptide sarcotoxin IA, which has been isolated from the flesh fly *Sarcophaga peregrina* and exists as a homologue in other insects (Mitsuhara, 2001). Although antimicrobial peptides with activity against *C. difficile* have not yet been observed in *M. domestica*, one called coprisin is present in the Korean dung beetle, *Copris tripartitus* and its antibiotic activity is thought to take place by disrupting the membrane of *C. difficile* (Kang *et al.*, 2011). As antimicrobial peptides are widely conserved it insects, it is possible that peptides exist in *M. domestica* which are similar to coprisin and have an antimicrobial effect against *C. difficile* but have yet to be discovered.

As *M. domestica* is often the most common fly in human occupied premises (Mallis, 1964) and can disperse for a number of miles (Greenberg, 1973, Busvine, 1980), it may also have the potential to be involved in community associated *C. difficile* cases.

C. difficile infection can be of veterinary concern and has been reported in horses, ostriches, companion animals, calves, pigs (Songer, 2004) and poultry (Zidaric *et al.*, 2008). There is also an overlap between *C. difficile* types present in animals and humans, with identical isolates of type 078 increasingly encountered in pigs and humans leading researchers to the conclusion that 'a common origin of animal and human strains should be considered' (Debast *et al.*, 2009). It is possible that because of their synanthropy and the fact that they can develop in human, calf, horse, pig, poultry and other animal faeces (Busvine, 1980), *M. domestica* may have a role to play in the potential interspecies transmission of *C. difficile* and represent the 'common origin' of infection in both livestock and humans. Indeed, this proposal of flies as a route of infection between livestock and humans is the opinion of Zurek and Ghosh (2014) who review the evidence for flies as the 'insect link between the agricultural and urban environment' regarding transmission of antibiotic-resistant bacteria. Furthermore, the principles of the first stages of interspecies transmission of *E. coli* have already been shown, in a study where *M. domestica* inoculated with an antibiotic resistant form of *E. coli* O157:H7 infected cattle via contamination of water and food, including direct contact with the

calves (Ahmad *et al.*, 2007). This potential for interspecies transmission has been shown in other flies, such as lesser houseflies, *Fannia canicularis* (some *Drosophila melanogaster* were included in this sample) and drain flies, *Psychoda alternata*, which have been collected from pig farms and been shown to be positive for *C. difficile* type 078 (Burt *et al.*, 2012). The fact that *C. difficile* has now been isolated from flies sampled from the environment goes some way toward proving the principle of the laboratory model of *C. difficile* transfer by *M. domestica* in the current study.

If climate change influences fly populations, there may be an impact on fly populations in hospitals, which requires consideration. Models have been produced, predicting that *M. domestica* populations could increase substantially under likely scenarios of climate change, with increases of up to 244% by 2080 when compared with current levels, with the greatest increases occurring in the summer months (Goulson, 2005). If this prediction holds true, it is possible that increases in the incidence of fly-borne diseases may occur, which may be of significance in terms of an increased reservoir of flies available to enter hospitals.

Isolation and identification of bacteria from laboratory M. domestica

The consistent finding of *S. aureus* in adult *M. domestica* indicates handling of specimens and rearing materials by insectary staff, as this bacterium is a common commensal of human skin (Kock *et al.*, 2010).

Providencia rettgeri and *Serratia marcescens* were isolated from adult *M. domestica*. This finding is to be expected, as *P. rettgeri* and *S. marcescens* have both been isolated from *M. domestica* supplied from insectaries, in a study where houseflies were used as the model organism (Grubel *et al.*, 1997). In the same study, experiments examining the transmission of a particular pathogen by flies were undertaken, which was not dissimilar to this study (Grubel *et al.*, 1997). *P. rettgeri* and *S. marcescens* might therefore be considered as a component of the natural flora of insectary-reared *M. domestica*.

B. circulans and *B. subtilis* that were isolated from the laboratory reared experimental adult flies in this study are numerous in nature and are typically environmental isolates found in soil (Hiroki, 1993, Dhas and Hena, 2012). *B. subtilis* was also found in wild type *M. domestica* in this study, so it is equally possible that this species may represent a component of the natural flora of houseflies or could be readily acquired from the environment. The same can also be said of the *M. domestica* association with *Enterobacter cloacae, Klebsiella oxytoca* and *Staphylococcus aureus*.

To the knowledge of the author this is the first example of *Bacillus circulans* isolation from *M. domestica*.

Bacillus circulans is a Gram-positive (in young cultures - inconstant in older cultures), aerobic and facultatively anaerobic, spore-forming (oval shaped spores that can be central subterminal or terminal in position), rod-shaped, motile bacterium (Cowan *et al.*, 2003) that is isolated from soil (Hiroki, 1993).

Following an operation in a case of ovarian cancer in a 78 year old woman, *B. circulans* was isolated from the ruptured wound (the infection subsequently cleared naturally) and has been described in a case of fatal meningitis, leading to it being classified as an opportunistic pathogen (Logan *et al.*, 1985). *B. circulans* has been isolated on three occasions from leukaemia patients and was susceptible to Vancomycin, re-iterating the status of this microorganism as an opportunistic pathogen (Banerjee *et al.*, 1988). An antibiotic resistant strain of *B. circulans* has recently been described, which caused fatal sepsis in an immunosuppressed man and is thought to be the only record of carbapenemase-production in this organism (Alebouyeh *et al.*, 2011).

The many different species of bacteria isolated in great quantities from laboratory / insectary-supplied adult *M. domestica* illustrated perfectly the need for selective CCFA plates, when examining the transfer of *C. difficile* by the model organism, the housefly.

2.5 CONCLUSION

In conclusion, *M. domestica* may harbour *C. difficile* for significant periods of time and transfer low numbers in the environment, potentially presenting a reservoir and infection risk to patients due to the low infective dose. This study highlights the potential for *M. domestica* to contribute to environmental persistence and spread of *C. difficile* and the need to consider pest control as part of infection control strategies.

3 CHAPTER 3: MECHANICAL TRANSFER OF *CLOSTRIDIUM DIFFICILE* BY *MUSCA DOMESTICA* LARVAE

3.1 INTRODUCTION

M. domestica adults may not be the only life stage able to transfer *C. difficile*, as housefly larvae can carry pathogenic bacteria sometimes throughout development. This means that the acquisition of pathogens in the larval stage could be an important consideration. For example, if *C. difficile* is acquired by *M. domestica* larvae and retained through to adulthood, the elimination of larval development sites as a source of contamination and use of larvicides would become even more important in fly control programmes.

Larvae are an active, feeding, mobile stage of the fly life cycle and could therefore present infection risks if they are acquiring and subsequently transferring bacteria from breeding sites that may be present in hospitals. Such breeding sites would include excrement, rotting organic matter associated with drains, animal carcasses and food spillage. For example, housefly larvae have been shown to harbour *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* (Banjo *et al.*, 2005), *Providencia rettgeri* and many other species of bacteria (Zurek *et al.*, 2000).

Some evidence shows that the vast majority of larval gut microorganisms are destroyed during metamorphosis and that at the point of emergence, approximately 20% of adult houseflies are sterile and that 'the adult fly has the most potential for disease transmission, as the maggot has limited motility and possesses autosterilization mechanisms which limit its capacity to carry pathogens over into the adult stage' (Greenberg, 1973). In contrast, a number of authors have shown that housefly larvae acquire and retain a considerable number of bacteria acquired at this stage, through to the pupal stage and finally adulthood (Glaser, 1923) and it is considered that retention of *E. coli* from larvae to pupae to adult houseflies could play a role in the transmission and spread of *E. coli* (Rochon *et al.*, 2005).

Non-spore-forming *Bacillus* spp are not found in association with adult flies derived from larvae experimentally exposed to these species but the spore-forming *Bacillus anthracis* does survive (Graham-Smith, 1914).

There appears to be no evidence in the literature of *C. difficile* being isolated from housefly larvae (or from larvae of any flies). However, other *Clostridium* species have been recovered from fly larvae.

For example, *Clostridium* spp have been found in association with non-biting midge larvae, *Chironomus plumosus* (Rouf and Rigney, 1993) and *Clostridium botulinum* with *Lucilia caesar* larvae (Greenberg, 1971, Greenberg, 1973).

As Gram-positive spore-forming rod-shaped bacteria such as *Bacillus anthracis* can be acquired by fly larvae, retained through metamorphosis and isolated from adult flies, it is hypothesised that the same may also hold true for the Gram-positive spore-forming rod-shaped *C. difficile*, especially as members of the same genus have been isolated from fly larvae.

In this chapter, the acquisition and retention of *C. difficile* through the life stages of *M. domestica*, from larvae to pupae to adults was explored.

3.2 MATERIALS AND METHODS

3.2.1 Larvae

Laboratory reared, housefly larvae, *M. domestica*, were provided by the Insect Supplies Unit at the Food and Environment Research Agency (FERA, York, UK). Larvae were stored for up to a maximum of two weeks at 4°C in refrigerator when not in use. The larger third instar larvae were selected as their size aided in dissection and handling. Only larvae that were observed to feed were used in the experiments. The later third instar larvae that had stopped feeding and were in the migratory stage were discarded. The larval medium supplied by FERA consisted of bran, grass meal, dried brewer's yeast, malt extract, dried milk powder and water.

3.2.2 Faecal emulsion

A faecal emulsion was prepared by suspending a human faecal sample (provided by a volunteer) in sterile distilled water (SDW) at a ratio of 1:20 w/v (using 0.1g of faecal matter with 0.9ml of SDW and 1ml of spore suspension) (Borriello and Barclay, 1986, Peach *et al.*, 1986). This emulsion was seeded with *C. difficile* spores using the spore suspension (1×10^6 CFU/ml). A faecal emulsion was used for larval experiments as the larvae were not observed to feed on the spore suspension used in 2.2.2. Just spores and not vegetative cells were examined in this series of experiments, as results in 2.3 showed that spores are the main form of *C. difficile* transferred by *M. domestica*.

3.2.3 Pre-treatment control

A pre-treatment control sample of housefly larvae (n=3) were macerated and analysed as per section 2.2.5.

3.2.4 Isolation of *C. difficile* from the external structures of *M. domestica* larvae

Housefly larvae (n=3) were exposed to *C. difficile* for 30 minutes, by being allowed to move over a sterile Petri dish that had been inoculated with 200µl of the faecal emulsion. Following exposure, individual larvae were transferred to their own sterile Petri dish and cooled to 4°C in a refrigerator, to aid subsequent handling and ligation. Fly larvae mouthparts and anus were then ligated with superglue

(Loctite super glue liquid, Henkel, Hempstead, UK), to prevent expulsion of gut contents during subsequent vortexing. Larvae were washed and analysed as per section 2.2.8. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15. This experiment was replicated three times to give n=9.

3.2.5 Isolation of *C. difficile* from *M. domestica* larvae alimentary canal

Housefly larvae (n=3) were exposed to *C. difficile* as per section 3.2.4 (note: this is not necessary if using larvae that were already exposed in the external structures experiment). Each larva was subsequently washed as per sections 2.2.8 and 2.2.9. The final set of PBS washings was retained and serially diluted down to 10^{-3} in sterile PBS and 0.1ml of each dilution used to inoculate the surface of a CCFA+Tc agar plate. The plates were incubated as per section 2.2.5. The larva alimentary canal and crop were then dissected aseptically (see section 3.2.6), macerated, mixed and analysed microbiologically as per section 2.2.10. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15. This experiment was replicated three times to give n=9.

3.2.6 Dissection of fly larvae

A dissection microscope, iridectomy scissors and fine entomological forceps were used for dissection. The scissors and forceps were sterilised before use as in section 2.2.6. Petri dishes were prepared by melting some wax and pouring it into the dishes. Larvae were dropped into sterile boiling water to kill them. The washed larvae were then transferred onto the wax using sterile forceps, placed ventral side down and fixed in place with 0.19mm entomological pins (Watkins and Doncaster, Kent, UK). These pins were handled with entomological pinning forceps (Watkins and Doncaster, Kent, UK). One of the pins was passed through the head segment and another one through the terminal segment by using the position of the posterior spiracles as a guide to the correct alignment of the pin. The larvae were then immersed in sufficient quantity of PBS (approximately 25ml, depending on the layer of wax deposited) so that the internal structures of the larvae would float freely during dissection. An incision was made at the terminal segment of the larvae with the iridectomy scissors and the incision continued to cut the cuticle upwards towards the head. The larvae were then opened up with the fine forceps and the cuticle pinned down to the side. During dissection, ethanol (70% $^{v}/_{v}$) was added drop-wise to the PBS to 'colour' and therefore enhance visibility of the internal structures of the fly to aid dissection. The exposed gut was then removed with the iridectomy scissors and then transferred to 1 ml of PBS in a sterile 1.5ml universal micro test tube.

This alimentary canal was analysed as per section 2.2.10. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

3.2.7 Retention of *C. difficile* through the life stages of *M. domestica*

Housefly larvae (n=24) were split into three groups of eight individuals and exposed to *C. difficile* for 30 minutes, by being allowed to move over a sterile dish containing 600µl of the faecal emulsion. Then one larva from each Petri dish was washed by vortexing for 30 seconds in 1ml PBS, macerated with the end of a sterile plate spreader, serially diluted down to 10^{-3} in sterile PBS and 0.1ml of each dilution used to inoculate the surface of a CCFA plus Tc agar plate. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15. This sample was considered as Day 0 and was used to confirm the ingestion of *C. difficile* spores.

The remaining larvae were incubated at 30°C in darkness in an incubator (Sanyo Gallenkamp, Loughborough, UK) in the same dishes to allow metamorphosis to proceed. Sterile substrate (sawdust) to allow burrowing and aid successful pupation was included in the sterile Petri dishes, as well as larval medium (see section 3.2.1). The substrate was moistened daily with 0.1ml SDW to prevent desiccation of larvae. These larvae were not ligated as this would prevent development.

From each group, one larva was extracted and examined (externally as per section 3.2.4 and internally as per 3.2.5 and 3.2.6) every alternate day during development, to assess the level of *C. difficile* isolated over time. The plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

As pupation occurred, the pupae were removed into fresh sterile Petri dishes referred to as ‘development plates’. The pupae were extracted (n = 3) each day post-pupation, for microbiological examination externally as per section 3.2.4 and internally via maceration as per section 2.2.9, to determine whether *C. difficile* was present. The plates were incubated as per section 2.2.5 and subsequently observed for characteristic *C. difficile* colony morphology.

Pupae were retained in separate sterile Petri dishes and stored at 30°C in an incubator until adult emergence. Adult flies and empty puparia were examined microbiologically by the described external washing (section 2.2.8) and maceration (section 2.2.9) techniques. The plates were incubated as per

section 2.2.5 and subsequently observed for characteristic *C. difficile* colony morphology. This method was designed to determine whether adult flies emerge with external contamination of *C. difficile* obtained from the puparium, or *C. difficile* had been retained throughout development. This experiment was replicated three times to give n=9 for each life stage of *M. domestica*.

3.2.8 Isolation of bacteria from laboratory *M. domestica* larvae

The laboratory reared, larval houseflies, *M. domestica*, provided by the Insect Supplies Unit at the Food and Environment Research Agency (FERA, York, UK), used as the model organism in aspects of this study, were examined microbiologically in accordance with the method in 2.2.13.

3.3 RESULTS

3.3.1 Pre-treatment control

No colonies were present on the pre-treatment control plates, confirming that the larvae were not contaminated with *C. difficile* prior to being exposed to the bacterial suspensions.

External vs internal control: No *C. difficile* was recovered, indicating that the washing method was sufficient to remove external bacteria prior to maceration.

3.3.2 Isolation of *C. difficile* from the external structures of *M. domestica* larvae

M. domestica larvae (n=9) exposed to the spore suspension and then washed, harboured the following mean *C. difficile* CFUs externally; 222.5 +/- 87.03 1st wash, 22.5 +/- 3.88 2nd wash, 16.67 +/- 1.72 3rd wash and 10 +/- 0 for the 4th wash. Further washes were negative for *C. difficile*. The mean of the total *C. difficile* CFUs isolated from external structures of the larvae was 262.5 +/- 91.79 and 306.25 +/- 103.77 internally.

3.3.3 Isolation of *C. difficile* from *M. domestica* larvae alimentary canal

The mean *C. difficile* CFUs isolated from the alimentary canals of *M. domestica* larvae (n=18) exposed to a 4 x 10^6/ml spore suspension for 30 minutes were 56.36 +/- 21.56. *C. difficile* was not isolated from the 4th wash.

3.3.4 Retention of *C. difficile* through the life stages of *M. domestica*

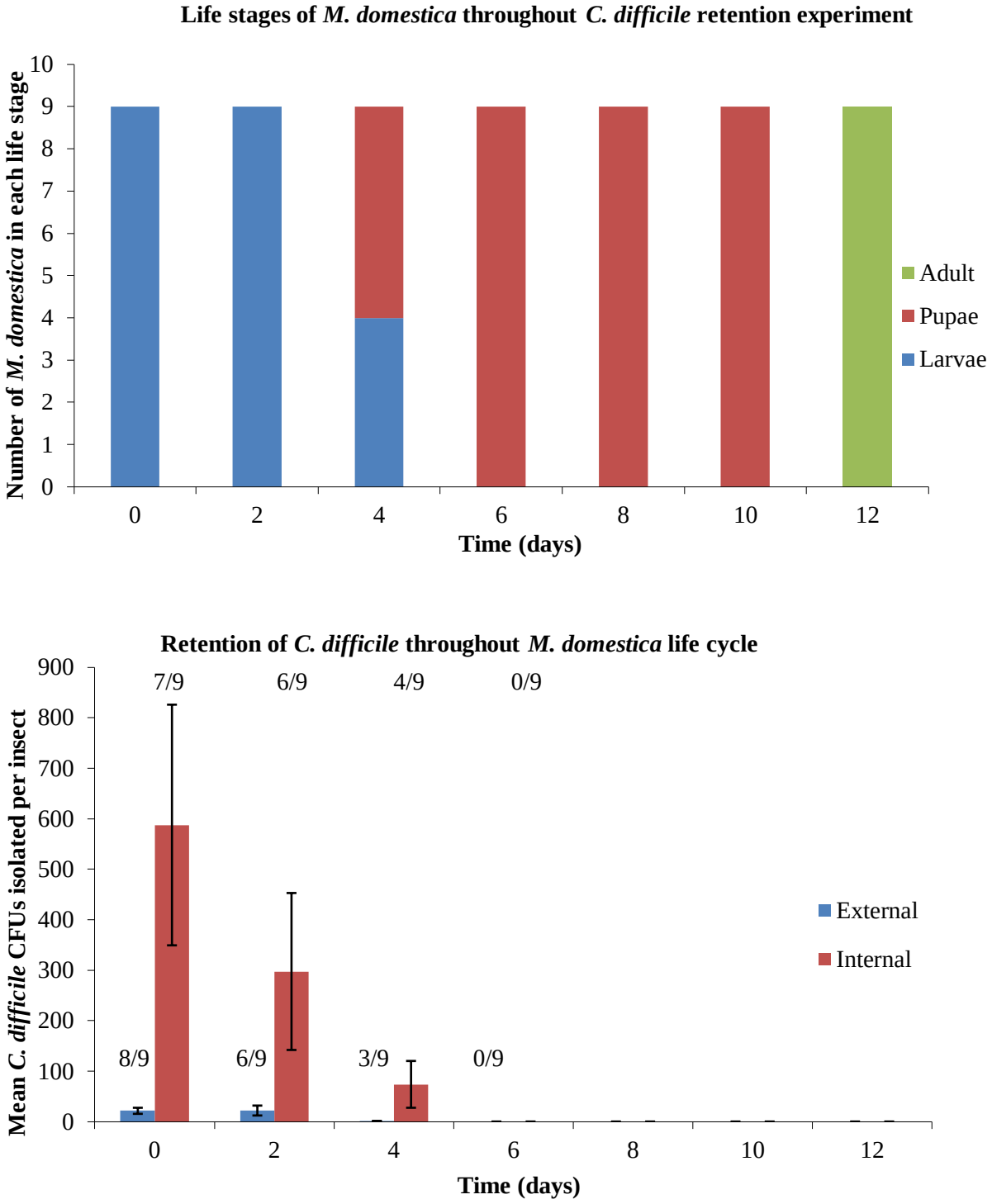

Figure 3.1 Retention of *C. difficile* throughout *M. domestica* life cycle (Mean ± SE CFUs isolated per insect), corresponding with life stages. Numbers above the columns are numbers of positive individuals / number of individuals tested in terms of external and internal isolation of *C. difficile*.

M. domestica larvae (n=9) exposed to the faecal emulsion harboured *C. difficile* externally, with means of 21.56 +/- 5.76 CFUs initially at day 0, 22.44 +/- 9.90 after 2 days, decreasing to 0.56 +/- 0.34 at day 4, with no *C. difficile* isolated thereafter (Figure 3.1). The same *M. domestica* larvae harboured *C. difficile* internally, with means of 587.33 +/- 238.29 CFUs initially at day 0, decreasing to 297.44 +/- 155.21 after 2 days, decreasing further still to 73.67 +/- 46.74 after 4 days, with no *C. difficile* isolated thereafter (Figure 3.1). The zero recovery of *C. difficile* coincided with the development of *M. domestica* larvae into pupae. From Day 6 onwards, all larvae had developed into the pupal stage and no *C. difficile* was isolated from any pupae (Figure 3.1). Adult flies emerged on day 12, from which no *C. difficile* was recovered (Figure 3.1). Empty puparia from which the flies emerged were negative for *C. difficile* spores.

3.3.5 Isolation and identification of bacteria from laboratory *M. domestica* larvae

Bacteria isolated from laboratory reared / insectary-supplied *M. domestica* larvae used as the model organism in this study are listed in Table 3.1.

Table 3.1 A checklist of bacteria isolated from laboratory stock of larval *M. domestica*

Bacteria isolated	**ID Kit code**	**Estimated CFUs per fly per ml (mean)**	**Batch (month & year)**
***Bacillus* sp**			
Bacillus licheniformis	76376276	2,862,500	01 2014
Bacillus licheniformis	77776177	2,862,500	01 2014
Bacillus lentus	76362000	1,500,000	03 2014
**Paenibacillus macerans*	77777420	1,500,000	03 2014
Bacillus licheniformis	16356067	4,820,000	05 2014
Enterobacteriaceae			
Enterobacter cloacae	3305573	3,272,500	01 2014
Citrobacter koseri/ amalonaticus	2344711	3,272,500	01 2014
Providencia rettgeri	0274301	1,408,889	03 2014
Klebsiella oxytoca	5255773	1,408,889	03 2014
Klebsiella oxytoca	1255773	1,408,889	03 2014
**Serratia ureilytica*	7317721 (ADH & URE +ve '*S. marcescens*')	8,406,667	05 2014
Staphylococci			
Staphylococcus aureus		3,060,000	01 2014
Staphylococcus aureus		1,936,667	03 2014
Staphylococcus aureus		1,483,333	05 2014

*Isolated from *M. domestica* for the first time, to the knowledge of the author.

3.4 DISCUSSION

M. domestica larvae, via contact with their external surfaces, may be able to mechanically transfer *C. difficile* in the clinical setting after initial exposure to a deposit of spores, as they harboured the bacterium externally, following contact with a seeded faecal emulsion under experimental conditions. *C. difficile* was isolated specifically from the alimentary canal of *M. domestica* larvae allowed to feed on a seeded faecal emulsion, showing that ingestion of the bacterium occurs. Other authors also report the isolation of pathogenic bacteria from fly larvae; *Providencia rettgeri* has been isolated from the gut of housefly larvae collected from turkey bedding and corn silage (Zurek *et al.*, 2000). In the same study, two mammalian pathogens, *Yersinia pseudotuberculosis* and *Ochrobactrum anthropi*, were isolated from the gut of the housefly larvae.

Although there appears to be no evidence in the literature of *C. difficile* being isolated from housefly larvae (or from larvae of any flies) in field or laboratory studies before, other *Clostridium* species have been recovered from fly larvae. For example, *Clostridium* spp have been found on external surfaces and in the gut of non-biting midge larvae, *Chironomus plumosus*, which were sampled from mud dredged from Lake Winnebago (Rouf and Rigney, 1993). Greenberg (1971) and Greenberg (1973) review other *Clostridium* spp associated with flies. Some of the associations found were; *Clostridium botulinum* with *Lucilia caesar* larvae on poultry farms and from bird carcasses, also *Cochliomyia macellaria* larvae from bird carcasses. These contaminated larvae were fed to healthy birds, which subsequently became infected with *C. botulinum*. Experimental observations have been made regarding housefly larvae and isolation of other bacteria similar to *C. difficile* (in that they are Gram-positive spore-forming rods), such as *Bacillus anthracis* survival through fly development (Graham-Smith, 1914).

Carriage of *C. difficile* continued both externally and predominantly internally through the larval stage, was at its greatest following initial exposure to spores, decreased up to the end of the larval stage, could not be isolated from pupae that developed thereafter and adult flies emerged free of *C. difficile*. These observations suggest that *C. difficile* spores associated with immature stages of *M. domestica* may be destroyed due to changes which occur during metamorphosis, by fly antimicrobial peptides, by other aspects of the fly immune system, or simply excreted.

The observation that *C. difficile* is not retained beyond the larval stage is not unexpected because it is known that larval gut microorganisms are destroyed during metamorphosis and that at the point of emergence, a percentage of adult flies are sterile (Greenberg, 1973). Specifically, the review by Greenberg and Klowden (1972) describes that from the mature larval stage to the prepupa stage of houseflies, there is a greater than 90% reduction in numbers of bacteria, mainly due to feeding ceasing and evacuation of bacteria from the larval gut continuing. A further reduction in the number of

bacteria occurs when the larval foregut and hindgut are shed during pupation and become deposited in the puparium upon adult fly emergence. It is also thought that destruction and synthesis of structures and general reorganisation of tissues in metamorphosis probably reduces the amount of bacteria present. These factors all contribute to 17% of houseflies being sterile upon emergence.

Competition with normal housefly gut flora appears to be a main reason why experimentally introduced bacteria are not retained during metamorphosis. Work by Greenberg, referred to in the aforementioned review (Greenberg and Klowden, 1972), confirms the studies of earlier researchers, that bacteria such as *Salmonella* and *Shigella*, when introduced to larvae with normal gut flora, are unable to survive pupation and were not isolated from any adults that emerged. In the same experiment, the introduced bacteria could not even be isolated from the majority of the fly larvae. Only by using aseptic rearing techniques and gnotobiotic flies could experimentally introduced bacteria at the larval stage be recovered from pupae and adults. Even then, although the bacteria survived metamorphosis, there was still a reduction in numbers. The conclusion of the review by Greenberg and Klowden (1972) was 'the adult fly has the most potential for disease transmission, as the maggot has limited motility and possesses autosterilization mechanisms which limit its capacity to carry pathogens over into the adult stage'.

Of the 'autosterilization mechanisms' described in the review by Greenberg and Klowden (1972), secretions of fly larvae and specifically antibacterial peptides from *M. domestica* and *Lucilia sericata* have been shown to be active against bacteria (Wang *et al.*, 2006, Liang *et al.*, 2006, Ratcliffe *et al.*, 2011). Larval secretions of the blowfly *L. sericata* have a bactericidal property against *Staphylococcus aureus*, Haemolytic streptococci and *Clostridium perfringens* (Simmons, 1935). An antibacterial peptide, seraticin, has been extracted from larvae of *L. sericata* and shows activity against MRSA and *C. difficile* as well as a range of Gram-positive (e.g. *Bacillus cereus*) and Gram-negative (e.g. *E. coli*) bacteria (Bexfield *et al.*, 2008). It is possible that the presence of antibacterial peptides in *M. domestica* larvae could be influential in the lack of retention of *C. difficile* through metamorphosis and although yet to be discovered in *M. domestica*, some insects (the Korean dung beetle, *Copris tripartitus*) do possess antimicrobial peptides (coprisin) with activity against *C. difficile* (Kang *et al.*, 2011).

However, some researchers present different evidence regarding the retention of bacteria through metamorphosis of flies. When *E. coli* is fed experimentally to housefly and stable fly larvae, it persists through the pupal stage (Rochon *et al.*, 2005). The *E. coli* population increased in the early stages of pupal development, before declining prior to emergence of adult houseflies. In stable flies, the *E. coli* population increased and remained at a high level during pupal development. The fly puparia were also examined for the presence of *E. coli* and the shed puparia of the stable fly usually contained more of the bacteria than that of the housefly. All housefly puparia were positive for *E. coli*. *E. coli* was

then isolated from the external surfaces of 72% of emerged adult houseflies and the internal structures of 66% (Rochon *et al.*, 2005). *Vibrio cholerae* is another species of bacteria that has recently been shown to be capable of surviving through fly metamorphosis. Larvae of the non-biting midge, family Chironomidae, were experimentally exposed to *V. cholerae* while in flasks of water and the flying adults that emerged were caught and found to be positive for the bacteria (Broza *et al.*, 2005). Further evidence shows that non-sporing bacteria, such as *Bacillus typhosus, B. enteritidis* and *B. dysenteriae* are not found in association with adult flies derived from larvae experimentally exposed to these species but the spore-forming *Bacillus anthracis* does survive (Graham-Smith, 1914).

Isolation of bacteria from laboratory M. domestica larvae

The consistent finding of *S. aureus* in *M. domestica* larvae indicates handling of specimens and rearing materials by insectary staff, as this bacterium is a common commensal of human skin (Kock *et al.*, 2010).

Serratia ureilytica was identified from *M. domestica* larvae, although the API20E identification kit gave a 'doubtful' identification of *Serratia marcescens*, with 'tests against' being listed as positive results for both ADH (arginine dihydrolase) and URE (urease). This doubtful result for *S. marcescens* prompted a literature search for *Serratia* species that are ADH and URE positive, which led to an identification of the urea-utilising novel species *Serratia ureilytica* that is isolated from water (Bhadra *et al.*, 2005) and is not included in the API20E database. The association of *Serratia ureilytica* from *M. domestica* larvae can be explained by the urea-utilisation of this bacterium, as it would be able to derive nutrition from urea and uric acid, which are excretory products of insects (Imms *et al.*, 1977). A further explanation is that *S. ureilytica* produces chitinase (an enzyme which metabolises chitin, a major component of the arthropod exoskeleton) and uses this to derive nutrition from shrimp shells (Wang *et al.*, 2009), so it may also be surviving by utilising chitin from *M. domestica*. To the knowledge of the author, the isolation of *Serratia ureilytica* from *M. domestica* larvae represents the first case of *Serratia ureilytica* isolated from insects.

Providencia rettgeri was isolated from larvae. This finding is to be expected, as *P. rettgeri* has been isolated from *M. domestica* supplied from insectaries, in a study where the houseflies were used as a model organism in experiments examining the transmission of a particular pathogen by flies, which was not dissimilar to this study (Grubel *et al.*, 1997).

To the knowledge of the author, *Paenibacillus macerans* was isolated from *M. domestica* for the first time. *Paenibacillus macerans* is a Gram-positive (in young cultures - inconstant in older cultures), aerobic and facultatively anaerobic, spore-forming (oval shaped spores that are terminal in position),

rod-shaped, motile bacterium (Cowan *et al.*, 2003). *P. macercans* is abundant in nature and has been noted as a contaminant of the hospital environment (Noskin *et al.*, 2001).

The fact that different species of bacteria are found in the insectary-supplied *M. domestica* larvae versus adults could be explained by evidence showing that the vast majority of larval gut microorganisms are destroyed during metamorphosis (Greenberg, 1973), perhaps allowing adult flies to acquire flora that is distinct to that of their immature stages. Some species of bacteria were similarly found in both the larval and adult stages of *M. domestica*, which points to either a similarity in the bacterial fauna of their respective environments, or retention through their life stages. For example, a number of authors have shown that housefly larvae retain a considerable number of bacteria acquired at this stage, through to the pupal stage and finally adulthood (Glaser, 1923) and it is considered that retention of *E. coli* from larval to adult houseflies could play a role in the transmission and spread of *E. coli* (Rochon *et al.*, 2005).

The many different species of bacteria isolated in great quantities from laboratory / insectary-supplied *M. domestica* larvae illustrated perfectly the need for selective CCFA plates, when examining the retention of *C. difficile* throughout the life stages of the model organism, the housefly.

3.5 CONCLUSION

From the observations made in this study, it is apparent that adult *M. domestica* are the most important life stage in the transfer of *C. difficile* and acquire this bacterium from the environment rather than via retention through larval and pupal stages. The potential antimicrobial action of *M. domestica* larvae and their extracts against *C. difficile* should form the basis of a future study.

4 CHAPTER 4: SURVIVAL OF *CLOSTRIDIUM DIFFICILE* IN ASSOCIATION WITH *MUSCA DOMESTICA* ELECTROCUTED IN AN ELECTRONIC FLY KILLER (EFK)

4.1 INTRODUCTION

It is recognised that adhesive light traps are an effective method of sampling flying insects in hospitals (Da Silva *et al.*, 2011). It is the author's personal experience that ultra-violet (UV) light flytraps in the form of professional sticky traps (adhesive traps) are present in hospitals in the UK, as well as Electronic Fly Killers (EFKs), both of which were used for sampling purposes in this thesis chapter.

As the effects of electricity are known to kill bacteria (Hülsheger *et al.*, 1981), it was deemed important to investigate the survival of *C. difficile* associated with flies electrocuted in EFKs. If EFKs were to be a viable method of collecting flying insects and then examining them for the presence of *C. difficile*, this pathogen would need to be able to survive the electrocution process.

EFKs and professional sticky traps are used as a component of integrated flying insect control in UK hospitals, which is why they are present at such sites. They also serve another purpose in that they were identified in this study as a useful tool for sampling flying insects in hospitals. Research suggests that EFKs in hospitals actually present their own problems in terms of transfer of pathogens. The flying insects captured by them are a potential source of bacterial contamination of the local environment, as the spread / release of *Serratia marcescens* during electrocution of houseflies has been reported (Urban and Broce, 2000). In the same study, each dead fly was potentially almost as contaminated as it was when it was alive. The survival of *S. marcescens* in association with houseflies and following their electrocution by an EFK has been determined (Cooke *et al.*, 2003). *S. marcescens* survived on and within the housefly corpses for up to five weeks after electrocution.

The results of the studies by Urban and Broce (2000) and Cooke *et al.* (2003) therefore show that EFKs, despite their electrocuting function, do permit survival of Enterobacteriaceae in association with electrocuted flies, so are a viable sampling method for the purposes of this thesis. However, during the time that this chapter was initiated and completed, there existed no available evidence that *C. difficile*, a focus of this thesis, would survive electrocution in an EFK.

The aim of this chapter was to determine the initial and long-term survival of *C. difficile* associated with flies that have been exposed to independent vegetative cell and spore suspensions and subsequently electrocuted in an Electronic Fly Killer (EFK). This was undertaken in order to assess the suitability of using EFKs as a sampling technique to recover viable *C. difficile* from flying insects in UK hospitals.

Since this chapter's study was concluded, *C. difficile* has been recovered from flies sampled 'in the field' via adhesive fly papers and electrocuting fly traps (Burt *et al.*, 2012).

4.2 MATERIALS AND METHODS

4.2.1 Electronic fly killer (EFK)

The EFK used in these experiments was a Titan 300 (PestWest Ltd, Ossett, UK), operated according to manufacturer's instructions.

Figure 4.1 Titan 300 (PestWest Ltd, Ossett, UK)

4.2.2 Initial survival of *C. difficile* spores

Control (pre-electrocution) houseflies (n=5) were exposed to *C. difficile* for 30 minutes, by being allowed to walk over a CCFA plus Tc agar plate that had been inoculated with 0.1ml of the $1x10^6$/ml spore suspension. The flies were inactivated by incubation in a sterile Petri dish in a -18°C freezer for two minutes. Control flies were then macerated in 1ml PBS with the end of a sterile plate spreader. A 0.1ml volume of the macerate was inoculated onto CCFA plus Tc agar and the plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

Treatment (post-electrocution) houseflies (n=5) were exposed to *C. difficile* for 30 minutes, by being allowed to walk over an agar plate that had been inoculated with 0.1ml of the $1x10^6$/ml spore suspension. The treatment flies were inactivated by incubation in a sterile Petri dish in a -18°C freezer for two minutes and introduced onto the 'killing grid' of the EFK. The flies were introduced into the EFK killing grid by hooking them up with a sterile disposable inoculation loop. The electrocuted flies were subsequently macerated with the end of a sterile plate spreader in 1ml PBS. A 0.1ml volume of the macerate was inoculated onto CCFA plus Tc agar and the plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

4.2.3 Long-term survival of *C. difficile* spores

This experiment used the same methodology as section 4.2.2 for treatment flies, except all flies were stored in the EFK catch tray, to assess *C. difficile* spore survival over time and were sampled (n=5) once every month for three months.

4.2.4 Initial survival of *C. difficile* vegetative cells

Control (pre-electrocution) houseflies (n=5) were exposed to *C. difficile* for 30 minutes by being allowed to walk over a CCFA agar plate that had been inoculated with 0.1ml the $1x10^6$/ml vegetative cell culture. After exposure, flies were not inactivated by incubation in a sterile Petri dish in a -18°C freezer for two minutes, as this may influence vegetative cell survival or cause sporulation. Control flies were then macerated in 1ml PBS. A 0.1ml volume of the macerate was inoculated onto the surface of CCFA and CCFA plus Tc agar and the plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

Treatment (post-electrocution) houseflies (n=5) were exposed to *C. difficile* vegetative cells in the same way as the control group. The treatment flies were then immediately introduced onto the 'killing grid' of the EFK. The electrocuted flies were subsequently macerated with the end of a sterile plate spreader in 1ml PBS. A 0.1ml volume of the macerate was inoculated onto CCFA and CCFA plus Tc agar and the plates were incubated as per section 2.2.5, subsequently observed for characteristic *C. difficile* colony morphology and an example of a presumptive colony identified as per section 2.2.15.

4.2.5 Long-term survival of *C. difficile* vegetative cells

This experiment used the same methodology as section 4.2.4 for the treatment flies, except all flies were stored in the EFK catch tray, to assess *C. difficile* vegetative cell and spore survival over time and were sampled (n=5) once every month for three months.

4.3 RESULTS

4.3.1 Initial and long-term survival of *C. difficile* spores

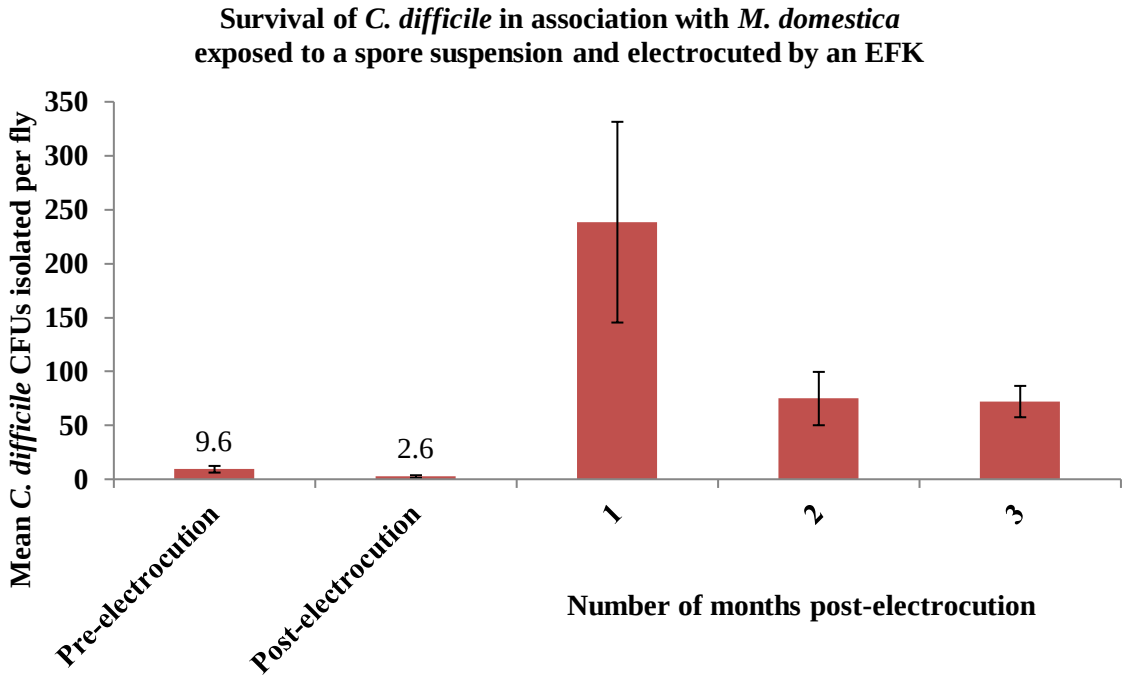

Figure 4.2 The survival of *C. difficile* in association with *M. domestica* over time, following fly exposure to a 1 x 10^5 spore solution and subsequent electrocution by an EFK. Shown as mean CFUs per fly ± Standard Error (SE) (n=5 for each data point).

At time point zero for the pre-electrocution group of flies, the mean CFUs isolated per fly and representing spore recovery were 9.6 +/- 3, which was higher than that of the post-electrocution flies at 2.6 +/- 1.4, showing a drop of 7 CFUs immediately following electrocution (Figure 4.2). Of the *M. domestica* sampled at 1, 2, and 3 months post-electrocution, the mean *C. difficile* CFUs isolated per fly were 238.4 +/- 92.8, 75.2 +/- 24.7 and 72 +/- 14.7 respectively (Figure 4.2).

4.3.2 Initial and long-term survival of *C. difficile* vegetative cells

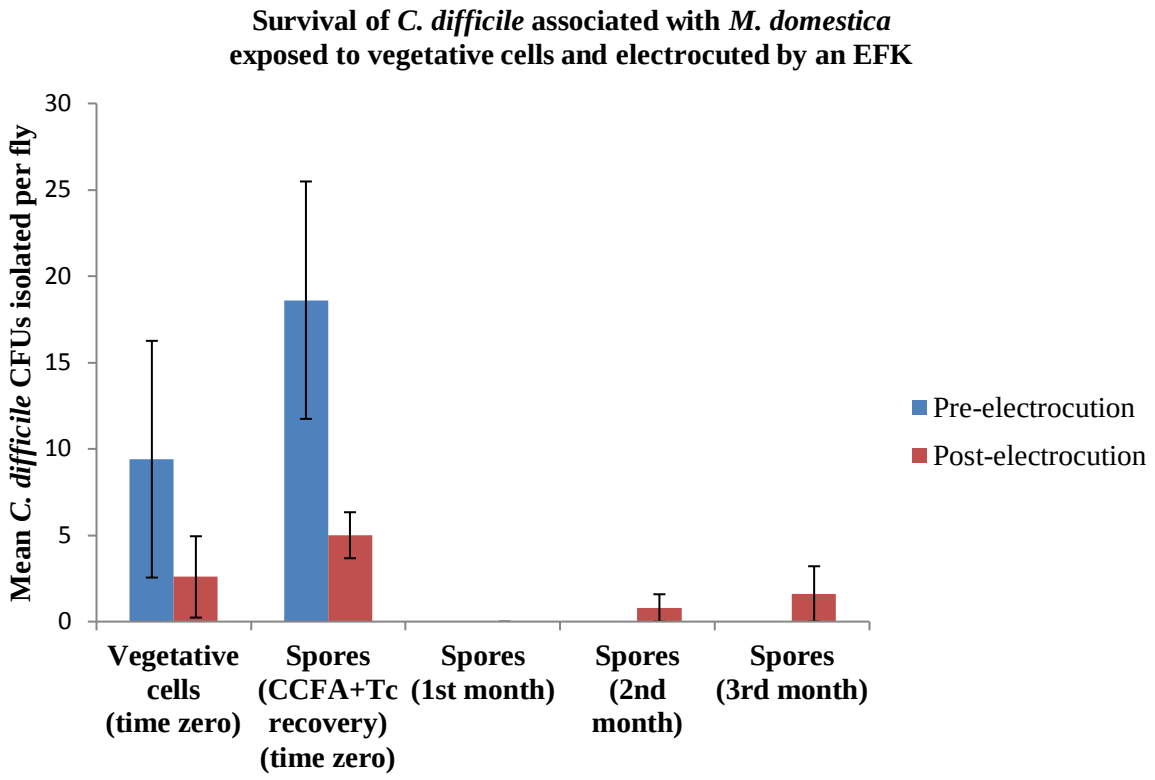

Figure 4.3 The survival of *C. difficile* in association with *M. domestica* over time, following fly exposure to a 1 x 10^5 vegetative cell solution and subsequent electrocution by an EFK. Shown as mean CFUs per fly ± SE (n=5 for each data point).

At time point zero for the pre-electrocution group of flies, the mean CFUs isolated per fly and representing vegetative cell recovery were 9.4 +/- 6.9, which was higher than that of the electrocuted flies at 2.6 +/- 2.4, showing a drop of 6.8 CFUs immediately following electrocution (Figure 4.3). At time point zero for the pre-electrocution group of flies, the mean CFUs isolated per fly and representing combined vegetative cell and spore recovery was 18.6 +/- 6.9, which was higher than that of the electrocuted flies at 5.0 +/- 1.3, showing an overall drop of 13.6 CFUs immediately following electrocution (Figure 4.3). As an approximation of spore recovery, the CFUs of vegetative cells (time zero) for pre and post-electrocution can be subtracted from the CFUs for the combined vegetative cell and spore recovery shown by CCFA+Tc recovery (time zero). The approximation of spore recovery is therefore 9.2 CFUs pre-electrocution and 2.4 CFUs post-electrocution, showing a drop of 6.8 CFUs immediately following electrocution.

After the initial electrocution of *M. domestica*, minimal *C. difficile* was isolated from flies that were kept in the catch tray and sampled monthly. No *C. difficile* was isolated from *M. domestica* sampled at 1 month post-electrocution (Figure 4.3). Of the *M. domestica* sampled at 2 months and 3 months post-electrocution, the mean *C. difficile* CFUs isolated per fly were 0.8 +/- 0.8 and 1.6 +/- 1.6 respectively (Figure 4.3).

4.4 DISCUSSION

Results of the laboratory work regarding the survival of *C. difficile* after *M. domestica* electrocution in an EFK are of significance because it has been shown that viable bacteria can be isolated under these experimental circumstances. The relevance of this is that the field sampling of flying insects from EKFs and subsequent microbiological analysis should be worthwhile based on the laboratory study and if *C. difficile* is present in field samples it is expected that it will be isolated using the described laboratory techniques. In some hospitals, EFK and sticky trap maintenance (and therefore sampling as part of this study) takes place once every three months. It is therefore possible that some field sampled flying insects will have been in the EFKs or sticky traps for up to three months prior to collection and analysis, so if *C. difficile* was initially present on the insects it would need to be viable for the extent of this period in order for successful isolation to occur. The laboratory studies have shown that *C. difficile* can be isolated from flies after they were exposed to vegetative cell and spore suspensions, subsequently electrocuted in the EFK and then remained in the catch-tray for up to three months. This suggests that if a similar situation were to occur in the field, then *C. difficile* could be isolated successfully.

Since this experiment was performed, *C. difficile* has been recovered from flies sampled 'in the field' via adhesive fly papers and electric fly traps, providing confirmatory results that viable *C. difficile* can be recovered from electrocuted flies (Burt *et al.*, 2012).

The variability of the data in Figure 4.2 is great, especially when comparing the low bacterial load of pre-electrocution flies and post-electrocution flies with the much higher bacterial load of flies sampled in the following months. This could be explained by the variability in the initial bacterial loading of flies when they were experimentally exposed to *C. difficile*. For example, the method of exposing *M. domestica*, described in 4.2.2 is imperfect, as the behaviour of individual flies is variable, so each fly may not have had the same amount of contact with the bacterial inoculum due to differential levels of walking behaviour. Although this method of exposure may be seen as imperfect, this technique was decided upon as it more closely represents bacterial exposure scenarios of flies in field conditions. The flies sampled at pre-electrocution versus those sampled in the following months were distinct from eachother and may simply have acquired differing levels of *C. difficile* contamination at the initial exposure stage, thus providing an explanation for the great variability in results. However, the initial bacterial loading of flies pre-electrocution was relatively consistent (as shown by small error bars), so differences in initial bacterial exposure may not be the only explanation. Bacteria are released from electrocuted flies (Urban and Broce, 2000) so the observed variability in the data could also be due to this, as *C. difficile* could have been released from electrocuted flies onto individuals already in the catch tray, thus adding to their levels of contamination. High levels of variability are

seen in the data from another study on EFKs, with levels of *S. marcescens* being '10- to 100-fold higher' in electrocuted flies compared to pre-electrocution samples, although this was attributed to subsequent bacterial replication on fly corpses (Cooke *et al.*, 2003), which would not take place with the strictly anaerobic *C. difficile*.

Electrocution of flies in EFKs results in the death of these insects, although the exact lethal mechanisms have not been determined in the scientific literature. It is likely that the heat resulting from electrocution leads to rapid dehydration of flies and the electricity produced causes fatal electrical disruption of the fly nervous system, resulting in insect mortality. The effects of electricity and heat appeared to impact on the viability of *C. difficile* associated with *M. domestica*. An immediate effect of electrocution was shown, in that levels of *C. difficile* in *M. domestica* were lower in flies sampled immediately post-electrocution compared to pre-electrocution flies. This difference was similar for vegetative cells and spores, so it is possible that the viability of *C. difficile* vegetative cells and spores are both reduced following electrocution. This could be explained by the fact that the effects of electricity are known to kill vegetative bacteria (Hülsheger *et al.*, 1981) and that *C. difficile* spores show heat resistance. For example, dormant *C. difficile* spores survive following heat shock at 80°C (Nerandzic *et al.*, 2009) and even when heated at 100°C for 10 minutes (Nakamura *et al.*, 1985), while vegetative *C. difficile* cells are killed when heated at 60°C (Sorg and Sonenshein, 2008).

Due to the fact that *M. domestica* remained contaminated with *C. difficile* following electrocution in an EFK, frequent removal of fly corpses from EFKs should be undertaken as an aid to infection control, as well as disinfection of such devices.

As bacteria are released from flies electrocuted in EFKs (Urban and Broce, 2000) they should not be the preferred form of UV light flytraps used in hospitals. Professional sticky traps should be used instead, as they retain the flies and therefore prevent expulsion of bacterially contaminated insect fragments onto food and fomites.

4.5 CONCLUSION

C. difficile was isolated from *M. domestica* after they were exposed to vegetative cell and spore suspensions and subsequently electrocuted in an EFK, even after the flies had remained in the catch-tray for up to three months. This suggests that if a similar situation were to occur in the field, then *C. difficile* could be isolated successfully, showing that the sampling of flies from EFKs and subsequent microbiological analysis of these insects is a viable method in terms of detecting contamination with *C. difficile*. This method of sampling gives a good assessment of the carriage of *C. difficile* by *M.*

domestica and therefore vector potential, even a number of months after an initial bacterial exposure event.

5 CHAPTER 5: KILLGERM CHEMICALS INSECT IDENTIFICATION SERVICE – DATABASE ANALYSIS

5.1 INTRODUCTION

Data from technical advisory services for the public health pest control industry are available in the form of databases, which detail the number of enquiries received that are related to insects and include information on their seasonality and location. Such data can be used to supplement and complement sampling efforts in the field. Data from these services can also act as a predictor of likely results that may be found in specific practical collection efforts instigated by researchers. This prior knowledge can help in the design of appropriate sampling techniques for use in the field.

A further benefit of technical advisory services is that they provide a valuable resource of longitudinal data collected over extended periods of time, often many years. This can be especially useful, as specific and targeted field sampling of insects for research purposes is often time-limited, due to constraints of the study, financially or otherwise. For example, the Urban Pest Advisory Service (UPAS) in Zurich is able to present data on the incidence of household arthropod pests over a period of 17 years, including information on temporal and locational changes in pest incidence (Mueller *et al.*, 2011). The Danish Pest Infestation Laboratory (DPIL) has data regarding the pest related enquiries received by their advisory service, for every month since 1965 (Kilpinen *et al.*, 2008).

The Killgerm Chemicals technical department operates a technical advisory service, which is similar in many aspects to the services provided by UPAS and DPIL. The Killgerm Chemicals insect identification service (KCIIS) has 13 years of data and provides over 4,000 insect identification reports annually, issuing detailed information on the insects identified, including their significance as pests and recommendations for their control. The source of the KCIIS data is insect samples submitted predominantly by pest controllers from private firms and local authorities and also environmental health practitioners. Submissions to the service are dated and include location details, which allows for their analysis alongside the species that are identified. The KCIIS is a general service for identification of all insect pests encountered in public health pest control and it was decided that exploring these data and extracting specific records of insects from hospitals would be of benefit to this study, in terms of guiding the study, as well as complementing and supplementing data from field sampling of insects in UK hospitals. Access to the KCIIS data was provided by the Killgerm Chemicals technical department.

The aim of this chapter was to analyse the pre-existing database containing data on insects identified in UK hospitals, in order to classify and enumerate the reports of insects and establish their seasonality and location in such premises, therefore complementing other aspects of this study with the end result of informing pest control measures.

5.2 MATERIALS AND METHODS

Data from the KCIIS database was analysed with Microsoft Access and Microsoft Excel. Data relating to insects identified from UK hospitals was extracted.

5.3 RESULTS

5.3.1 Checklist of flying insects found associated with UK hospitals (KCIIS database 2000 – 2013)

Table 5.1 A checklist of flying insects found associated with UK hospitals, from KCIIS data from the year 2000 to the year 2013.

ORDER	FAMILY	SPECIES NAME	COMMON NAME
Coleoptera	Scarabaeidae	*Melolontha melolontha*	Maybug
		Serica brunnea	Brown chafer
Diptera	Anisopodidae	*Sylvicola fenestralis*	Window gnat
	Asilidae	Asilidae	Robber fly
	Bibionidae	*Bibio* sp	Bibionid fly
		Dilophus febrilis	Fever fly
	Calliphoridae	*Calliphora* sp	Blowfly / bluebottle
		Calliphora vicina	Blowfly / bluebottle
		Calliphora vomitoria	Blowfly / bluebottle
		Lucilia caesar	Greenbottle
		Pollenia rudis	Cluster fly
	Cecidomyiidae	Cecidomyiidae	Gall midge
		Jaapiella veronicae	Gall midge
	Ceratopogonidae	Ceratopogonidae	Biting midge
	Chironomidae	Chironomidae	Non-biting midge
		Chironomus plumosus	Non-biting midge
		Chironomus sp	Non-biting midge
	Culicidae	*Culex pipiens*	Mosquito
		Culiseta annulata	Mosquito
	Drosophilidae	*Drosophila* sp	Fruit fly
	Fanniidae	*Fannia canicularis*	Lesser housefly
	Muscidae	*Dasyphora cyanella*	Green cluster fly
		Dasyphora cyanicolor	Blue cluster fly
		Mesembrina meridiana	Noon fly
		Musca autumnalis	Autumn fly
		Musca domestica	Housefly
		Phaonia viarum	Muscid fly

		Polietes lardaria	Muscid fly
	Mycetophilidae	Mycetophilidae	Fungus gnat
	Phoridae	*Megaselia* sp	Phorid fly
		Phoridae	Phorid / scuttle fly
	Psychodidae	*Psychoda alternata*	Owl midge
		Psychoda sp	Owl midge
		Psychodidae	Owl midge
	Scathophagidae	Scathophagidae	Dung fly
	Scatopsidae	*Scatopse notata*	Scatopsid fly (dung midge)
		Scatopsidae	Scatopsid fly (dung midge)
	Sciaridae	*Sciara* sp	Mushroom fly
		Sciara thomae	Mushroom fly
		Sciaridae	Sciarid fly
	Sepsidae	Sepsidae	Black scavenger flies
	Sphaeroceridae	Sphaeroceridae	Lesser dung flies
	Syrphidae	Syrphidae	Hoverfly
	Tipulidae	Tipulidae	Tipulid fly (daddy-long-legs)
Hemiptera	Aphididae	Aphididae	Greenfly
Hymenoptera	Apidae	*Andrena* sp	Solitary mining bee
		Anthophora sp	Solitary bee
		Osmia rufa	Red mortar bee
	Bethylidae	Bethylidae	Parasitic wasp
	Braconidae	Braconidae	Parasitic wasp
	Cynipidae	Cynipidae	Gall wasps
	Formicidae	*Hypoponera punctatissima*	Roger's ant
		Lasius niger	Black ant
	Ichneumonidae	Ichneumonidae	Ichneumon fly (parasitic wasp)
		Pimpla instigator	Ichneumon fly (parasitic wasp)
	Pteromalidae	Pteromalidae	Parasitic wasp
	Vespidae	*Vespa crabro*	Hornet
		Vespula vulgaris	Common wasp
Lepidoptera	Geometridae	Geometridae	Geometer moth
	Noctuidae	*Autographa gamma*	Silver Y moth
		Noctuidae	Night flying moth
	Oecophoridae	*Hofmannophila pseudospretella*	Brown house moth
	Pyralidae	*Ephestia elutella*	Warehouse moth

		Plodia interpunctella	Indian meal moth
	Tineidae	*Nemapogon granella*	Grain moth
		Tinea pellionella	Case-bearing clothes moth
		Tineola bisselliella	Clothes moth
Psocoptera	Liposcelidae	*Lachesilla pedicularia*	Winged booklouse
Thysanoptera	Thysanoptera	Thysanoptera	Thrip
Trichoptera	Trichoptera	Trichoptera	Caddis fly
		No evidence	Unconfirmed report of insects - possible illusory parasitosis case.

Table 5.1 shows the great diversity of flying insects recorded in UK hospitals as part of the KCIIS records from 2000 – 2013, while serving as an example of the fauna that is expected to be encountered in future field-sampling work and subsequently assessed for bacterial carriage.

The KCIIS data shows that flying insects from eight Orders were recorded in UK hospitals from 2000-2013. Beetles (Order Coleoptera) were represented by one family, the scarab beetles (Scarabaeidae), containing two species, the maybug *Melolontha melolontha* and the summer chafer *Serica brunnea*. The true flies / two-winged flies (Order Diptera) were represented by 21 families, containing an estimated 42 species, meaning that this Order was the most recorded and the most speciose. The number of species is an estimate, as in some cases speciation was not possible, so a reference to genus or family level is counted as a separate record. The true bugs (Hemiptera) were represented by one family, the aphids / greenfly (Aphididae). Wasps, ants and bees were represented by eight families, containing an estimated 13 species. There were three species of solitary bees, six species of parasitic wasp, two species of ant and two species of social wasps. Moths (Order Lepidoptera) were represented by five families, containing nine species. Three Orders were represented by one species each; winged booklouse *Lachesilla pedicularia* (Order Psocoptera, family Liposcelidae), thrip (Order Thysanoptera) and caddis fly (Order Trichoptera).

A total of approximately 70 species of flying insect were recorded in UK hospitals from 2000 – 2013 as part of the KCIIS. The use of word ‘approximate’ refers to the fact that a number of individuals were identified to Order or family or genus level only.

5.3.2 Flies associated with UK hospitals (KCIIS database 2000 – 2013)

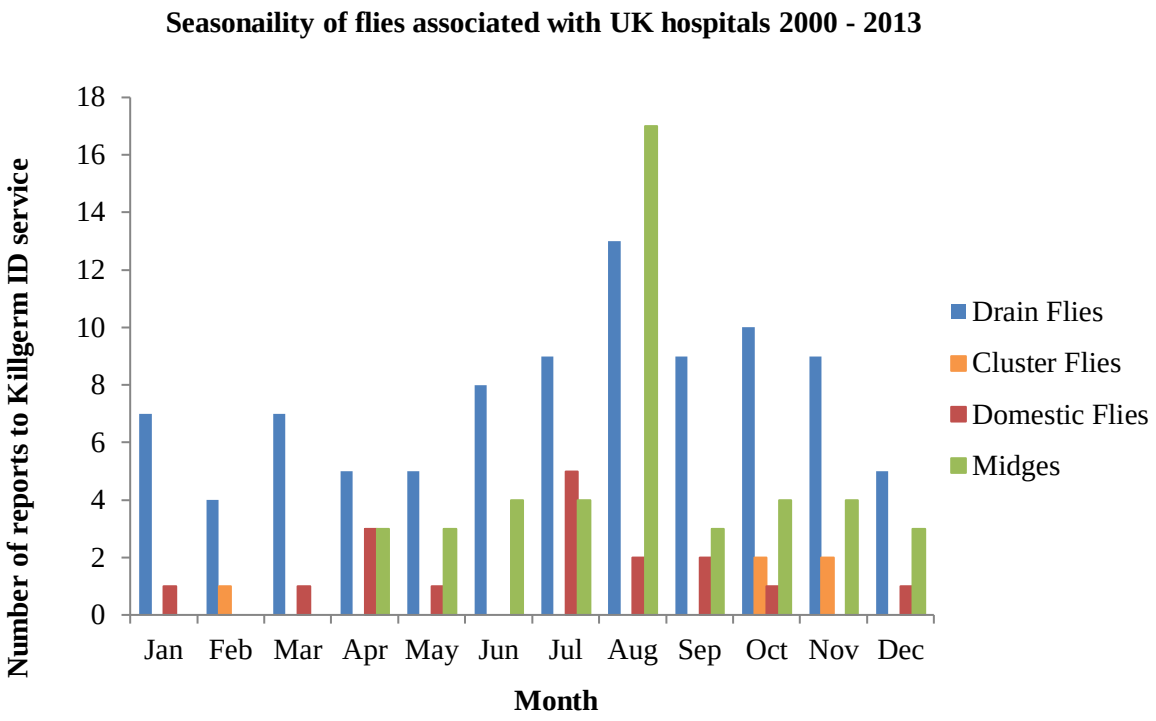

Figure 5.1 Seasonality of flies associated with UK hospitals 2000 – 2013. The number of reports to the KCIIS (pooled monthly) of different groups of flies associated with UK hospitals, from the year 2000 to the year 2013.

'Drain Flies' category pools records of the rotting organic matter breeding *Drosophila* sp, Phoridae, Mycetophilidae, Psychodidae, Sciaridae, Scatopsidae, Sphaeroceridae and Sepsidae.

'Cluster Flies' category pools records of the overwintering and clustering *Pollenia rudis, Dasyphora cyanella, Dasyphora cyanicolor Thaumatomyia notata* and *Musca autumnalis*.

'Domestic Flies' category pools records of the rotting organic matter breeding houseflies *Musca domestica* and *Fannia canicularis*. Flesh breeding *Calliphora* sp and *Lucilia* sp are also included.

'Midges' category pools the records of various swarming flies and flying insects whose breeding media is not likely to be on site. Includes mosquitoes (*Culex pipiens, Culiseta annulata*), Chironomidae, Ceratopogonidae, Cecidomyiidae, Cynipidae, Tipulidae and *Sylvicola fenestralis*.

Flies are indeed found in UK hospitals, as shown by Table 5.1 and Figure 5.1.

'Drain flies' were present in UK hospitals throughout the year and they were consistently the most commonly reported flies in every month apart from August, when 'midges' were most numerous with 17 records, which was also their peak. Records of 'drain flies' peaked in August (13) and they were the most commonly recorded group of flies in total throughout the entire time period for the data (the years 2000 – 2013), with 91 records, compared to 45, 17 and 5 records for 'midges', 'domestic flies' and 'cluster flies' respectively (see Figure 5.1). The number of 'domestic flies' peaked in July, with five records. Cluster flies were recorded once in February and twice in both October and November. The greatest total number of records of flies was in August (32), followed by July (18), October (17) and November (15) respectively. When the data are looked at seasonally, total records of flies peaked in summer (62), were second highest in autumn (46), lower in spring (28) and lowest in winter (22).

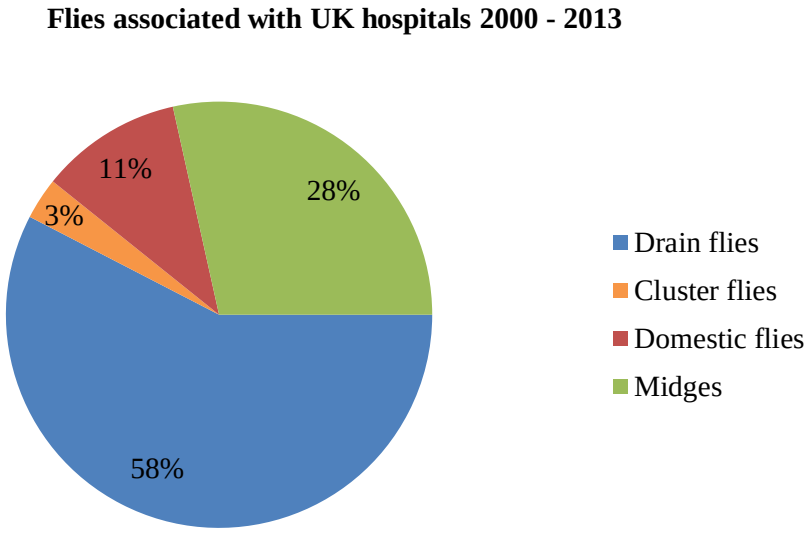

Figure 5.2 Flies associated with UK hospitals 2000 – 2013 (the percentage of different groups of flies associated with UK hospitals from 2000 – 2013, from KCIIS data). The fly groups are defined in Figure 5.1.

The data in Figure 5.2 are an adaptation of the data shown in Figure 5.1 previously, in order to give a clearer visual picture of the overall prevalence of fly groups. This shows that 'drain flies' were the most commonly recorded group of flies in total throughout the entire time period for the data (the years 2000 – 2013), comprising 58% of records, compared to 28%, 11% and 3% of records for 'midges', 'domestic flies' and 'cluster flies' respectively.

5.3.3 Specific locations of flying insect activity in UK hospitals

Table 5.2 Specific location records of flying insect activity in UK hospitals from KCIIS data, from 2000 – 2013

Location category	Specific location	Flying insect species
Food preparation	Food production area of hospital.	Phoridae (*Megaselia* sp)
	Ward kitchens.	Chironomidae
Non-hospital healthcare	Dental hospital.	Mycetophilidae, *Psychoda* sp, Tipulidae
	Nursing home kitchens, hallways and bedrooms.	Phoridae
	Resident's room at a care home.	Pteromalidae
Non-patient areas	Hospital linen.	*Anthophora* sp
	Hospital office	*Bibio* sp, Chironomidae, Mycetophilidae, *Psychoda* sp
	Light fittings	*Pollenia rudis*
	Staff room of an eye clinic.	*Psychoda* sp
Operating / surgery	Children's hospital operating theatre.	Phoridae
	Operating theatres.	*Culex pipiens*
	Surgery	Phoridae
Treatment areas	General ward	*Psychoda* sp
	Hospital wards	*Calliphora vomitoria*
		Chironomidae
		Culiseta annulata
		Hypoponera punctatissima
		Musca domestica
		Phaonia viarum
		Pollenia rudis
		Sciaridae
	Labour ward	*Psychoda* sp
	Maternity ward	*Culex pipiens*
	MRI and A&E departments	Phoridae
	Neonatal & Maternity wards	Psychodidae, Sciaridae & Sphaeroceridae

	Renal Department	*Hypoponera punctatissima*
	Renal units	Chironomidae, Mycetophilidae

Detailed in Table 5.2 are some specific records of the locations of flying insects in potentially sensitive areas in UK hospitals. Flying insects were found throughout a wide range of areas in the hospital environment and other healthcare environments.

The most reports came from the location category ‘treatment areas’, which includes hospital wards (among other areas where hospital patients are treated), with 18 flying insect species reports being recorded. There were three reports of flying insect activity in hospital operating / surgery areas. Two reports of flying insect activity were from food preparation areas of hospitals. Five reports came from non-hospital healthcare facilities, such as nursing / care homes and dental hospitals. Seven reports were from non-patient areas of hospitals i.e. areas where patients do not routinely have access.

5.3.4 Checklist of crawling insects found associated with UK hospitals (KCIIS database 2000 – 2013)

Table 5.3 Checklist of crawling insects (and other arthropods) found associated with UK hospitals (KCIIS database 2000 – 2013)

ORDER	FAMILY	SPECIES NAME	COMMON NAME
(Acari) Astigmata	Glycyphagidae	*Glycyphagus domesticus*	House furniture mite
(Acari) Mesostigmata	Dermanyssidae	*Dermanyssus gallinae*	Bird mite
	Macrochelidae	*Macrocheles muscaedomesticae*	Macrochelid mites
(Acari) Parasitiformes	Gamasidae	Gamasidae	Gamasid mites
(Acari) Trombidiformes	Tetranychidae	*Bryobia* sp	Clover mite
	Trombidiidae	*Eutrombidium rostratus*	Velvet mites
Araneae	Agelenidae	*Tegenaria* sp	House spider
	Araneae	Araneae	Spider
	Clubionidae	Clubionidae	Foliage spider
	Linyphiidae	Linyphiidae	Money spider
Chilopoda	Chilopoda	Chilopoda	Centipede
Coleoptera	Anobiidae	*Lasioderma serricorne*	Cigarette beetle
		Stegobium paniceum	Biscuit beetle
	Anthicidae	*Anthicus floralis*	Narrow-necked grain beetle
	Apionidae	*Ceratapion* sp	Weevil
	Carabidae	*Amara* sp	Ground beetle
		Carabidae	Ground beetle
		Carabus nemoralis	Ground beetle
		Harpalus aenus	Ground beetle
		Harpalus rufipes	Ground beetle
		Harpalus sp	Ground beetle
		Pterostichus madidus	Ground beetle
		Pterostichus sp	Ground beetle
		Trechus quadristriatus	Swarming ground beetle

	Cerambycidae	*Arhopalus rusticus*	Longhorn beetle
		Mesosa nebulosa	Longhorn beetle
	Chrysomelidae	*Phyllotreta* sp	Leaf beetle
	Curculionidae	*Sitona* sp	Clover weevil
		Sitophilus granarius	Grain weevil
		Sitophilus oryzae	Lesser rice weevil
	Dermestidae	*Anthrenus verbasci*	Carpet beetle
		Dermestes peruvianus	Leather beetle
	Elateridae	Elateridae	Click beetles
	Geotrupidae	*Geotrupes stercorarius*	Dor beetle
	Lathridiidae	*Aridius nodifier*	Plaster beetle
		Cartodere constricta	Plaster beetle
		Corticaria sp	Plaster beetle
		Dienerella sp	Plaster beetle
		Lathridiidae	Plaster beetle
	Nitidulidae	*Glischrochilus quadripunctatus*	Sap beetle / ‘beer bugs’
		Meligethes aeneus	Sap beetle / pollen beetle
	Oedermidae	*Nacerdes melanura*	Wharf borer
	Ptinidae	*Gibbium psylloides*	Humped spider beetle
		Ptinus tectus	Spider beetle
	Scarabaeidae	*Aphodius* sp	Scarab beetle
		Colobopterus fossor	Scarab beetle
		Melolontha melolontha	Cockchafer
		Serica brunnea	Summer chafer
	Silphidae	*Necrodes littoralis*	Burying beetle
		Nicrophorus investigator	Burying beetle
		Nicrophorus vespillo	Burying beetle
		Nicrophorus vespilloides	Burying beetle
	Silvanidae	*Oryzaephilus mercator*	Merchant grain beetle
		Oryzaephilus surinamensis	Saw-toothed grain beetle
	Staphylinidae	*Anotylus* sp	Rove beetle
		Philonthus sp	Rove beetle
		Staphylinidae	Rove beetle
	Tenebrionidae	*Lagria hirta*	Garden beetle
		Tribolium confusum	Confused flour beetle
Collembola	Collembola	Collembola	Springtail
	Entomobryidae	*Entomobryia nivalis*	Springtail

	Tomoceridae	*Tomocerus vulgaris*	Springtail
Dictyoptera	Blaberidae	*Panchlora nivea*	Cuban cockroach
	Blattellidae	*Blattella germanica*	German cockroach
		Ectobius lapponicus	Dusky cockroach
		Supella longipalpa	Brown-banded cockroach
	Blattidae	*Blatta orientalis*	Oriental cockroach
		Periplaneta americana	American cockroach
		Periplaneta australasiae	Australian cockroach
Diplopoda	Diplopoda	Diplopoda	Millipedes
Hemiptera	Acanthosomatidae	*Acanthosoma haemorrhoidale*	Hawthorn shield bug
	Cicadellidae	Cicadellidae	Leaf hopper
	Cimicidae	*Cimex lectularius*	Bedbug
	Lygaeidae	*Heterogaster urticae*	Ground nettle bug
	Miridae	Miridae	Mirid bug
	Pentatomidae	*Pentatoma rufipes*	Forest bug
		Piezodorus lituratus	Shield bug
Hymenoptera	Formicidae	Formicidae (pupae)	Ant pupae
		Hypoponera punctatissima	Roger's ant
		Lasius flavus	Yellow meadow ant
		Lasius niger	Garden ant
		Monomorium pharaonis	Pharaoh ants
		Tapinoma melanocephalum	Ghost ant
Orthoptera	Gryllidae	Gryllidae	Cricket
Phthiraptera	Pediculidae	*Pediculus humanus*	Human louse
Psocoptera	Liposcelidae	*Liposcelis bostrychophila*	Booklouse
	Psocoptera	Psocoptera	Booklouse
	Trogiidae	*Lepinotus patruelis*	Booklouse
Siphonaptera	Pulicidae	*Ctenocephalides felis*	Cat flea
		Pulex irritans	Human flea
		Siphonaptera (larva)	Flea larva
Thysanura	Lepismatidae	*Lepisma saccharina*	Silverfish

Table 5.3 shows the great diversity of crawling insects (and other arthropods) recorded in UK hospitals as part of the KCIIS.

Of the 'other arthropods' i.e. non-insects, the KCIIS data shows; mites (Acari) represented by four orders, six families and six species, spiders (Order Araneae) represented by three families containing four species, centipedes (Class Chilopoda) with one species and one record of millipedes (Class Diplopoda).

There were 11 orders of insects (Class Insecta). The beetles (Order Coleoptera) were the most numerous in terms of records and the most speciose, represented by 19 families containing 48 species. The most commonly encountered beetles were ground beetles, family Carabidae, accounting for nine species records. Three different species of springtails (Order Collembola) were recorded. Cockroaches (Order Dictyoptera) were represented by three families containing seven species. Bugs (Order Hemiptera) were present, represented by six families containing seven species. Hymenoptera were represented by approximately six species of ants (family Formicidae). Three species of booklice (Order Psocoptera) and fleas (Order Siphonaptera) were recorded. The remaining records were a cricket (Order Orthoptera, family Gryllidae), the human louse *Pediculus humanus* (Order Phthiraptera, family Pediculidae) and silverfish *Lepisma saccharina* (Order Thysanura, family Lepismatidae).

A total of approximately 92 species of crawling insect (and other arthropods) were recorded in UK hospitals from 2000 – 2013 as part of the KCIIS.

5.3.5 Crawling insects associated with UK hospitals (KCIIS database 2000 – 2013)

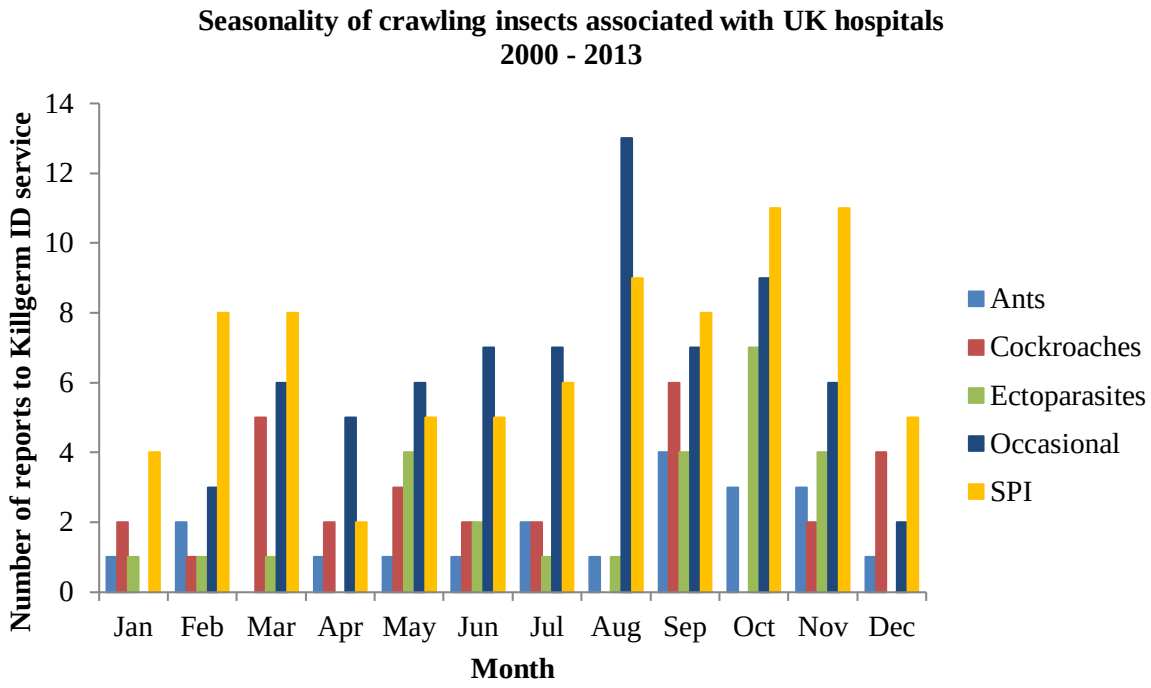

Figure 5.3 Seasonality of crawling insects associated with UK hospitals 2000 – 2013. The number of reports to the KCIIS (pooled monthly) of different groups of crawling insects (and other arthropods) associated with UK hospitals, from the year 2000 to the year 2013.

'Ants' category pools records of *Hypoponera punctatissima, Lasius niger, Lasius flavus, Monomorium pharaonis* and *Tapinoma melanocephalum*.

'Cockroaches' category pools records of *Blatta orientalis, Blattella germanica, Periplaneta americana, Periplaneta australasiae, Supella longipalpa, Panchlora nivea* and *Ectobius lapponicus*

'Ectoparasites' category pools records of *Cimex lectularius, Ctenocephalides felis, Pulex irritans, Dermanyssus gallinae* and *Pediculus humanus*.

'Occasional' category pools records of arachnids and 'casual intruder' type Coleoptera, Hemiptera, Myriapoda and Orthoptera.

'SPI' category pools records of stored product insects and 'fungus feeders' such as Anobiidae, Curculionidae, Dermestidae, Ptinidae, Silvanidae, Tenebrionidae and Lathridiidae, Collembola, Psocoptera, Thysanura.

Crawling insects are indeed found in UK hospitals, as shown by Figure 5.3.

Crawling insects are present in UK hospitals throughout the year, at a reasonably consistent level. Stored product insects (SPI) are the most reported group with 82 reports during the data period (2000 – 2013). SPI numbers peaked in October and November, with 11 reports for each month. SPI were also reported (number of reports in parentheses) as the most numerous insect group in January (4), February (8), March (8), September (8) and December (5). 'Occasional' insects are the casual intruder group, which features insects whose breeding media is not likely to be on site. The occasional / casual intruder group of insects are the second most commonly reported group in hospitals, with 71 reports. The incidence of occasional / casual intruder insects peaks in August, with 13 reports, which is the highest number of records for any of the studied crawling insect groups in a month. The occasional / casual intruder insects were the most numerous insect group in April (5), May (6), June (7) and July (7). Cockroaches were the next most frequently reported in the sample period with 29 records, followed by ectoparasites (26) and ants (20). Cockroaches peaked in September (6), ectoparasites in October (7) and ants in September (4). The greatest total number of records of crawling insects was in October (30), followed by September (29), November (26) and August (24) respectively. When the data are looked at seasonally, total records of crawling insects peaked in autumn (85), were second highest in summer (59), lower in spring (49) and lowest in winter (35).

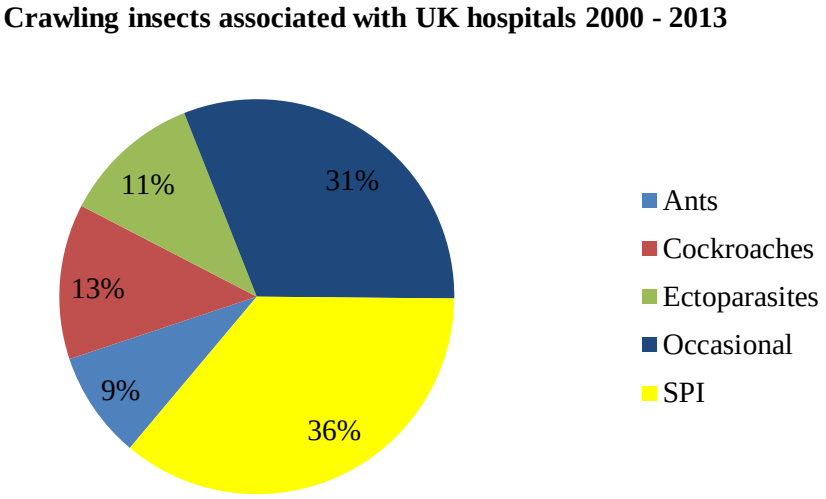

Figure 5.4 Crawling insects associated with UK hospitals 2000 – 2013. The percentage of different groups of crawling insects (and other arthropods) associated with UK hospitals from 2000 – 2013, from KCIIS data. The crawling insect groups are defined in Figure 5.3.

The data in Figure 5.4 are an adaptation of the data shown in Figure 5.3 previously, in order to give a clearer visual picture of the overall prevalence of crawling insect groups. This shows that 'SPI (stored product insects)' were the most commonly recorded group in total throughout the entire time period for the data (the years 2000 – 2013), comprising 36% of records, compared to 31%, 13%, 11% and 9% of records for 'occasional', 'cockroaches', 'ectoparasites' and 'ants' respectively.

5.3.6 Specific locations of crawling insect activity in UK hospitals

Table 5.4 Specific location records of crawling insect activity in UK hospitals from KCIIS data, from 2000 – 2013

Location category	Specific location	Crawling insect species
Food preparation	Bag of rice	*Sitophilus oryzae*
	Dining area of hospital	*Nicrophorus investigator*
	Hospital dry food store	*Blattella germanica*
		Corticaria sp
		Liposcelis bostrychophila
	Hospital kitchen	Anthicus floralis
		Clubionidae
		Dermestes peruvianus
		Lagria hirta
		Linyphiidae
	Hospital restaurant	*Periplaneta australasiae*
	Hospital ward kitchen	*Carabus nemoralis*
	In a patient's meal	*Pterostichus* sp
	In bananas	*Periplaneta australasiae*
	In hospital kitchen and dining room	*Amara* sp
		Pterostichus sp
	Pallet in hospital kitchen	*Panchlora nivea*
	Trays in hospital restaurant	*Meligethes aeneus*
	Under vending machine	*Blatta orientalis*
Non-hospital healthcare	Care home	*Lepisma saccharina*
		Pediculus humanus
	Dental practice	Elateridae
	Nursing home	*Dermanyssus gallinae*
		Pterostichus sp
		Serica brunnea
	Nursing home bedroom lights	*Stegobium paniceum*
	Residential home	*Blatta orientalis*
		Ectobius lapponicus
	Speech therapy clinic	*Ptinus tectus*
Non-patient area	Accommodation at children's	*Tapinoma melanocephalum*

	hospital	
	Cytology labs	*Entomobryia nivalis*
	Hospital accommodation	*Monomorium pharaonis*
	Hospital accommodation block & kitchens	*Blattella germanica*
	Hospital admin desk	*Tomocerus vulgaris*
	Hospital fluorescent light	*Lepinotus patruelis*
	Hospital light fitting	*Hypoponera punctatissima*
		Liposcelis bostrychophila
	Hospital office	*Eutrombidium rostratus*
	Hospital pharmacy	Miridae
	Hospital reception	Chilopoda
		Diplopoda
		Pediculus humanus
	Hospital residence	*Lasioderma serricorne*
	Hospital staff accommodation	*Supella longipalpa*
	Hospital sterile products store room	*Glischrochilus quadripunctatus*
	Lift and lift shaft	*Liposcelis bostrychophila*
	Medical records department	*Tomocerus vulgaris*
	MRI control room	*Hypoponera punctatissima*
	Nurses accommodation	*Oryzaephilus surinamensis*
	Staff canteen at maternity unit	*Dermestes peruvianus*
Operating theatre / surgery	Hospital theatre	*Anotylus* sp
		Pentatoma rufipes
		Philonthus sp
		Staphylinidae
	Inside light diffusers in surgery	Diplopoda
	Sterile operating theatre	*Anotylus* sp
Treatment area	Cardiology ward	*Arhopalus rusticus*
		Ceratapion sp
	Consulting room	*Dermanyssus gallinae*
	Critical care	*Cimex lectularius*
	Diabetic clinic	*Dermanyssus gallinae*
	Hospital ante-natal clinic	*Dermanyssus gallinae*
	Hospital bed	*Ptinus tectus*
	Hospital bedroom	*Periplaneta americana*

	Hospital ward	*Blatta orientalis* (ootheca)
		Colobopterus fossor
		Ctenocephalides felis
		Dermanyssus gallinae
		Hypoponera punctatissima
		Lasius niger
		Liposcelis bostrychophila
		Stegobium paniceum
	Intensive care for neonates	*Dermanyssus gallinae*
	Maternity unit	*Periplaneta australasiae*
	Maternity ward	*Tomocerus vulgaris*
	Neonatal ward	*Cimex lectularius*
	On a hospital chair	*Sitophilus granarius*
	On a pillow in a hospital ward	Siphonaptera (larva)
	Patient bedding	*Sitona* sp
	Radiotherapy	*Ctenocephalides felis*
	Renal department	*Hypoponera punctatissima*
	Within physiotherapy unit heating pads	*Sitophilus oryzae*

Detailed in Table 5.4 are some specific records of the locations of crawling insects in potentially sensitive areas in UK hospitals. Crawling insects were found throughout a wide range of areas in the hospital environment and other healthcare environments. The most reports came from the location category ‘treatment areas’, which includes hospital wards (among other areas where hospital patients are treated), with 26 flying insect species reports being recorded. There were six reports of crawling insect activity in hospital operating / surgery areas. There were 19 reports of crawling insect activity from food preparation areas of hospitals. Ten reports came from non-hospital healthcare facilities, such as nursing / care homes and dental hospitals. There were 21 reports from non-patient areas of hospitals i.e. areas where patients do not routinely have access.

5.4 DISCUSSION

Flying insects

It is important to note a potential limitation of the KCIIS, in that it is not a mandatory reporting scheme. Users of the service tend to submit insects (and sometimes other arthropods) that they cannot identify, so there exists perhaps the potential for a skew towards unfamiliar insects in the data, rather than readily identifiable ones. However, as probably the longest running and most extensive dataset on insects in hospitals in the UK, the benefits of interpreting this outweigh any limitations.

Results from the KCIIS illustrate a key point relevant to the hypothesis that flying insects, particularly *M. domestica*, may transfer *C. difficile* in hospitals (based on the described laboratory studies in this work); the key point being that these insects are indeed found in association with such premises. A more detailed examination of information regarding the abundance, diversity, location and seasonality of flying insect fauna in UK hospitals is required in order to develop a deeper understanding of the potential role that flying insects may play in the transfer of *C. difficile* and other pathogenic bacteria. This information will be used to help assess associated risks to public health and to make recommendations regarding 'integrated pest management as infection management'. The following interpretation of the KCIIS data (and the field sampling and analysis of UV light flytrap data in section 6) forms a key part of the relevant recommendations.

Results from the KCIIS study, as well as confirming that flies and other flying insects are indeed present in UK hospitals, show that there is a great diversity of such insects in these premises, not just *M. domestica*, which is regularly quoted as the most common fly species found indoors (Mallis, 1964). This simple yet important finding should be a key recommendation to those involved in infection control and pest control – *M. domestica* is not the only fly species of concern in hospitals, so consider other species in integrated pest management programs.

The fact that 'Drain flies' are present in UK hospitals throughout the year and are the most commonly reported flies in every month (apart from August) and in total should change the way that infection control and pest control deal with fly problems. These findings go against perceived wisdom that larger flies like houseflies are the most common and that fly problems peak in summer. From personal experience, 'drain flies' are not the typical fly species that infection control and pest control staff expect to see in hospitals and there is a general lack of awareness regarding their identification, source / breeding media, public health significance and control measures. This should now change based on the findings of this study, in that 'drain flies' should be at the forefront of the education of pest controllers and hospital staff, with control measures being tailored more specifically towards this

group of flies, the most common in UK hospitals according to this dataset. Furthermore, the contents of EFKs and professional sticky traps in terms of flying insect species should be identified, in order to provide more targeted advice and control measures, not simply ignored or recorded only as a simple quantity of flies. It is often the presence of different species that are the most significant finding relevant to control, rather than simply counting the total number of flies as a whole. An expert entomologist should be sought out to provide accurate identification of flying insect species, when infection control and pest control staff are unable to provide this.

An example of 'drain flies' recorded in this study are flies of the family Psychodidae, which are known by a number of other common names such as 'bathroom flies', 'filter flies' and 'sewage flies' (Mallis, 1990), in reference to their development areas, which can be anywhere where there is a preponderance of wet rotting organic matter. The larvae actually feed on the bacteria within the biological film of filter beds (Busvine, 1980). Despite this close relationship with bacteria, there are few references which detail their associated bacterial species. Bacteria isolated from Psychodidae include many species of Enterobacteriaceae from a study in German hospitals (Faulde and Spiesberger, 2013), *Nocardia* sp (Pelli *et al.*, 2007), *C. difficile* (Burt *et al.*, 2012), presumptive *Salmonella* sp and other unknown Enterobacteriaceae (Sparkes and Anderson, 2010). Although there are few references to bacterial carriage by these flies, there is a threat to public health. In terms of simple identification in the field, psychodid flies are often referred to as 'moth flies' or 'owl midges' on account of their appearance. They are minute flies with pointed hairy wings, which are held 'roofwise' when at rest (Chinery, 2012). There are approximately 80 species of Psychodidae in Britain (Chinery, 1993), of which *Psychoda alternata* is common.

Another example of 'drain flies' recorded in this study are flies of the family Phoridae, also known as 'coffin flies', referring to their development areas. The common name of 'coffin fly' refers to *Conicera tibialis*, which breeds in human corpses (Chinery, 1993). The larvae of other species of Phorid fly develop in animal carcasses (including attacking insects), fungi and other decaying organic matter (Chinery, 1993), which may build up in drains and this can include human excrement (Colyer and Hammond, 1951). Presumptive *Salmonella* sp and other unknown Enterobacteriaceae have been isolated from phorid flies (Sparkes and Anderson, 2010). They are sometimes called 'scuttle flies' in reference to the distinctive running movement of adults (Chinery, 2012). In terms of simple identification in the field, these flies are recognised by their small humpbacked appearance, black or brown or yellowish colour and characteristic wing venation, where the first three veins are short and thick and the rest are weak and not connected by cross-veins (Chinery, 1993). There are approximately 280 species of Phoridae in Britain (Chinery, 1993).

'Drain flies' include flies of the family Sphaeroceridae recorded in this study, which develop in dung (Chinery, 1993) and are sometimes referred to as 'lesser dung flies'. They can also develop in

decaying organic matter in general, such as leaves and fungi (Colyer and Hammond, 1951). Enterobacteriaceae (Greenberg, 1971) and presumptive *Salmonella* sp and other unknown Enterobacteriaceae (Sparkes and Anderson, 2010) have been isolated from Sphaeroceridae. These small dark flies are recognised by the faint appearance of wing veins 4 and 5 which commonly do not reach the posterior cross-vein (Chinery, 1993). There are almost 100 species in Britain (Colyer and Hammond, 1951).

Also referred to as 'drain flies' are *Drosophila* spp, which are synanthropic flies of the family Drosophilidae recorded in this study. They are more commonly called 'fruit flies' and are famous for developing in decaying fruit (Greenberg, 1971). *Drosophila* spp are also called 'beer flies' and 'vinegar flies'. This refers to their ability to develop in residues of fermenting alcoholic beverages and vinegar (Busvine, 1980). The ability of *Drosophila* spp to develop on rotting vegetables means that they can be a problem in kitchens, especially from a public health point of view as they also feed on faeces (Busvine, 1980). Fruit flies have been shown to be able to mechanically transfer *E. coli* to apples (Janisiewicz *et al.*, 1999) and harbour various multi-drug resistant bacteria (Nmorsi *et al.*, 2007). *Drosophila* spp are recognised by their bright red eyes and 'feathered' antennae which are forked at the tip (Chinery, 1993). There are approximately 50 species of Drosophilidae in Britain (Chinery, 1993).

As a general point regarding 'drain flies', they breed in rotting organic matter, typically associated with food residues and drainage faults in buildings. The continued presence of 'drain flies' throughout the year, as recorded in this study, can be explained by their biology. The year-round availability of their breeding media and the constant temperatures provided by the centrally heated hospital institutions provide the requisite conditions for their survival throughout the year. As a recommendation for infection control and pest control measures, repair of drainage faults and scrupulous hygiene should be a priority in order to limit the activity of this group of flies and therefore minimise the risk to public health. A further control recommendation is the use of ultra-violet (UV) light flytraps in the form of professional sticky traps to capture adult 'drain flies'. The recommendation that professional sticky traps should be used to capture these flies is made because of the small size of these flies. Their small size means that the electrocuting 'kill-grid' of EFKs may not be activated by contact but the flies stick perfectly well to glue boards, which have the added benefit of retaining the flies and preventing expulsion of bacterially contaminated insect fragments onto food and fomites.

Results from the KCIIS showed that August was the only month in which 'drain flies' were not the most common fly group in hospitals, the most common actually being non-biting midges of the family Chironomidae. Chironomidae develop in water or decaying matter and include the well-known *Chironomus* spp of which the larval stages are called 'bloodworms' and are found in stagnant water

(Chinery, 1993). The 'bloodworms' are red in colour due to the presence of haemoglobin in their haemolymph which allows them to absorb oxygen (Chinery, 1993). *Clostridium* spp have been isolated from chironomid larvae (Rouf and Rigney, 1993) and *Vibrio cholerae* has been detected in egg masses and adults (Broza *et al.*, 2005). Bronchial asthma patients are known to be hypersensitive to chironomids (Hirabayashi *et al.*, 1997), which is a further health risk. Adult chironomids are recognised in the field by their small size, delicate appearance, humped thorax, long front legs, plumose antennae in males, narrow wings shorter than the body with weak venation and the striking green body colour in some species (Chinery, 1993). There are almost 400 species in Britain (Chinery, 1993). Adults can also form dense swarms (Chinery, 2012) typically in the summer months (explaining their described peak in August) and experience shows that chironomids are able to enter premises in large numbers due to inadequate proofing, such as a lack of flyscreening on open windows, or doors being left open. Those involved in pest control typically disregard 'midges' as being unimportant due to their small size and the fact that their breeding material is usually outdoors. This perception should change, due to the relatively recent evidence of bacterial isolation from such flies and the fact that this study recognises them as the most common fly family present in hospitals in August. Those involved in pest control and infection control should be prepared for an expected peak in reports of chironomids in summer months, typically August, when they are likely be the most common species in UK hospitals. It should be ensured that hospital buildings are adequately proofed against chironomid entry, by installing and maintaining flyscreens and that fly activity internally is minimised by the use of UV light flytraps in the form of professional sticky traps.

As expected due to their typical behaviour, cluster flies were found predominantly in hospitals in autumn, according to data from the KCIIS. *Pollenia rudis* of the family Calliphoridae, commonly called the 'cluster fly' is a parasite of earthworms *Allobophora* spp or *Eisenia* spp (Erzinclioglu, 1996). The adult female *P. rudis* lays eggs on soil and the first instar larvae emerge, then find and parasitise the earthworm host (Erzinclioglu, 1996). It is typically September when *P. rudis* lays eggs and the resulting larvae that parasitise the earthworm remain within the host over winter, with further development usually beginning in May (Richards and Davies, 1977). Adult *P. rudis*, which visit flowers and fruits (Mallis, 1990) are present during the summer months until late autumn and early winter and have acquired their common name the 'cluster fly' as a result of their clustering habits in attics in late autumn in preparation for overwintering (Colyer and Hammond, 1951). This clustering behaviour, when a great number of individuals cluster together, is sometimes called 'swarming' and is a source of nuisance in premises, mainly when the flies characteristically enter in autumn and leave in spring, something which can occur at the same premises year after year (Busvine, 1980). Typical clustering sites include roof spaces, vacant and unheated rooms, belfries, lofts (Busvine, 1980), under clothing in wardrobes, under curtains, in wall angles, behind pictures and behind furniture (Mallis, 1990). *P. rudis* can cause consternation in premises when adult flies appear sporadically because they

are temporarily 'awakened' due to intermittent heating, resulting in the sluggish flies falling down on to occupants (Busvine, 1980).

P. rudis sampled from a hospital in Germany were found to harbour opportunistic pathogens such as *Pseudomonas aeruginosa* and *Erwinia* spp which are also known by the synonym *Pantoea* spp (Faulde *et al.*, 2001).

Adult *P. rudis* are recognised firstly by their clustering behaviour, secondly by the characteristic golden hairs on their thorax and the black and white tessellated square 'chequerboard' appearance of the abdomen (Erzinclioglu, 1996). Cluster flies and relevant control techniques are well known in the pest control industry (Killgerm, 2013).

While on the topic of seasonality of fly species in hospitals, it should be noted that the KCIIS showed that 'domestic flies' peaked in the summer months. 'Domestic flies' recorded in this study included the houseflies, *M. domestica* and *Fannia canicularis*, plus *Calliphora* sp and *Lucilia* sp which are the bluebottles and greenbottles respectively. The observation that numbers of 'domestic flies' peaked in the summer can be explained by their biology, because their breeding cycle and development is accelerated in warmer temperatures.

The key points regarding public health significance, biology and identification of 'domestic flies' relevant to infection control and pest control in hospitals are now discussed (*M. domestica* is well-covered in chapter 1).

F. canicularis, the lesser housefly, is a synanthropic fly of the family Fanniidae, which develops preferentially in poultry manure but can also develop in other moist organic matter (Busvine, 1980) and harbours a number of species of pathogenic bacteria (Greenberg, 1971) including *C. difficile*, which was isolated from samples at a pig farm (Burt *et al.*, 2012). Although similar in appearance, it is slighter and smaller than *M. domestica*, the 4th wing vein is almost straight and it exhibits characteristic flight behaviour, with males circling constantly beneath light fittings etc., making jerky abrupt turns (Chinery, 2012).

C. vicina, the bluebottle fly, is a synanthropic fly of the family Calliphoridae, which typically develops on animal carcasses such as birds and rodents, can feed on faeces (Erzinclioglu, 1996) and harbours many species of pathogenic bacteria (Greenberg, 1971). There are approximately 35 species of Calliphoridae in Britain (Chinery, 1993). *C. vicina* is identified in the field by the following key features: dull metallic blue in colour, bristly in appearance, a length of approximately 11mm, with a wing span of about 25mm and reddish jowls (Busvine, 1980). Suitable entomological keys are available to confirm the identification of *C. vicina* (Erzinclioglu, 1996).

Lucilia sericata, the greenbottle fly, is a fly of the family Calliphoridae, which develops on animal carcasses and these flies can be recognised by their metallic bluish green to copper colour and their size of approximately 10mm long and 18mm wing span (Busvine, 1980). *L. sericata* can carry a wide range of bacteria, including many members of the family Enterobacteriaceae, such as *E. coli*, *Salmonella* sp, *Klebsiella* sp and *Shigella* sp (Greenberg, 1971). *L. sericata* also carries cocci such as *Staphylococcus* sp, as well as spore-forming bacteria *Bacillus cereus* Group, *Bacillus subtilis*, *Clostridium botulinum* and *Clostridium perfringens* (Greenberg, 1971). *Lucilia* species are rarely enticed indoors and as well as the aforementioned animal carcasses, can also develop in excrement (Colyer and Hammond, 1951).

Continuing with the discussion of fly seasonality and therefore highlighting the times of year when hospital patients would be most at risk from bacteria carried by flying insects, the study showed that the total records of flies were highest in the summer months. The highest risk month was August as flies peaked at this time, closely followed by July, October and November. This shows that flies are not just a summer problem, which is a key point in educating those planning a fly control and therefore infection control strategy.

A further consideration that should be made regarding risks to health posed by flying insects in hospitals is not just the time of year that they are present or particular characteristics of certain species but also the locations in which they are found. The KCIIS results showed records of flies in a wide range of sensitive locations in hospitals. Most reports of flies came from 'treatment areas' areas where patients would be most at risk from infection from bacteria carried by flying insects, with reports also coming from operating and surgery areas and food preparation areas. It follows that recommendations are made to focus fly control efforts at these aforementioned areas, which are higher-risk locations where flies are predominantly found in hospitals.

Crawling insects

Results from the KCIIS also highlighted the presence of crawling insects in hospitals and this is considered from the point of view of being inclusive due to varying definitions and because this area may represent an opportunity for further research, therefore complementing this thesis. Although one of the original aims of this thesis was to collect, identify and examine microbiologically the flying insects associated with UK hospitals, crawling insects are being included in this section, as the varying definitions of flying versus crawling insects are often unclear, with many insects typically defined as 'crawling' being capable of flight. Consideration of crawling insects in this section therefore covers any conflicts in definitions of insects. As the main focus of the thesis is on flying insects, the level of detail in this section regarding crawling insects is not as high. For example, detailed species accounts are omitted. These data were also looked at retrospectively and in response

to predominantly crawling insects that are also capable of flight being sampled from ultra-violet light flytraps in the field study in section 6.3.11.

Data from the KCIIS confirms that crawling insects are reported in hospitals throughout the year rather than being a seasonal problem and are found in great diversity, with beetles being the most commonly reported Order. From experience, perception of hospital and pest control staff is that ants and cockroaches are the most important crawling insects in hospitals, with seasonal peaks in summer, while the data from this study challenges these views. There is evidence that ants and cockroaches are the most common crawling insects in hospitals in Brazil (Cintra-Socolowski *et al.*, 2011) but it is important that pest control professionals and hospital staff understand that other insects such as beetles are the most commonly reported in UK hospitals and that activity continues throughout the year, so that pest control and therefore infection control measures are designed appropriately.

Many of the reported beetle species fall into the 'stored product insect' category or 'SPI', meaning that they are capable of damaging stored products, along with other insects as defined in Figure 5.3. Insects in the SPI category were the most common category of crawling insect reported in this study, peaking in October and November, again challenging the traditional view of a seasonal peak of general insect activity in summer. The relatively high level of reports of SPI throughout the year and their consistent presence suggests that incidence of SPI in hospitals is independent of weather conditions. The reasoning here is that their activity is predominantly indoors, which is a constant environment in terms of climate and their breeding material (stored food) is also indoors.

The findings regarding SPI in hospitals are important enough to lead to the recommendation that education and awareness of pest control and infection control staff regarding crawling insects in hospitals should focus on SPI in particular. Recent understanding has been updated regarding the threat to public health posed by SPI. SPI were often considered to present little risk to human health by way of bacterial carriage and transfer but research has emerged reporting the isolation of pathogenic and antibiotic resistant *Enterococcus* sp from rust red flour beetles *Tribolium castaneum* collected from feed mills (Larson *et al.*, 2008).

Of the crawling insects in hospitals that were recorded from the KCIIS data, the occasional / casual intruder group were the second most commonly encountered. The occasional / casual intruder group are often disregarded by many, as illustrated by the commonly used definition of 'occasional' / 'casual', implying their non-importance. However, this group should be given due consideration seeing as they were so common and were the most numerous of all groups in August, which conflicts with the 'occasional' definition. The 'occasional' / 'casual' intruder group features insects whose breeding media is not likely to be on site. Their breeding media will generally be external to the hospital, so these insects are invading the hospital sites from the outside where their development is

under the influence of climatic conditions. As expected, their incidence peaks in August, in correspondence with peak summer temperatures, which are a key driver for insect development. Proofing deficiencies in hospital buildings are often the route in for such insects and these should be highlighted to relevant staff such as facilities management / estates and remedial action taken.

The study showed that the total records of crawling insects were highest in the autumn months, as well as being present throughout the year as discussed. It follows that integrated pest management programs for crawling insects in hospitals should be in place throughout the year, with efforts focusing on the autumn months when these insects are most numerous.

A further consideration that should be made regarding risks to health posed by crawling insects in hospitals is not just the time of year that they are present or particular characteristics of certain species but also the locations in which they are found. The KCIIS results showed records of crawling insects found in a wide range of sensitive locations in hospitals. Most reports came from 'treatment areas' areas where patients would be most at risk from infection from bacteria carried by crawling insects, with reports also coming from operating and surgery areas and food preparation areas. It follows that recommendations are made to focus crawling insect control efforts at these aforementioned areas, which are higher-risk locations where such insects are predominantly found in hospitals.

5.5 CONCLUSION

Regarding the numbers of certain species, interpretation of the KCIIS data for flying insects revealed that 'drain flies' were the flying insect group of greatest importance in UK hospitals and chironomid midges were the most numerous flies in August and should not be ignored as they do pose a threat to public health. Regarding seasonality, reports of flies peaked in the summer months but they were also numerous in October and November with some species being present all year round. Based on the findings regarding location of insects in hospitals, fly control measures should focus on 'treatment areas' of hospitals which is where flies were most frequently reported.

This study updates the knowledge base regarding flies in hospitals and contrasts with the general wisdom that houseflies *M. domestica* are the most numerous in such premises and that flies are mainly a summer problem. These findings should therefore inform the design of pest control and infection control procedures required to protect public health.

Regarding the numbers and seasonality of certain species, interpretation of the KCIIS data for crawling insects revealed that SPI were the crawling insect group of greatest importance in UK

hospitals, were present all year round and peaked in October and November. Furthermore, 'occasional' / 'casual' intruders were the most numerous group in August and reports of crawling insects were throughout the year and peaked in the autumn months. Based on the findings regarding location of insects in hospitals, crawling insect control measures should focus on 'treatment areas' of hospitals which is where such insects were most frequently reported.

This study updates the knowledge base regarding crawling insects in hospitals and contrasts with the general wisdom such insects are mainly a summer problem and that ants and cockroaches are the main pests. These findings should therefore inform the design of pest control and infection control procedures required to protect public health

It is recommended that future work should be undertaken regarding field sampling and microbiological analysis of crawling insects in hospitals, to further determine the threat to public health and consider in more detail the role of pest control as infection control.

6 CHAPTER 6: COLLECTION AND IDENTIFICATION OF FLYING INSECTS FROM UK HOSPITALS – AN ENTOMOLOGICAL STUDY

6.1 INTRODUCTION

Previous studies exist in the literature regarding the species of insects found in hospitals, which include data on their numbers, prevalence and location. Such studies provide an insight into the kind of observations that may be made in this thesis chapter, regarding flying insects sampled from UK hospitals. These studies also act as a point of comparison and provide information on which sampling techniques should be the most appropriate for collecting insects in UK hospitals.

There are limitations regarding previous studies on insects present in hospitals. Typically, just one hospital was sampled for insects and only for a short period of time, while few studies have been done in the UK. Of the studies that have been done in the UK, none focus on flying insects and the data in some cases are over 30 years old. This thesis chapter addresses the limitations of previous works, as it presents recent results regarding insects sampled from a number of UK hospitals over a greater period of time and is the most in-depth study of its kind.

Cockroaches and ants were the most common insects in a hospital in Brazil (Cintra-Socolowski *et al.*, 2011). The American cockroach, *Periplaneta americana* was recorded the most (25% of observations), followed by ants (21% of observations with *Brachymyrmex* sp the most commonly encountered genus). Also recorded were mosquitoes *Culex* spp (14%). The remainder of the insects and arthropods recorded at the hospital consisted of spiders, flies, bugs and the Brazilian yellow scorpion, *Tityus serrulatus*. In total, the hospital recorded 95 instances of pests in 2010.

Although some studies show that ants and cockroaches are the most common insects in hospitals in Brazil (Cintra-Socolowski *et al.*, 2011), other studies show that flies are the predominant type of insect in hospitals in Brazil (Da Silva *et al.*, 2011). Flying and crawling insects were sampled from a surgical centre of a hospital in São Paulo, Brazil from May to September 2010. Adhesive light traps were used to capture flying insects in the passage leading to the surgical centre and crawling insects were sampled manually, twice weekly in the central internal passageway of the surgical centre. This is an important piece of information, showing that adhesive light traps are an effective and recognised method for sampling flying insects in hospitals, as utilised in this chapter along with EFKs. Studies focusing on crawling insects should also include manual sampling techniques. A total of 146

individual insects (and arachnids) were collected and identified using the described techniques (Da Silva *et al.*, 2011). These individuals were from six Orders: Diptera, Hymenoptera, Lepidoptera, Hemiptera, Coleoptera and Araneae. Although synanthropic ants generally cause the greatest concern in hospitals in Brazil because they can be one of the most common pests, it was actually flies (Order Diptera) that were the most numerous insects and they constituted 66.1% of all insects and arachnids sampled from the hospital (Da Silva *et al.*, 2011). The prevalence of flying insects in hospitals according to the literature is described in more detail in Table 6.1.

Table 6.1 A review of the prevalence of flying insect species in hospitals

Insect Group	**Species**	**Prevalence**	**Reference**
Flies	Order Diptera	35.2% of sampled areas	(Gliniewicz *et al.*, 2006)
	Order Diptera	66.1% of all arthropods	(Da Silva *et al.*, 2011)
	Musca domestica	3.1% of insects sampled	(Sramova *et al.*, 1992)
	Fannia canicularis	2.5% of insects sampled	
	Fannia scalaris	0.6% of insects sampled	
	Sarcophagidae	1.9% of insects sampled	
	Piophilidae	0.6% of insects sampled	
	Tachinidae	1.2% of insects sampled	
	Lauxaniidae	0.6% of insects sampled	
	Drosophila melanogaster	0.6% of insects sampled	
	Drosophila sp	0.6% of insects sampled	
	Culex pipiens molestus	5% of insects sampled	
	Chironomidae	12.4% of insects sampled	
	Culicidae	<5% of sampled areas	(Gliniewicz *et al.*, 2006)
		14% of observations	(Cintra-Socolowski *et al.*, 2011)
Moths	*Agrotis exclamationis*	0.6% of insects sampled	(Sramova *et al.*, 1992)
	Nemapogon cloacellus	0.6% of insects sampled	
Wasps	*Paravespula vulgaris*	0.6% of insects sampled	

Only Sramova *et al.* (1992) listed specific locations that the flying insects referred to in Table 6.1 were found. Mosquitoes *Culex pipiens molestus* were sampled from a dermatology ward and urology ward, flies from outdoors, dermatology, urology and infectious diseases wards, wasps from outdoors, non-biting midges Chironomidae from dermatology and urology wards and moths from the urology ward (Sramova *et al.*, 1992).

Sramova *et al.* (1992) collected spiders (Order Arachnida) and a number of crawling insects, including mealworm beetles (*Tenebrio molitor*), German cockroaches (*Blattella germanica*), a hemipteran bug, a ladybird (*Coccinella septempunctata*), a pollen beetle (*Meligethes* sp), a sap-sucking beetle (Nitidulidae) and garden ants (*Lasius niger* and *Lasius emarginatus*) from a hospital premises in Prague.

The issue of cockroaches in UK hospitals has been examined by a number of authors (Burgess and Chetwyn, 1979, Baker, 1982, Peck, 1985). The cockroach species recorded in hospitals were *Blatta orientalis, Blattella germanica, Periplaneta americana* and *Supella longipalpa*. Pharaoh ants (*Monomorium pharaonis*) in UK hospitals have also been described (Beatson, 1972).

Cockroaches and Pharaoh ants, *Monomorium pharaonis* are the main pests in hospitals in Poland (Gliniewicz *et al.*, 2006). *Blattella germanica* is the cockroach species most prevalent in hospitals in Turkey (Kutrup, 2003). The prevalence of these crawling insect species and others is described in Table 6.2.

Table 6.2 A review of the prevalence of crawling insect species recorded in hospitals in the literature

Insect Group	Species	Prevalence	Reference
Cockroaches	*Blattella germanica*	41% of insects sampled	(Sramova *et al.*, 1992)
		50.7% of wards 'infested'.	(Lee, 1995)
		4% of units 'infested'	Baker, pers comm in Murphy and Oldbury (1996)
		98.25% of cockroach species	(Kutrup, 2003)
		45.7% of sampled areas	(Gliniewicz *et al.*, 2006)
	Blatta orientalis	56% of units 'infested'	Baker, pers comm in Murphy and Oldbury (1996)
		1.12% of cockroach species	(Kutrup, 2003)
		31.9% of sampled areas	(Gliniewicz *et al.*, 2006)
	Periplaneta americana	Reported	(Baker, 1982)
		0.63% of cockroach species	(Kutrup, 2003)
		25% of observations	(Cintra-Socolowski *et al.*, 2011)
	Supella longipalpa	1% of units 'infested'	Baker, pers comm in Murphy and Oldbury (1996)
Ants	*Monomorium pharaonis*	11.6% of hospitals 'infested'	(Edwards and Baker, 1981)
		21.1% of sampled areas	(Gliniewicz *et al.*, 2006)
	Lasius niger	4% of hospitals requesting advice	(Baker, 1982)
		7% of arthropods sampled	(Sramova *et al.*, 1992)
	Lasius emarginatus	8% of arthropods sampled	
	Brachymyrmex sp	21% of observations	(Cintra-Socolowski *et al.*, 2011)
Beetles	Dermestidae	1.2% of hospitals requesting advice	(Baker, 1982)
	Stegobium paniceum	0.6% of hospitals	

		requesting advice	
	Ptinidae	0.6% of hospitals requesting advice	
	Athous niger	0.6% of arthropods sampled	(Sramova *et al.*, 1992)
	Coccinella 7-punctata	0.6% of arthropods sampled	
	Meligethes sp	0.6% of arthropods sampled	
	Nitidulidae	0.6% of arthropods sampled	
	Tenebrio molitor	1.2% of arthropods sampled	
Bugs	Hemipteran bug	0.6% of arthropods sampled	
	Cimex lectularius	1.8% of hospitals requesting advice	(Baker, 1982)
Crickets		1.8% of hospitals requesting advice	
Firebrats and Silverfish		2.4% of hospitals requesting advice	
Fleas		Reported	
		Reported	(Gliniewicz *et al.*, 2006)
Spiders	*Steatoda bipunctata*	0.6% of arthropods sampled	(Sramova *et al.*, 1992)
	Tegenaria domestica	1.2% of arthropods sampled	
	Nuctenea umbratica	2.5% of arthropods sampled	
	Araneus sp	0.6% of arthropods sampled	
	Mitopus morio	0.6% of arthropods sampled	
Mites		1.2% of hospitals requesting advice	(Baker, 1982)

In hospitals in Poland, *B. germanica* was reported mainly in kitchens (31.4% of hospitals), followed by utility rooms (22.3% of all tested), store rooms (14.3% of hospitals) and laundries (13.1% of areas) according to Gliniewicz *et al.* (2006). *B. germanica* was also recorded in patient's rooms (10% of all tested), consulting rooms (3.4% of areas), sterilisation rooms (4% of areas tested) and operating theatres (1.7% of hospitals) as reported by Gliniewicz *et al.* (2006).

In hospitals in Turkey, *B. germanica* was found throughout all hospital areas, while *Blatta orientalis* and *Periplaneta americana* were caught exclusively in kitchen areas (Kutrup, 2003). *B. germanica* nymphs were the most commonly sampled life stage (67.28 – 88.26%) and their locations included patient rooms, nurse rooms and doctor rooms. Local Authority environmental health departments rank the following areas (from most likely to least likely) as being 'most likely to support infestations

within the hospital'; kitchens, ducting, boiler house, laundries, wards, theatres (Murphy and Oldbury, 1996).

Based on the literature, hospital wards and kitchens should be sampled, as these are likely areas for insect activity and light traps are considered to be an appropriate method of undertaking this sampling, with many different species of flies (Order Diptera) being the type of flying insects most likely to be found. Crawling insects may also be encountered as part of the sampling.

The aim of this thesis chapter was to build on the analysis of the KCIIS data by collecting and identifying the flying insects associated with a number of UK hospitals, in order to classify and enumerate the insects found and establish their seasonality and location in such premises, therefore informing pest control measures.

6.2 MATERIALS AND METHODS

6.2.1 Collection of flying insects from hospitals – National Health Service and pest control approval

Killgerm Chemicals customers providing a pest control service to National Health Service (NHS) hospitals were approached to assist with the collection of flying insects. Permission was then sought in principle from Facilities Management who managed the pest control contracts at the relevant NHS premises.

6.2.2 Ethics approval from the NHS

The required NHS Ethics approval was sought for the study. An ethics application was prepared using the Integrated Research Application System (IRAS) and submitted to an NHS Research Ethics Committee (REC) for review.

6.2.3 Research and Development (R&D) approval from the NHS

After the NHS Ethics review was completed, a Research and Development (R&D) application was submitted via IRAS to the R&D department for each NHS trust in order to gain R&D approval from all participating trusts.

6.2.4 Collection of flying insects from hospitals – field sampling

Flying insects were collected from pre-existing ultra-violet (UV) light flytraps in the form of Electronic Fly Killers (EFKs) and professional sticky traps located throughout seven hospitals 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.

6.2.5 Identification of flying insects sampled from hospitals

The samples were identified to species where possible and by genus or family otherwise, by using a dissection microscope and entomological references (Colyer and Hammond, 1951, Unwin, 1981, Chinery, 1993, Chinery and Falk, 2007, Chinery, 2012). Entomological tweezers sterilised as in section 2.2.6, were used to handle the flying insect samples. Once the insects were identified, they were sorted into 30ml screw-top sterile sample tubes (King Scientific, Liversedge, UK) and stored at 4°C in a domestic refrigerator, awaiting microbiological analysis.

6.2.6 Statistical techniques

A measure of species diversity was calculated seasonally for all insects collected, using Simpson's diversity index (*D*) (Begon *et al.*, 1996). However, because insect families rather than species were being analysed and there was an unequal sample size (i.e. between seasons, as the number of insects sampled from certain hospitals at different times of year was not equal), equitability (E_D) was calculated (Begon *et al.*, 1996).

Simpson's diversity:

$$D = 1 - \frac{\Sigma\, n\,(n-1)}{N\,(N-1)}$$

D = Simpson's diversity index

N = total number of individuals of all species

n = number of individuals of a specific species

i = subscript to denote the number of different species

Equitability:

$$D = \frac{1}{\sum_{i=1}^{s} p_i^2} \qquad E_D = \frac{D}{D_{max}} = \frac{1}{\sum_{i=1}^{s} p_i^2} \times \frac{1}{S}$$

D = Simpson's diversity index

S = total number of species in the community (richness)

p_i = proportion of S made up of the ith species

E_D equitability (evenness)

6.3 RESULTS

6.3.1 Collection of flying insects from hospitals – National Health Service and pest control approval

Initially, ten NHS trusts were identified as potential collaborators. Permission in principle from both pest control and Facilities Management was subsequently obtained from six of the identified trusts.

6.3.2 Ethics approval from the NHS

Full ethics approval was granted for the study, approval reference 09/H0408/99.

6.3.3 Research and Development (R&D) approval from the NHS

Full R&D approval was granted for the study, reference EX10/9215.

6.3.4 Collection and identification of flying insects from hospitals

A total of ***19,937 individual insects*** (and other arthropods) were collected from seven UK hospitals from March 2010 to August 2011 in this study. Of these individuals, approximately 114 arthropod species were identified. The use of word 'approximate' refers to the fact that a number of individuals were identified to Order or family or genus level only. Key species of importance are illustrated in Figure 6.1.The full results are listed in Table 6.3 in the form of a species checklist and are described in the following sections of this chapter.

The housefly *Musca domestica*, life cycle. Model laboratory organism.

Right: Adults. Top Centre: Eggs.

Bottom: Larvae. Top left: Pupae.

Clemson University - USDA Cooperative Extension Slide Series, Bugwood.org

Calliphora vicina, blowfly, family Calliphoridae.

The most common synanthropic fly in UK hospitals.

Gary Alpert, Harvard University, Bugwood.org

Non-biting midge, family Chironomidae.

The most common fly in UK hospitals.

Joseph Berger, Bugwood.org

Psychoda sp, family Psychodidae.

The most common 'drain fly' in UK hospitals.

Whitney Cranshaw, Bugwood.org

Figure 6.1 Flies of importance in UK hospitals.

Table 6.3 Full checklist of arthropod species identified in UK hospitals

ORDER	FAMILY	SPECIES	COMMON NAME	Number
Araneae	Dysderidae	*Dysdera crocata*	Woodlouse spider	1
Coleoptera	Anobiidae	*Stegobium paniceum*	Biscuit beetle	16
	Anthribidae	Anthribidae unknown	Fungus weevil	1
	Cantharidae	*Rhagonycha fulva*	Red soldier beetle	1
	Carabidae	Carabidae unknown	Ground beetle	9
	Chrysomelidae	*Gastrophysa viridula*	Green dock beetle	1
	Coccinellidae	*Adalia bipunctata*	Two-spot ladybird	9
		Adalia-10-punctata	Ten-spot ladybird	1
		Calvia-14-guttata	Cream-spot ladybird	8
		Coccinellidae unknown	Ladybird	3
		Coccinella-7-punctata	Seven-spot ladybird	1
		Harmonia axyridis	Harlequin ladybird	51
		Propylea 14-punctata	14-spot ladybird	1
		Psyllobora-22-punctata	22-spot ladybird	1
	Curculionidae	*Phyllobius pomaceous*	Nettle weevil	5
		Polydrusus formosus	Broad-nosed weevil	2
		Sitona sp	Pea leaf weevil	5
	Dermestidae	*Anthrenus verbasci*	Varied carpet beetle	47
		Attagenus pellio	Fur beetle	48
		Dermestes peruvianus	Leather beetle	113
		Reesa vespulae	Dermestid beetle	19
	Mycetophagidae	Mycetophagidae unknown	Fungus beetle	1

	Scarabaeidae	*Amphimallon solstitialis*	Summer chafer	1
	Staphylinidae	Staphylinidae unknown	Rove beetle	41
	Tenebrionidae	*Lagria hirta*	Tenebrionid beetle	13
		Tribolium castaneum	Rust red flour beetle	1
Diptera	Anisopodidae	*Sylvicola fenestralis*	Window gnat	9
	Anthomyiidae	Anthomyiidae unknown	Anthomyid fly	1
		Eustalomyia festiva	Anthomyid fly	1
	Bibionidae	Bibionidae unknown	March fly	1
		Dilophus febrilis	Fever fly	6
	Calliphoridae	*Calliphora vicina*	Bluebottle	1914
		Lucilia sericata	Greenbottle	64
		Pollenia rudis	Cluster fly	96
	Cecidomyiidae	Cecidomyiidae unknown	Gall midge	383
		Jaapiella veronicae	Gall midge	154
	Ceratopogonidae	Ceratopogonidae unknown	Biting midge	19
	Chironomidae	Chironomidae unknown	Non-biting midge	8442
	Chloropidae	Chloropidae unknown	Frit flies	3
		Thaumatomyia notata	Small yellow cluster fly	4
	Culicidae	*Culex pipiens*	Mosquito	3
		Culicidae unknown	Mosquito	7
		Culiseta annulata	Mosquito	1
	Dixidae	Dixidae unknown	Meniscus midges	5
	Dolichopodidae	Dolichopodidae unknown (likely *Dolichopopus popularis*)	Long-legged fly	71
		Sciapus platypterus	Long-legged fly	1
	Drosophilidae	*Drosophila* sp	Fruit fly	79
	Fanniidae	*Fannia canicularis*	Lesser housefly	169
	Heleomyzidae	Heleomyzidae unknown	Heleomyzid fly	14
		Suilia variegata	Heleomyzid fly	3
		Tephroclamys rufiventris	Heleomyzid fly	15
	Lonchaeidae	Lonchaeidae unknown	Lonchaeid fly	1

	Lonchopteridae	Lonchopteridae unknown	Pointed-wing fly	10
	Muscidae	*Helina reversio*	Muscid fly	2
		Helina sp	Muscid fly	2
		Musca autumnalis	Autumn fly / face fly	6
		Musca domestica	Housefly	89
		Muscina stabulans	False stable fly	12
		Phaonia sp (*subventa* or *rufiventris* or similar)	Muscid fly	5
		Phaonia sp	Muscid fly	17
		Polietes lardaria	Muscid fly	3
		Polietes sp	Muscid fly	3
	Opomyzidae	Opomyzidae unknown	Opomyzid fly	2
	Phoridae	Phoridae unknown	Phorid fly, scuttle fly, coffin fly	1131
	Psychodidae	Psychodidae unknown	Owl midge	1315
	Rhagionidae	*Chrysopilus cristatus*	Snipe fly	1
	Sarcophagidae	*Sarcophaga carnaria*	Flesh fly	16
	Scathophagidae	*Scathophaga stercoraria*	Yellow dung fly	11
	Scatopsidae	Scatopsidae unknown	Dung midge	1
	Sciaridae	Sciaridae unknown	Fungus gnat	222
	Sepsidae	Sepsidae unknown	Black scavenger fly	3
		Sepsis fulgens	Black scavenger fly	1
	Simuliidae	Simuliidae unknown	Blackfly	12
	Sphaeroceridae	Sphaeroceridae unknown	Lesser dung fly	712
		Trichiaspis sp	Lesser dung fly	9
	Stratiomyidae	*Beris vallata*	Common orange legionnaire	5
	Syrphidae	*Episyrphus balteatus*	Marmalade hoverfly	17
		Eristalis tenax	Drone fly	2
		Syrphidae unknown	Hoverfly	4
		Syrphus ribesii	Hoverfly	3
	Tabanidae	*Haematopota pluvialis*	Cleg / horse fly	1

	Tephritidae	Tephritidae unknown	Fruit fly	1
	Tipulidae	*Nephrotoma appendiculata*	Spotted crane fly	62
		Tipulidae unknown	Crane fly	69
Hemiptera	Aphididae	Aphididae unknown	Aphid	1977
	Cicadellidae	Cicadellidae unknown	Leafhopper	650
	Cercopidae	Cercopidae unknown	Froghopper	111
	Lygaeidae	Lygaeidae unknown	Ground bug	1
	Psyllidae	Psyllidae unknown	Jumping plant louse	30
	Pentatomidae	Pentatomidae unknown	Shield bug	5
Hymenoptera	Apidae	*Andrena cineraria*	Mining bee	3
		Bombus lucorum	White-tailed bumblebee	2
		Colletes sp	Mortar bee	4
		Osmia rufa	Red mason bee	1
	Chalcidae	Chalcidae unknown	Chalcid wasp	1
	Cynipidae	Cynipdae unknown	Gall wasp	13
	Formicidae	*Hypoponera punctatissima*	Roger's ant	504
		Lasius niger	Garden ant	235
	Ichneumonidae	Ichneumonidae unknown	Ichneumon wasp	61
	Sphecidae	Sphecidae unknown	Digger wasp	1
	Vespidae	*Dolichovespula sylvestris*	Tree wasp	2
		Vespidae unknown	Wasp	2
		Vespula germanica	German wasp	8
		Vespula vulgaris	Common wasp	96
Lepidoptera	Noctuidae	Noctuidae unknown	Night-flying moth	574
	Oecophoridae	*Endrosis sarcitrella*	White shouldered house moth	2
		Hofmannophila pseudospretella	Brown house moth	7

	Pterophoridae	Pterophoridae unknown	Plume moth	4
Neuroptera	Chrysopidae	Chrysopidae unknown	Green lacewing	19
Psocoptera	Psocoptera	Psocoptera unknown	Booklouse / Psocid	2
Symphyta	Symphyta	Symphyta unknown	Sawfly	1
Thysanoptera	Thysanoptera	Thysanoptera unknown	Thrip / thunder bug	4
Trichoptera	Trichoptera	Trichoptera unknown	Caddis fly	2

Table 6.4 Arthropod orders sampled from hospitals

Arthropod order	**Number of individuals (%)**
Diptera	15,215 (76.3%)
Hemiptera	2,774 (13.9%)
Hymenoptera	933 (4.7%)
Lepidoptera	587 (2.9%)
Coleoptera	399 (2%)
Neuroptera	19 (0.1%)
Thysanoptera	4 (<0.1%)
Psocoptera	2 (<0.1%)
Trichoptera	2 (<0.1%)
Symphyta	1 (<0.1%)
Araneae	1 (<0.1%)

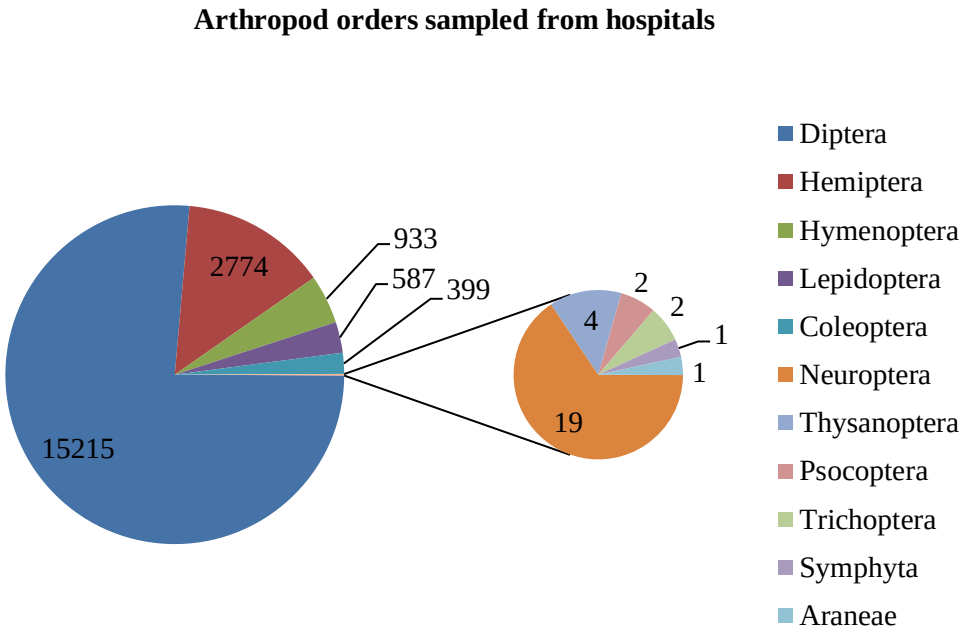

Figure 6.2 Arthropod orders sampled from seven UK hospital sites from March 2010 to August 2011. The numbers of individuals from each order are presented, as are the percentages in the form of pie charts.

Table 6.4 and Figure 6.2 show that true flies of the order Diptera were the most commonly identified of all insect (and other arthropod) orders sampled from hospitals, accounting for 76.3% of all samples. This was followed by true bugs (order Hemiptera) at 13.9%, wasps, ants and bees (Hymenoptera) at 4.7%, moths and butterflies (order Lepidoptera) at 2.9% and beetles (order Coloeptera) at 2%. The remainder was made up of lacewings (Neuroptera), thrips (Thysanoptera), booklice (Psocoptera), caddis flies (Trichoptera), saw flies (Symphyta) and spiders (Araneae).

Table 6.5 Diptera families sampled from hospitals

Diptera family	**Number of individuals (%)**		**Diptera family**	**Number of individuals (%)**
Chironomidae	8442 (55.5%)			
Calliphoridae	2074 (13.6%)		Culicidae	11 (<0.1%)
Psychodidae	1315 (8.6%)		Scathophagidae	11 (<0.1%)
Phoridae	1131 (7.4%)		Lonchopteridae	10 (<0.1%)
Sphaeroceridae	721 (4.7%)		Anisopodidae	9 (<0.1%)
Cecidomyiidae	537 (3.5%)		Bibionidae	7 (<0.1%)
Sciaridae	222 (1.5%)		Chloropidae	7 (<0.1%)
Fanniidae	169 (1.1%)		Dixidae	5 (<0.1%)
Muscidae	139 (0.9%)		Stratiomyidae	5 (<0.1%)
Tipulidae	131 (0.9%)		Sepsidae	4 (<0.1%)
Drosophilidae	79 (0.5%)		Anthomyiidae	2 (<0.1%)
Dolichopodidae	72 (0.5%)		Opomyzidae	2 (<0.1%)
Heleomyzidae	32 (0.2%)		Lonchaeidae	1 (<0.1%)
Syrphidae	26 (0.2%)		Rhagionidae	1 (<0.1%)
Ceratopogonidae	19 (0.1%)		Scatopsidae	1 (<0.1%)
Sarcophagidae	16 (0.1%)		Tabanidae	1 (<0.1%)
Simuliidae	12 (<0.1%)		Tephritidae	1 (<0.1%)

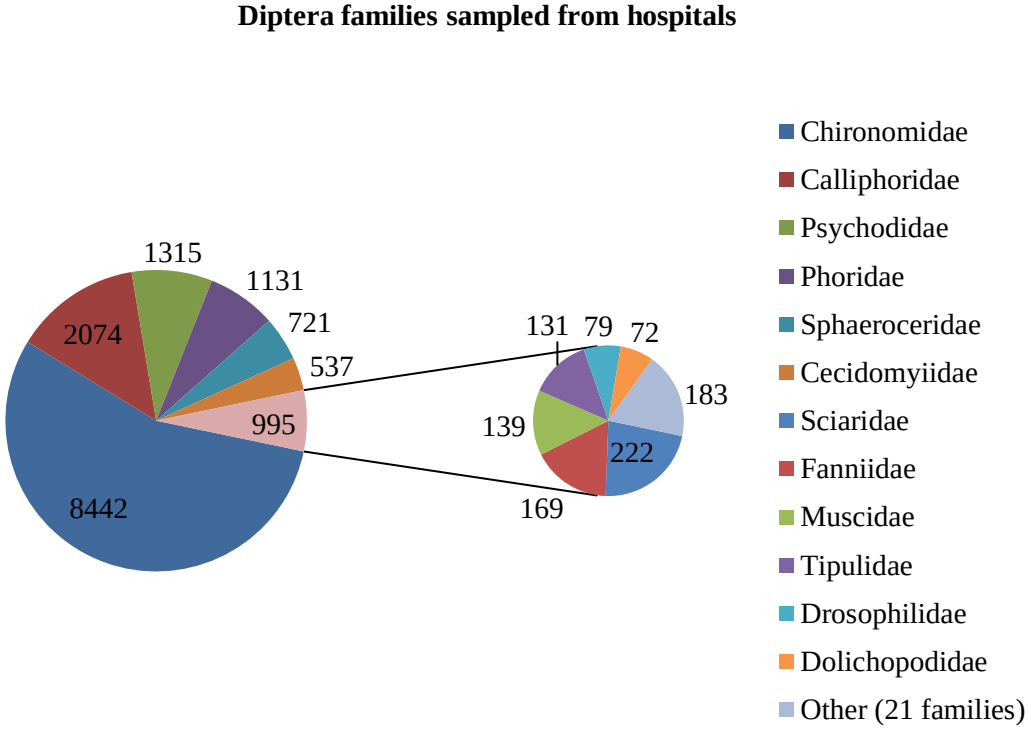

Figure 6.3 Diptera families sampled from seven UK hospitals from March 2010 to August 2011. The numbers of individuals from each family are presented, as are the percentages in the form of pie charts.

Table 6.5 and Figure 6.3 show that non-biting midges of the family Chironomidae were the most commonly encountered flies, accounting for 55.5% of all Diptera samples from hospitals. Blowflies of the family Calliphoridae were the most common synanthropic fly, comprising 13.6% of all Diptera samples. The next most commonly sampled families were owl midges of the Family Psychodidae, scuttle flies of the family Phoridae and lesser dung flies of the family Sphaeroceridae, representing 8.6%, 7.4% and 4.7% of Diptera samples respectively. Flies of these three families can be classed as 'drain flies', due to their propensity to breed in decaying organic matter that is often associated with drains. Fungus gnats of the Family Sciaridae, fruit flies of the family Drosophilidae, black scavenger flies of the family Sepsidae and dung midges of the family Scatopsidae can also be classed as 'drain flies' for the same reason. The seven families of 'drain flies' contribute collectively to 22.8% of all Diptera sampled from hospitals. Fly families Fanniidae and Muscidae, which contain the synanthropic flies the lesser housefly *F. canicularis* and housefly *M. domestica* respectively, accounted for a surprisingly low 1.1% and 0.9% of Diptera sampled from hospitals. The remainder of fly families sampled from hospitals that per family accounted for 0.5% or higher of all Diptera samples were 'casual intruder' type flies with external breeding media. These were gall midges of the family Cecidomyiidae, crane flies of the family Tipulidae and long-legged flies of the family Dolichopodidae, representing 3.5%, 0.9% and 0.5% of Diptera samples respectively. The remainder

of fly families all contributed 0.2% or less per family to Diptera samples as a whole. There were 21 fly families that fell into this category and are described as 'other' in Figure 6.3.

Table 6.6 Hemiptera families sampled from hospitals

Hemiptera family	Number of individuals (%)
Aphididae	1977 (71%)
Cicadellidae	650 (23%)
Cercopidae	111 (4%)
Psyllidae	30 (1%)
Pentatomidae	5 (<1%)
Lygaeidae	1 (<1%)

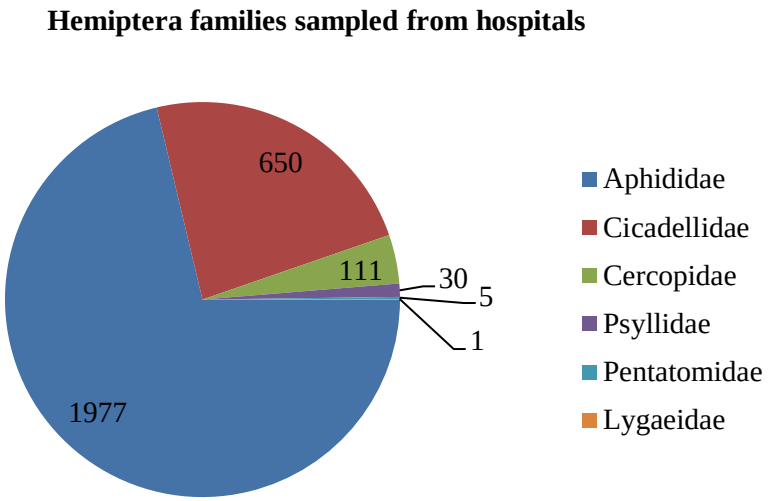

Figure 6.4 Hemiptera families sampled from seven UK hospitals from March 2010 to August 2011. The numbers of individuals from each family are presented, as are the percentages in the form of a pie chart.

Table 6.6 and Figure 6.4 show that aphids of the family Aphididae were the most commonly encountered family from the true bugs, Order Hemiptera, accounting for 71% of all Hemiptera samples from hospitals. Leafhoppers of the family Cicadellidae were the next most common, comprising 23% of all Hemiptera samples from hospitals. The remaining 6% of Hemiptera consisted of planthoppers of the family Cercopidae, plant lice of the family Psyllidae, shield bugs of the family Pentatomidae and ground bugs of the family Lygaeidae.

Table 6.7 Hymenoptera families sampled from hospitals

Hymenoptera family	Number of individuals (%)
Formicidae	738 (79.1%)
Vespidae	109 (11.7%)
Ichneumonidae	61 (6.5%)
Cynipidae	13 (1.4%)
Apidae	10 (1.1%)
Chalcidae	1 (0.1%)

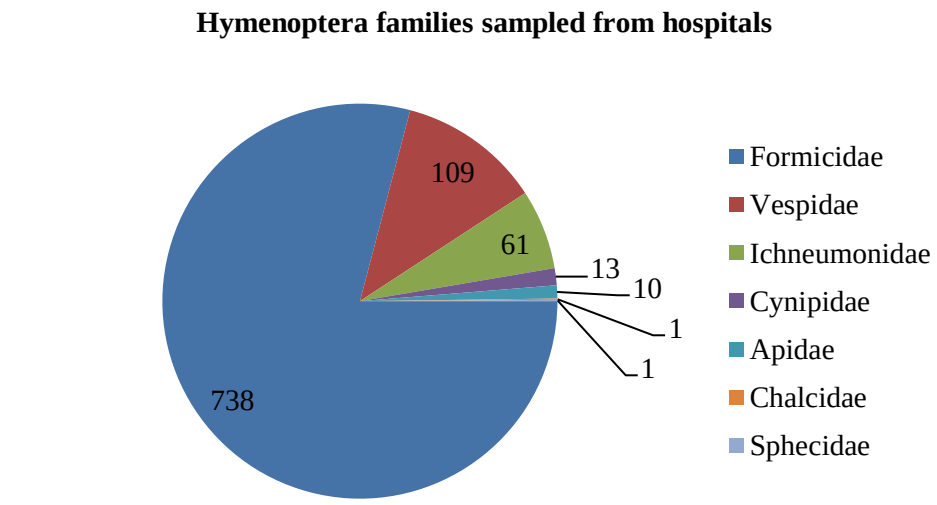

Figure 6.5 Hymenoptera families sampled from seven UK hospitals from March 2010 to August 2011. The numbers of individuals from each family are presented, as are the percentages in the form of a pie chart.

Table 6.7 and Figure 6.5 show that ants of the family Formicidae were the most commonly encountered family from the Order Hymenoptera (wasps, ants and bees), accounting for 79.1% of all Hymenoptera samples from hospitals. Social wasps of the family Vespidae were the next most common, comprising 11.7% of all Hymenoptera samples from hospitals, followed by parasitic wasps or 'ichneumon flies' of the family Ichneumonidae that contributed 6.5%. The remaining 2.6% of Hymenoptera consisted of gall wasps of the family Cynipidae, bees of the family Apidae and chalcid / parasitic wasps of the family Chalcidae.

Table 6.8 Lepidoptera families sampled from hospitals

Lepidoptera family	Number of individuals (%)
Noctuidae	574 (97.8%)
Oecophoridae	9 (1.5%)
Pterophoridae	4 (0.7%)

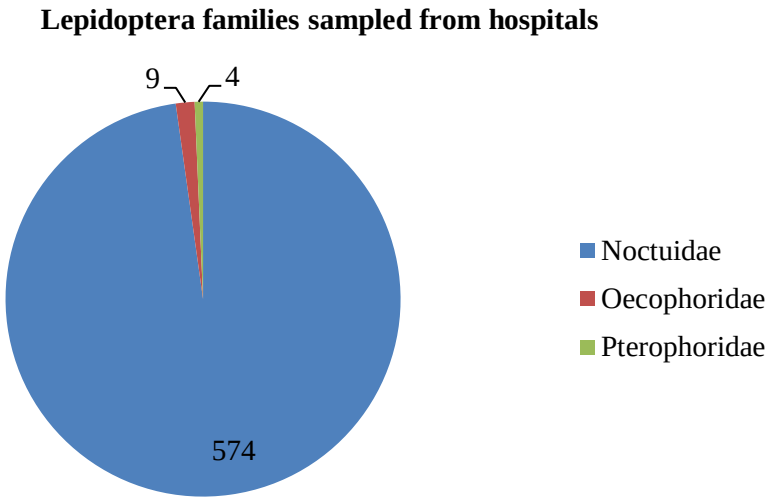

Figure 6.6 Lepidoptera families sampled from seven UK hospitals from March 2010 to August 2011. The numbers of individuals from each family are presented, as are the percentages in the form of a pie chart.

Table 6.8 and Figure 6.6 show that night-flying moths of the family Noctuidae were the most commonly encountered family from the Order Lepidoptera (moths and butterflies), accounting for 97.8% of all Lepidoptera samples from hospitals. The remaining 2.2% of Lepidoptera consisted of moths of the family Oecophoridae and plume moths of the family Pterophoridae.

Table 6.9 Coleoptera families sampled from hospitals

Coleoptera family	Number of individuals (%)		Coleoptera family	Number of individuals (%)
Dermestidae	227 (56.9%)			
Coccinellidae	74 (18.5%)		Anthribidae	1 (0.3%)
Staphylinidae	41 (10.3%)		Cantharidae	1 (0.3%)
Anobiidae	16 (4%)		Chrysomelidae	1 (0.3%)
Tenebrionidae	14 (3.5%)		Mycetophagidae	1 (0.3%)
Curculionidae	13 (3.3%)		Scarabaeidae	1 (0.3%)
Carabidae	9 (2.3%)			

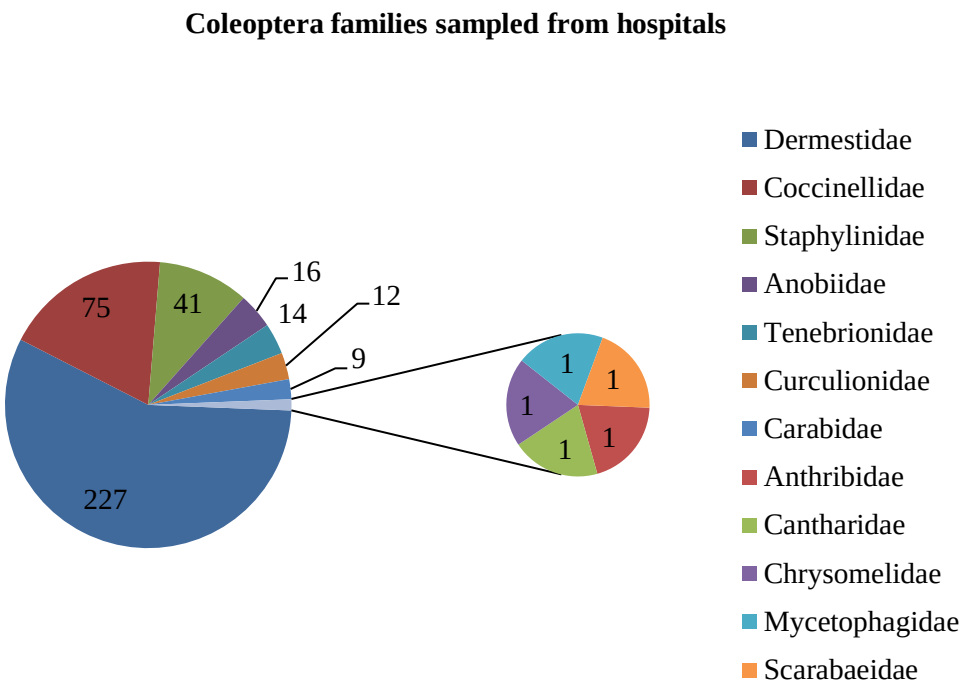

Figure 6.7 Coleoptera families sampled from seven UK hospitals from March 2010 to August 2011. The numbers of individuals from each family are presented, as are the percentages in the form of pie charts.

Table 6.9 and Figure 6.7 show that skin feeding beetles of the family Dermestidae were the most commonly encountered beetles, accounting for 56.9% of all Coleoptera samples from hospitals. Ladybirds of the family Coccinellidae, were the next most common, comprising 18.5% of all Coleoptera samples from hospitals, followed by rove beetles of the family Staphylinidae, wood-boring and stored product beetles of the family Anobiidae, darkling beetles of the family Tenebrionidae, weevils of the family Curculionidae and ground beetles of the family Carabidae, accounting for 10.3%, 4%, 3.5%, 3.3% and 2.5% respectively. The remaining 1.2% of Coleoptera consisted of fungus weevils of the family Anthribidae, soldier beetles of the family Cantharidae, leaf beetles of the family Chrysomelidae, fungus beetles of the family Mycetophagidae and chafer beetles of the family Scarabaeidae.

Table 6.10 Other orders / families sampled from hospitals

Order	**Family**	**Number of individuals**
Neuroptera	Chrysopidae	19
Thysanoptera	-	4
Psocoptera	-	2
Trichoptera	-	2
Symphyta	-	1
Araneae	Dysderidae	1

Table 6.10 shows the other orders / families sampled from hospitals. The percentage that the number of individuals represents for their given order / family is not provided as the sampled individuals are the sole representatives of their order / family.

Lacewings of the family Chrysopidae numbered 19 individuals in the sampled hospitals, followed by 4 thrips of the order Thysanoptera, 2 booklice of the order Psocoptera, 2 caddis flies of the order Trichoptera, 1 sawfly of the order Symphyta and 1 spider from the family Dysderidae of the order Araneae.

6.3.5 Types of insects sampled according to synanthropy classification

Sramova *et al.* (1992) used the following categories to define insects sampled in their hospital study; *Parasites* (mosquitoes, Ceratopogonidae, Simuliidae, etc.), *Eusynanthropic* (synanthropic flies, wasps, SPI beetles), *Hemisynanthropic* (ants, spiders) and occasionally encountered insects (non-biting midges & other flies, moths, beetles). Using this system of categorisation, results from the current study are presented in Table 6.11.

Table 6.11 Types of insects sampled from hospitals, according to synanthropy classification

Synanthropy classification, according to Sramova *et al.* (1992)	**Number of individuals (%)**
Occasionally encountered insects	12,936 (64.9%)
Eusynanthropic	6,217 (31.2%)
Hemisynanthropic	739 (3.7%)
Parasites	45 (0.2%)

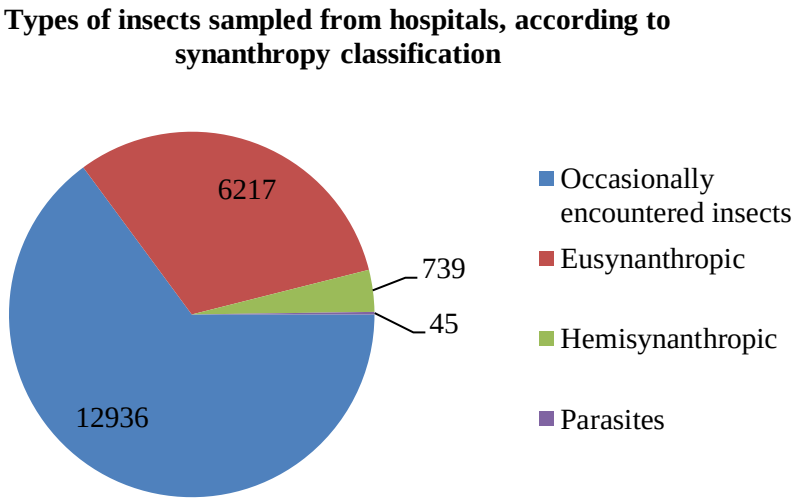

Figure 6.8 Insect groups sampled from seven UK hospitals from March 2010 to August 2011, arranged according to the synanthropy classification of insects used by Sramova *et al.* (1992) The numbers of individuals from each group are presented, as are the percentages in the form of a pie chart.

Examining the data in Table 6.11 and Figure 6.8, which are presented according to the synanthropy classification of insects used by Sramova *et al.* (1992), it is shown that 'occasionally encountered

insects' were the insect group most commonly sampled from hospitals, accounting for 64.9% of all insects. Eusynanthropic insects comprised 31.2% of insects sampled from hospitals, while hemisynanthropic insects and parasites contributed 3.7% and 0.2% respectively.

6.3.6 Life stages of sampled insects

The life stages of insects collected in the current study are described.
Number of **adults** collected; 19,839
Number of **larvae** collected; 98.
All the collected larvae were of the family Dermestidae and included 50 *Dermestes peruvianus* larvae and 48 *Attagenus pellio* larvae. Presumably these developed from eggs laid by winged adults in the catch trays (all larvae were found in UV light flytrap catch trays) and were feeding on dead insects.

Of the adults collected, Hymenopteran **queens** numbered; 386.
The majority of these were *Hypoponera punctatissima* and numbered 302. There were also 67 *Lasius niger* queens, 16 *Vespula vulgaris* queens and 1 *Bombus lucorum* queen.

Of the adults collected, Hymenopteran **workers** numbered; 67. The majority of these were *Vespula vulgaris* workers and numbered 62. There were also 5 *Vespula germanica* workers.

Of the adults collected, Hymenopteran **males** numbered; 168. All of these were *Lasius niger*, collected from 1 sticky board in a hospital coffee shop on 05/07/2011.
Scathophaga stercoraria were the only non-hymenopteran insects sexed; Males 5 Females 3.

Live insects were collected and numbered 64. One of these was a live *Calliphora vicina* that had presumably just been electrocuted in an electronic fly killer (EFK) type of ultra-violet light flytrap but perished soon thereafter. *Attagenus pellio* larvae, numbering 48, were collected live from EFK catch trays. The only other live insects that were collected were 15 *Calliphora vicina*, which were killed with Sorex super fly spray (BASF, Cheadle Hulme) in medical illustration department toilets.

6.3.7 Species diversity

Table 6.12 Species diversity (equitability E_D) for all insects collected from all hospitals

Total number of individuals	19937
Sum of pi squared reciprocal	4.686840
E_D	0.069952838
Number of species or families	67

Complete equitability is classed as 1, so the equitability here is very low.

Table 6.13 Species diversity (equitability E_D) for all insects (and other arthropods) from Sramova *et al*. (1992)

Total number of individuals	161
Sum of pi squared reciprocal	4.693283
E_D	0.187731
Number of species or families	25

Complete equitability is classed as 1, so the equitability here is very low.

The equitability of insects sampled in the current study was lower than that of the previous study by Sramova *et al.* (1992).

Table 6.14 Seasonal species diversity (expressed as equitability E_D) for all insects

Season	**Spring**	**Summer**	**Autumn**	**Winter**
Number of individuals	7605	9165	1015	2152
Number of families	41	59	26	30
Equitability (E_D)	0.066002	0.096933	0.086586	0.154611

Equitability is the evenness of individuals' distribution among families in this community. Using this method, complete equitability is classed as 1, so the equitability (E_D) figures here are actually very low. Table 6.14 shows that equitability for all insects was highest in winter, followed by summer and autumn and was lowest in spring. This can be interpreted as insect populations in hospitals being most diverse in spring and least diverse in winter.

6.3.8 Seasonality of insect numbers

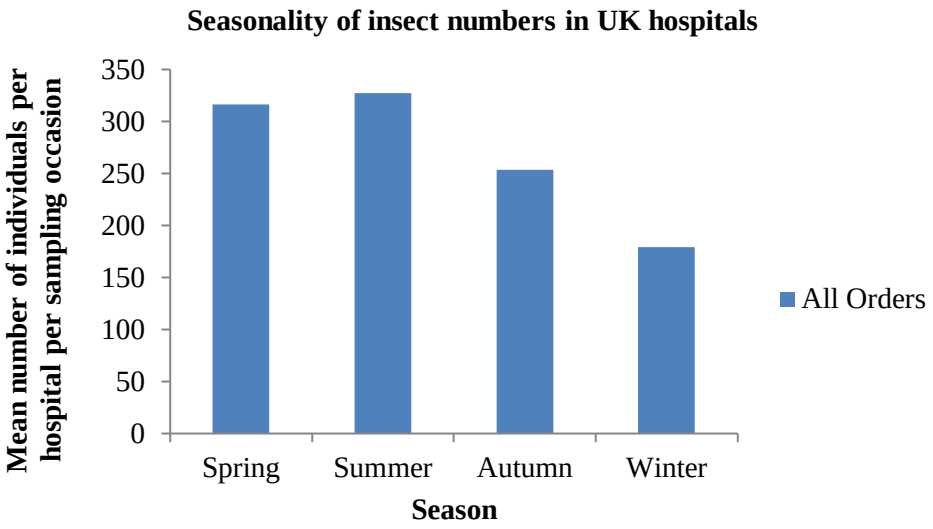

Figure 6.9 Seasonality of insects in UK hospitals. The mean seasonal number of all insect (and other arthropod) individuals per hospital per sampling occasion, sampled from seven UK hospitals from March 2010 to August 2011.

Figure 6.9 shows the mean seasonal number of all insect (and other arthropod) individuals per hospital per sampling occasion, sampled from seven UK hospitals from March 2010 to August 2011. The data are adjusted in this way, to compensate for the unequal sample sizes between hospitals and seasons i.e. some hospitals were sampled more than others and there were more sampling occasions in certain seasons. This gives a reflection of the number of insects a pest controller or member of an infection control team may expect to find in a typical hospital during a visit in one of the given seasons. The data presented in figures Figure 6.10, Figure 6.11, Figure 6.12, Figure 6.13 and Figure 6.14 is also adjusted in this way, for the same reason. Figure 6.9 shows that the greatest numbers of insect individuals per hospital per sampling occasion were sampled in summer, followed by spring, autumn and winter, with respective adjusted figures of 327, 317, 254 and 179.

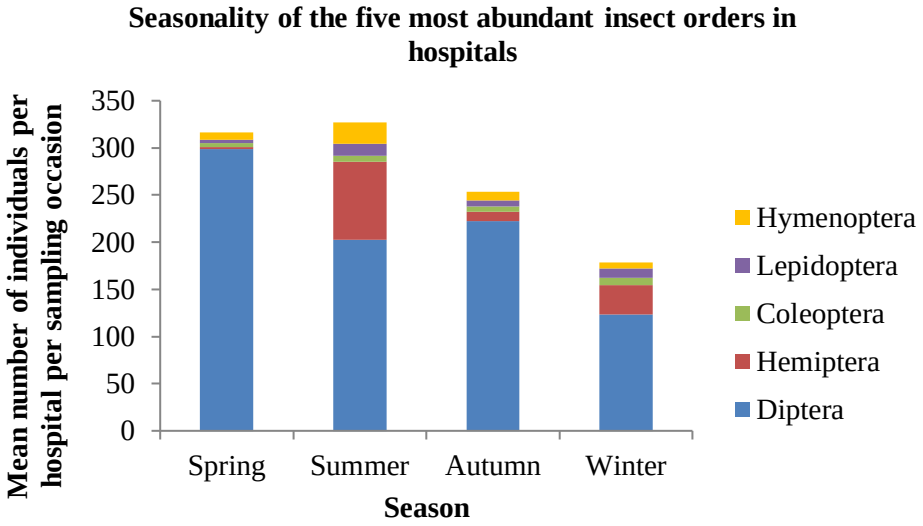

Figure 6.10 Seasonality of the five most abundant insect orders in hospitals. The mean seasonal number of individuals from the five most abundant insect Orders, per hospital per sampling occasion, sampled from seven UK hospitals from March 2010 to August 2011.

Figure 6.10 shows that flies (Order Diptera) were the most abundant insect Order in all seasons. Flies (Order Diptera) peaked in spring with a mean of approximately 299 individuals per hospital per sampling occasion, were second highest in autumn with a value of 223, third highest in summer with a value of 202 and lowest in winter with 124. True bugs (Order Hemiptera) peaked in summer with a mean of approximately 83 individuals per hospital per sampling occasion, were second highest in winter with a value of 31, third highest in spring with a value of eight and lowest in autumn with six. Beetles (Order Coleoptera) showed little variation through the seasons, with a mean of approximately eight individuals per hospital per sampling occasion in winter, seven in summer, six in autumn and four in spring. Moths (Order Lepidoptera) peaked in summer with a mean of approximately 13 individuals per hospital per sampling occasion, were second highest in winter with a value of 10, third highest in autumn with six and lowest in spring with four. Wasps, ants and bees (Order Hymenoptera) showed a distinctive peak in numbers in summer, with a mean of approximately 22 individuals per hospital per sampling occasion, followed by 10 in autumn, eight in spring and seven in winter.

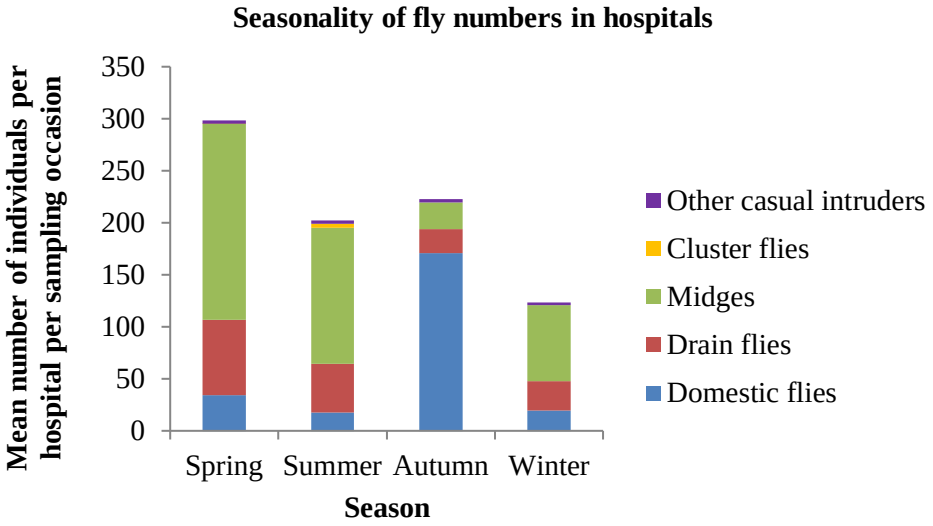

Figure 6.11 Seasonality of fly numbers in hospitals. The mean seasonal number of all fly (Order Diptera) individuals per hospital per sampling occasion, sampled from seven UK hospitals from March 2010 to August 2011.

'Drain Flies' category pools records of the rotting organic matter breeding *Drosophila* sp, Phoridae, Psychodidae, Sciaridae, Scatopsidae, Sphaeroceridae and Sepsidae.

'Cluster Flies' category pools records of the overwintering and clustering *Pollenia rudis*, *Thaumatomyia notata* and *Musca autumnalis*.

'Domestic Flies' category pools records of the rotting organic matter breeding houseflies *Musca domestica* and *Fannia canicularis*. Flesh breeding *Calliphora* sp, *Lucilia* sp and *Sarcophaga* sp are also included.

'Midges' category pools the records of various swarming flies and flying insects whose breeding media is not likely to be on site. Includes mosquitoes (*Culex pipiens*, *Culiseta annulata*), Chironomidae, Ceratopogonidae, Cecidomyiidae, Tipulidae, *Sylvicola fenestralis*, Simuliidae, Dixidae.

'Other casual intruder' category pools all other Diptera not covered by the above categories.

Figure 6.11 shows that 'midges' peaked in spring with a mean of approximately 189 individuals per hospital per sampling occasion, were second highest in summer with a value of 131, third highest in winter with a value of 73 and lowest in autumn with 25. 'Domestic flies'(fly categories defined in Figure 6.11) peaked in autumn with a mean of approximately 171 individuals per hospital per sampling occasion, were second highest in spring with a value of 35, third highest in winter with a value of 20 and lowest in summer with 18. 'Drain flies' peaked in spring with a mean of approximately 72 individuals per hospital per sampling occasion, were second highest in summer with

a value of 47, third highest in winter with a value of 28 and lowest in winter with 23. 'Cluster flies' were only found in numbers during summer with a mean of approximately 3 individuals per hospital per sampling occasion, a mean of less than one in spring and no cluster flies reported in autumn and winter.

'Other casual intruders' showed no real seasonal differences, with a mean of approximately 3 individuals per hospital per sampling occasion for each season.

A total of 1,914 adult *Calliphora vicina* were collected via sticky board and electronic fly killer units from all 7 hospital sites during the sampling period of March 2010 to August 2011, meaning that it was the most common synanthropic fly in this study. *C. vicina* were present in spring, summer, autumn and winter. The numbers of *C. vicina* collected seasonally were; spring 694, summer 405, autumn 648 and 167 in winter. As the number of sampling occasions and number of hospitals was not equal for each season, the adjusted figures for the seasonality of *C. vicina* are shown in Figure 6.12.

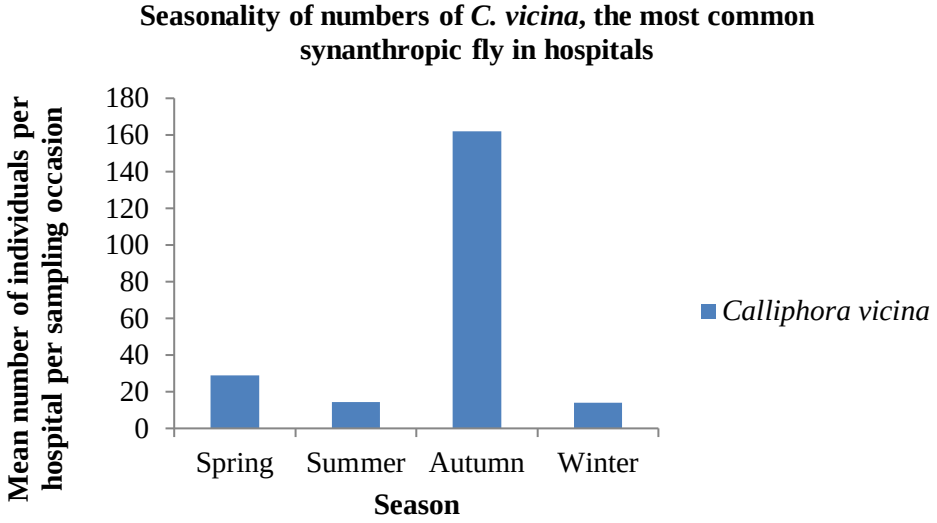

Figure 6.12 Seasonality of numbers of *C. vicina*, the most common synanthropic fly in hospitals. The mean seasonal number of *C. vicina* individuals per hospital per sampling occasion, sampled from seven UK hospitals from March 2010 to August 2011.

C. vicina peaked in autumn with a mean of approximately 162 individuals per hospital per sampling occasion, were second highest in spring with a value of 29, third highest in summer and winter with values of 14.

Table 6.15 Specific location records of *C. vicina* activity in hospitals

Fly species	Location	Specific location (n)
Calliphora vicina	Food preparation	Café (2)
		Catering (4)
		Coffee shops (5)
		Cooked food store
		Dry Food Store
		Dry stores
		Dry stores corridor
		Kitchen (9)
		Kitchen Sluice
		Laundry mess room kitchen
		Main kitchen stores
		Mental Health Kitchen A
		Patient Hotel Kitchen
		Postgrad Kitchen
		Raw food veg store
		Restaurant (3)
		Ward Kitchen (16)
	Treatment areas	Maternity
		Mental Health ward
		Neonatal
		New Neonatal & Maternity
		New Neonatal & Maternity (central delivery)
		Theatre waiting room
		Ward
	Non-patient areas	Leisure centre
		Mortuary
		Mortuary Bays
		Physiotherapy reception
		Ward office toilets (medical illustration)
		Workmen's room

Table 6.15 shows that *C. vicina* were found throughout a wide variety of hospital areas. The most reports came from the location category 'food preparation' with 50 cases of *C. vicina* activity in these areas, such as ward kitchens and food stores. There were seven reports from 'treatment areas', which includes hospital wards (among other areas where hospital patients are treated). Six reports came from non-patient areas of hospitals i.e. areas where patients do not routinely have access.

6.3.9 Specific locations of insect activity in hospitals

Locations that insects were sampled from in the current study included ward kitchens, catering units, cafés, café kitchens, restaurants, coffee shops, cooked food stores, dry food stores, raw food stores, reception areas, laundry, leisure centre, maternity wing, neonatal, mental health wing kitchens, mortuary, nursery, patient hotel kitchen, plant room, theatre waiting room, wards, ward toilets and a workmen's' room. Sampling locations are analysed in more detail for selected species throughout this chapter.

6.3.10 Insect vectors of *C. difficile*; numbers, seasonality and location in hospitals

The current study has shown that *M. domestica* has the potential to transfer *C. difficile*. *Fannia canicularis*, *Drosophila melanogaster* and *Psychoda alternata* collected from pig farms have all been shown to be positive for *C. difficile* (Burt *et al.*, 2012). These species of flies can therefore be classed as potential vectors of *C. difficile* and their prevalence, seasonality and locations within hospitals are shown in Table 6.16 and Figure 6.13.

Table 6.16 Number of individuals of fly species with vector potential for *C. difficile* sampled from hospitals

Fly species with vector potential for *C. difficile*	**Number of individuals (%)**
Psychodidae	1,315 (79.6%)
Fannia canicularis	169 (10.2%)
Musca domestica	89 (5.4%)
Drosophila sp	79 (4.8%)

A larger number of Psychodidae were sampled compared to other fly species with vector potential for *C. difficile* with these flies accounting for 79.6% of this group.

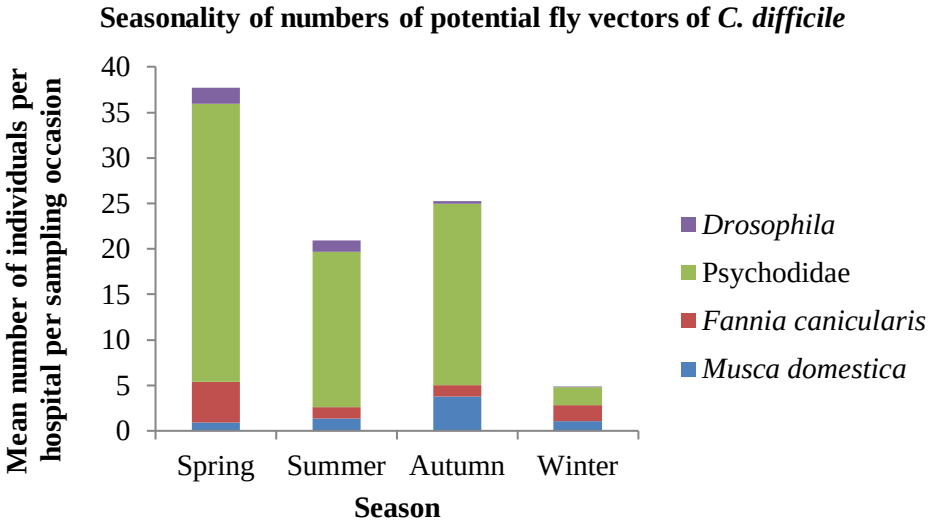

Figure 6.13 Seasonality of numbers of potential fly vectors of *C. difficile*. The mean seasonal number of fly individuals with *C. difficile* vector potential, per hospital per sampling occasion, sampled from seven UK hospitals from March 2010 to August 2011.

Of the potential fly vectors of *C. difficile* that were sampled, Psychodidae were the most commonly encountered in each season. Psychodidae peaked in spring with a mean of approximately 31 individuals per hospital per sampling occasion, were second highest in autumn with a value of 20, third highest in summer with 17 and lowest in winter with 2. Lesser houseflies *F. canicularis* peaked in spring with a mean of approximately 5 individuals per hospital per sampling occasion, second highest in winter with a value of 2, equal third highest in summer and autumn with values of 1. Houseflies *M. domestica* peaked in autumn with a mean of approximately 4 individuals per hospital per sampling occasion, equal highest in spring, summer and winter with values of 1. Fruit flies *Drosophila* sp were present in spring and summer, with respective means of 2 and 1 individual/s per hospital per sampling occasion. Means of less than one fruit fly were noted in autumn and winter.

Table 6.17 Specific location records of activity of fly species with vector potential for *C. difficile* in hospitals

Fly species with vector potential for *C. difficile*	**Location**	**Specific location**
Psychodidae	Food preparation	Café Bar
		Catering
		Cooked food store
		Dry food stores
		Ground Floor Kitchen
		Kitchen Trap door drain
		Main kitchen EFK
		Postgrad Kitchen
		Restaurant
		Restaurant / Café kitchen
		Ward Kitchen (4 instances)
	Treatment area	Maternity
		Neonatal
		Neonatal incubator room
		Neonatal incubator store (New)
		Neonatal & Maternity
		New Neonatal & Maternity (central delivery)
	Non-patient areas	Ward
		Plant room
		Workmen's' room
Fannia canicularis	Food preparation	Café Bar
		Catering unit (2)
		Coffee Shop (2)
		Ground Floor Kitchen
		Kitchen
		Kitchen (regeneration)
		Laundry mess room kitchen
		Main kitchen (2)
		Patient Hotel Kitchen
		Restaurant

		Ward kitchen (5)
	Treatment areas	Cardio
		Maternity
		Mental Health – eating disorders
		New Neonatal
		Ward C1
	Non-patient areas	Leisure centre
		Mortuary
		Workmen's room
Musca domestica	Food preparation	Café
		Café bar
		Café kitchen
		Delicatessen
		Catering facilities
		Coffee shop
		Dry food store
		Cooked food store
		Ground floor kitchen
		Mental health kitchen
		Patient hotel kitchen
		Restaurant
		Ward kitchens (6)
	Treatment areas	Neonatal unit
		Nursery
	Non-patient areas	Mortuary
Drosophila sp	Food preparation	Catering
		Cooked food store
		Kitchen
		Kitchen raw food stores
		Kitchen stores
		Main kitchen
		Ward Kitchen
	Treatment areas	Ward
	Non-patient areas	Leisure centre

Table 6.17 shows that fly species with known vector potential for *C. difficile* were found throughout a wide range of areas in the hospital environments that were sampled. The most reports came from the location category 'food preparation areas', with 57 instances of potential *C. difficile* vectors being sampled from food preparation areas. There were 14 instances of potential *C. difficile* vectors being sampled from 'treatment areas', which includes hospital wards (among other areas where hospital patients are treated). There were seven instances of potential *C. difficile* vectors being sampled from non-patient areas of hospitals i.e. areas where patients do not routinely have access. The limitation of the location data is that the data are a function of ultra-violet light flytrap placement.

In this thesis study, *M. domestica* was used as the model experimental organism, has been shown to have vector potential for *C. difficile* (see section 2.3) and hospital-sampled individuals harboured pathogenic bacteria (see 7.3.1), therefore it is important to take a closer look at this species in terms of its status in hospitals. A total of 89 adult *M. domestica* were collected via sticky board and electronic fly killer units from all 7 hospital sites, during the sampling period of March 2010 to August 2011 (Table 6.3).

In terms of the location of *M. domestica* within hospitals, the most reports came from the location category 'food preparation' with 18 cases of activity in these areas, such as ward kitchens and food stores (Table 6.17). There were two reports from 'treatment areas', which includes hospital wards (among other areas where hospital patients are treated). One report came from a non-patient area i.e. areas where patients do not routinely have access. The specific locations that *M. domestica* were sampled from (Table 6.17) included; café, café bar, café kitchen, delicatessen, catering facilities, coffee shop, dry food store, cooked food store, ground floor kitchen, mental health kitchen, mortuary, neonatal unit, nursery, patient hotel kitchen, restaurant and 6 different ward kitchens.

M. domestica were present in spring, summer, autumn and winter (Figure 6.13). The numbers of *M. domestica* collected seasonally were; spring 22, summer 39, autumn 15 and 13 in winter, although this is biased due to differences in the number of sampling occasions and hospitals sampled. The adjusted figures for the seasonality of *M. domestica* (and other potential vectors of *C. difficile*) are shown in Figure 6.13 and reflect the mean number of individuals per hospital per sampling occasion. *M. domestica* peaked in autumn with a mean of approximately 4 individuals per hospital per sampling occasion and was equal highest in spring, summer and winter with values of 1 (Figure 6.13).

M. domestica is frequently referred to as the most common synanthropic fly in human occupied premises, which is why it is a focus of this study (Mallis, 1964). However, in the current study, this was found not to be the case and *Calliphora vicina* was actually the most common synanthropic fly in UK hospitals, numbering 1,914 individuals.

6.3.11 Collection and identification of crawling insects from hospitals

A number of insects were recorded that are typically classed as 'crawling insects'. This may be seen as a surprise because ultra-violet light flytraps were the source of insect samples in this study, which are a component of integrated pest management of flying insects. The reason that some crawling insects were sampled from the ultra-violet light flytraps is that although they are classed as 'crawling insects' (because they crawl predominantly) many are capable of flight.

The realisation that crawling insects were identified from ultra-violet light flytraps in the current study of this chapter led to a re-examination of data from the KCIIS database (section 5). Data relating to crawling insects identified from UK hospitals arising from this chapter study is shown in Table 6.18 and Figure 6.14.

Table 6.18 A checklist of crawling insect species identified in UK hospitals

ORDER	FAMILY	SPECIES	Number
Araneae	Dysderidae	*Dysdera crocata*	1
Coleoptera	Anobiidae	*Stegobium paniceum*	16
	Anthribidae	Anthribidae unknown	1
	Cantharidae	*Rhagonycha fulva*	1
	Carabidae	Carabidae unknown	9
	Chrysomelidae	*Gastrophysa viridula*	1
	Coccinellidae	*Adalia bipunctata*	9
		Adalia-10-punctata	1
		Calvia-14-guttata	8
		Coccinellidae unknown	3
		Coccinella-7-punctata	1
		Harmonia axyridis	51
		Propylea 14-punctata	1
		Psyllobora-22-punctata	1
	Curculionidae	*Phyllobius pomaceous*	5
		Polydrusus formosus	2
		Sitona sp	5
	Dermestidae	*Anthrenus verbasci*	47
		Attagenus pellio	48
		Dermestes peruvianus	113
		Reesa vespulae	19
	Mycetophagidae	Mycetophagidae unknown	1
	Scarabaeidae	*Amphimallon solstitialis*	1
	Staphylinidae	Staphylinidae unknown	41
	Tenebrionidae	*Lagria hirta*	13
		Tribolium castaneum	1
Hemiptera (crawling)	Cercopidae	Cercopidae unknown	111
	Lygaeidae	Lygaeidae unknown	1
	Psyllidae	Psyllidae unknown	30
	Pentatomidae	Pentatomidae unknown	5
Hymenoptera	Formicidae	*Hypoponera punctatissima*	504
		Lasius niger	235

Of the crawling insects sampled from hospitals, beetles (Order Coleoptera) provided the greatest number of species, with 400 individuals representing 25 species within 12 families. The greatest numbers of individuals were represented by ants, family Formicidae, numbering 739 in total, provided by two species Roger's ants *H. punctatissima* and the garden ant *Lasius niger*. True bugs (Order Hemiptera) were represented by 147 individuals in four families. Spiders (Order Araneae) were represented by one individual, *Dysdera crocata*, from the family Dysderidae.

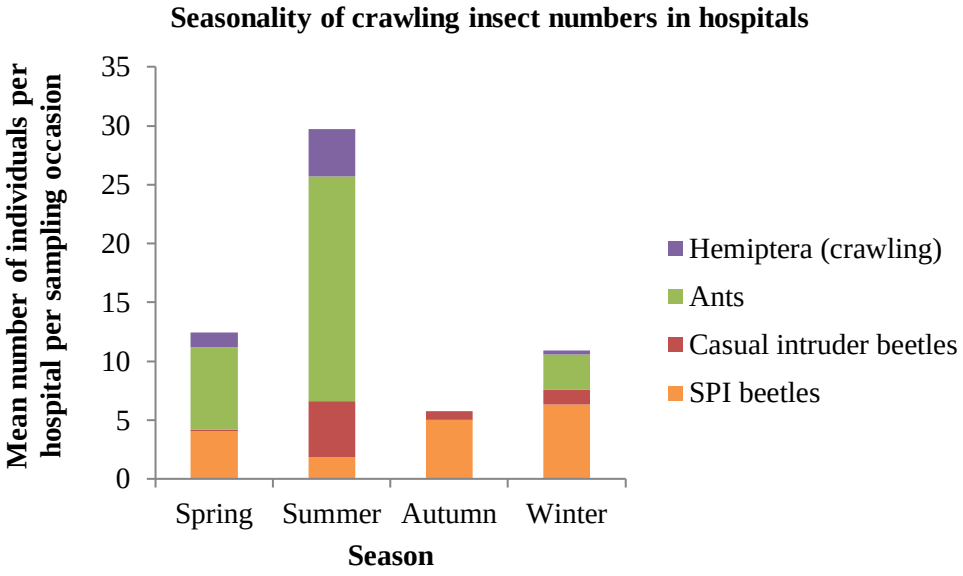

Figure 6.14 Seasonality of crawling insect numbers in hospitals. The mean seasonal number of crawling insect individuals per hospital per sampling occasion, sampled from seven UK hospitals from March 2010 to August 2011.

'Ants' category pools records of *Hypoponera punctatissima and Lasius niger*

'Hemiptera' pools records of this Order as per the families listed in Table 6.3.

'Casual intruder beetles' includes beetles from the families Anthribidae, Cantharidae, Carabidae, Chrysomelidae, Coccinellidae, Curculionidae, Scarabaeidae, Staphylinidae and *Lagria hirta*.

'SPI' category pools records of Stored Product Insects and 'fungus feeders' such as Anobiidae, Dermestidae, Mycetophagidae and *Tribolium castaneum*.

Crawling insects peaked in summer, with a mean of approximately 30 individuals per sampling occasion, were second highest in spring with a value of 12, third highest in winter with a value of 10 and lowest in autumn with 6. Ants peaked in summer with a mean of approximately 19 individuals per hospital per sampling occasion, were second highest in spring with a value of seven, third highest in winter with a value of three and no ants were recorded in autumn. SPI peaked in winter with a mean of approximately six individuals per hospital per sampling occasion, were second highest in autumn with a value of five, third highest in spring with a value of 4 and lowest in summer with a value of two. Casual intruder beetles peaked in summer with a mean of approximately five individuals per hospital per sampling occasion, were equal second highest in autumn and winter with a value of one and lowest in spring with a mean value of less than one. True bugs (Hemiptera) peaked in

summer with a mean of approximately four individuals per hospital per sampling occasion, were second highest in spring with a value of one, had a mean value of less than one in winter and were not recorded in autumn.

6.3.12 Case study: insects in a neonatal unit

In the 'old' neonatal unit of one hospital, 242 individual insects were sampled over three sampling occasions. When the new neonatal unit was built for that particular hospital, it was sampled over three sampling occasions and 200 individual insects were collected, which is a reduction in numbers compared to the old unit.

6.4 DISCUSSION

It is important to note the limitations of the sampling method used in this study, in that insects were collected from pre-existing UV light flytraps in the form of EFKs and professional sticky traps located throughout hospitals, which provides a potential bias in some of the data. For example, the data regarding location of insects in hospitals is actually a function of UV light flytrap placement.

UV light flytraps are designed to sample flying insects and not crawling insects, which is acceptable as the main focus of this study was on flying insects. However, UV light flytraps do capture predominantly crawling insects that are also capable of flight, which is why data regarding these species is presented and discussed in this thesis. From hereafter, any references to 'crawling insects' should be taken to mean 'predominantly crawling insects that are also capable of flight'.

Differential attraction to UV light in insect species may have influenced results also. The sampling method is clearly biased towards the mobile adult life stages of insects and adults were of course the most frequently sampled life stage in this study. Despite the limitations of the sampling method, the benefits and knowledge that this study brings are manifold and are outlined in this discussion.

This study represents the longest, most extensive and thorough study of flying insects in hospitals, with the greatest number of individual insects and species recorded in any available work in the literature. Therefore the conclusions and recommendations made in this work are the most authoritative currently available.

True flies of the order Diptera were the most commonly identified of all insect (and other arthropod) orders sampled, illustrating that they are the most important of all the flying insects in UK hospitals and that control of such insects should be a priority over other groups. This finding corresponds with another study, also showing that flies were the predominant type of insect in hospitals, this time in Brazil (Da Silva *et al.*, 2011). In terms of seasonality and assessing the time of year that fly problems are most likely in hospitals in the UK, this study showed that flies were the most abundant insect Order in all seasons. Fly numbers peaked in spring and were found in decreasing numbers in autumn then summer, being lowest in winter, information which should be used to guide fly control and therefore infection control.

Echoing the findings of the KCIIS database analysis, non-biting midges of the family Chironomidae were also commonly encountered in this study. Whereas chironomids were the most common flies in August in the KCIIS study, they were the most common fly family of all in this chapter by some distance, with numbers peaking in spring. Chironomids were also the most common flies in a Prague

hospital, accounting for 12% of sampled flies (Sramova *et al.*, 1992), although not to the same extent as this study where they constituted 55% of Diptera. With this further weight of evidence, it is clear that these flies should no longer be ignored by pest control and infection control staff, which can be the case according to experience. As the most commonly encountered fly in hospitals in the UK, their significance should be made apparent to all relevant parties who should be referred to section 5.4, detailing the public health significance, identification features and control recommendations for non-biting midges of the family Chironomidae.

Of the synanthropic flies, blowflies of the family Calliphoridae, specifically *C. vicina* were most common. Levels of *C. vicina* showed a pronounced peak in autumn, so at this time of year the risk of transmission of pathogenic bacteria by *C. vicina* is most likely. A peak in numbers of *C. vicina* can be explained by their biology and is most likely due to increased availability of their preferred breeding matter, such as rodent or bird carcasses or waste meat. The most reports of *C. vicina* came from the location category 'food preparation' and it is these locations that are at greatest risk of harbouring bacteria deposited by these flies. The presence of *C. vicina* in food preparation areas can be explained by their biology, in that they are attracted to foodstuffs such as meat and fish, especially for purposes of oviposition.

The high numbers of *C. vicina* could also be a function of the sampling method of using UV light flytraps because *Calliphora* sp show a response to UV light (Hardie, 1984) and it is the author's personal experience of testing the efficacy of UV light flytraps that flies of this genus are caught extremely readily, much more so than *M. domestica* as an example. In fact, it is the author's experience that *Calliphora* sp are caught so well by UV light flytraps that they are considered to be of little practical value as a model organism when testing efficacy of such traps, as catch rates show no real differentiation between different models of traps.

Statistical models have been produced, predicting that *C. vicina* populations could increase substantially under likely scenarios of climate change, with increases of up to 85% by 2080 when compared with current levels, with the greatest increases occurring in the summer months (Goulson, 2005). If this prediction holds true, it is possible that increases in the incidence of fly-borne diseases may occur, which may be of significance in terms of an increased reservoir of *C. vicina* available to enter hospitals. A summary of the public health significance and identification features of *C. vicina* are provided in section 5.4, as a guide for those involved in pest control.

It starts to become apparent that this work provides pest control and infection control staff with knowledge of the key flying insect species that are likely to be present in hospitals at certain times of year and in which hospital locations, therefore guiding plans for integrated pest management programs, in order to minimise the risk of disease transmission.

The next most commonly encountered flies were the various families of 'drain flies', showing similarities with results of the KCIIS analysis, where this group of flies were the most commonly recorded. In this study, 'drain flies' peaked in spring while being present throughout the year. With two separate studies now showing that 'drain flies' are of greater significance in UK hospitals than has been realised before, the evidence is clear and education regarding the role of these flies in such premises should be a priority. Details regarding the public health significance, identification and control recommendations for 'drain flies' are covered in 5.4. The evidence showing the importance of 'drain flies' in UK hospitals is especially interesting, as *Drosophila melanogaster* and *Drosophila* sp were the only 'drain fly' representatives in a Prague hospital, accounting for just 1.6% of insects sampled (Sramova *et al.*, 1992). There are also only three other reports in the literature of 'drain flies' in hospitals; in Nigeria where *D. melanogaster* were reported and only accounted for 5% of flies (Nmorsi *et al.*, 2007), *Telmatoscopus albipunctatus* in Brazil (Pelli *et al.*, 2007) and *Clogmia albipunctata* Germany (Faulde and Spiesberger, 2013), which means that this study provides evidence that 'drain flies' in hospitals are an emerging problem.

Of the 'drain flies', the family Psychodidae is particularly significant in terms of threat to public health, the main reason being that Psychodidae are known carriers of *C. difficile* (Burt *et al.*, 2012). Of the potential fly vectors of *C. difficile* that were sampled, Psychodidae were the most commonly encountered in total and in each season, peaking in spring.

Combined numbers of Psychodidae, *F. canicularis*, *M. domestica* and *Drosophila* sp, the known fly vectors of *C. difficile* (Burt *et al.*, 2012), peaked in spring, suggesting that the risk of *C. difficile* transfer by flying insects is highest at this time of year, with Psychodidae probably being the most important flies in this respect. These points are central to fly control as an aspect of *C. difficile* infection control. The described fly species with known vector potential for *C. difficile* were found throughout a wide range of areas in the hospital environments that were sampled. The most reports came from the location category 'food preparation areas'. It is therefore prudent to recommend that fly control, hygiene measures, proofing and use of UV light professional sticky traps is focused in these areas, to minimise the risk of *C. difficile* dissemination by the fly species with known vector potential for this microorganism.

The well known synanthropic flies with vector potential for *C. difficile*, the housefly *M. domestica* and the lesser housefly *F. canicularis* accounted for a surprisingly low number of Diptera sampled from hospitals, being the 11th and eighth most common flies respectively. This is especially surprising considering that one study recorded *M. domestica* and *F. canicularis* as being the third and fourth most common Diptera respectively (Sramova *et al.*, 1992). While these species should never be discounted in control programs, the evidence presented in this study suggests that chironomids 'drain flies' and *C. vicina* represent greater significance in hospitals.

M. domestica, *F. canicularis* and *C. vicina* are categorised as 'domestic flies' in this study, along with *L. sericata* and *Sarcophaga carnaria*. Numbers of 'domestic flies' showed a pronounced peak in autumn, highlighting this time of year as being the period when hospitals are at the greatest risk of such fly activity and therefore transfer of bacteria from these species to the hospital environment. A species account for the members of the 'domestic flies' has already been given, apart from *S. carnaria*.

Sarcophaga carnaria is a synanthropic fly of the family Sarcophagidae, commonly called the 'flesh fly' and develops in carcasses, such as those of birds (Colyer and Hammond, 1951). *S. carnaria* frequents domestic waste bins and can enter houses (Busvine, 1980) and flies from the same family can be a pest around dog kennels as the larvae consume meat and dog faeces (Mallis, 1990). Pathogenic strains of enteroaggregative *E. coli* have been recovered from *S. carnaria* at a dog pound (Forster *et al.*, 2007). *S. carnaria* is identified by features such as its large size (although size is rather variable), large tarsal claws and pulvilli (giving the appearance of 'big feet'), red eyes, grey colour, dark longitudinal stripes on the thorax and a tessellated pattern on the abdomen (Colyer and Hammond, 1951).

Aphids of the family Aphididae were the most commonly encountered family from the true bugs, Order Hemiptera. Hemipterans in general peaked in summer. Although their public health significance is probably negligible, as the most common 'true bugs' in this study it is still relevant to be informed regarding aphids, even if it is only to separate them out from species of greater public health significance by identifying them correctly. Aphids, which are hemipteran bugs of the family Aphididae, are referred to as 'greenfly' or 'blackfly' on account of the colour and winged forms and they develop in association with plants by feeding on sap (Chinery, 1993). Aphids are not known to carry bacteria of public health significance. Key recognition features for aphids are their small size with many species being 2-3mm long, their mainly green or brown coloration, pear shaped body, piercing and sap-sucking mouthparts and two pairs of membranous wings with reduced venation that are often held roof-wise when at rest (Chinery, 2012). There are almost 500 species of British Aphididae (Chinery, 1993). Their presence in hospitals is indicative of inadequate proofing measures.

Ants, bees and wasps (Order Hymenoptera) showed a distinctive peak in numbers in summer. Of the predominantly crawling insects that are also capable of flight, ants of the family Formicidae also peaked in summer and represented the greatest numbers of individuals sampled, showing that they are among the most important. Ants of the family Formicidae were the most commonly encountered family from the Order Hymenoptera. The species of ant most commonly encountered in hospitals in this study was Roger's ant, *Hypoponera punctatissima*. This species is potentially neglected by pest controllers and infection control staff, possibly due to a lack of familiarity and difficulty of identification when compared to other ant species such as the black / garden ant *L. niger*, so

awareness needs to be raised. This is especially important considering *H. punctatissima* has the ability to sting and also carries bacteria. *H. punctatissima* are non-trail forming predatory tropical ants that feed on other insects and are usually only found indoors in the UK in centrally heated premises, examples being hospitals, conservatories, bakeries and hotels, where they are associated with damp areas like drains and toilets (Gray *et al.*, 1995). Female *H. punctatissima* possess stings and it is often the winged Queen ants that are encountered by humans, with stings resulting in a 'dermal weal and flare reaction followed by development of a 1cm erythematous, pruritic papule that lasts several days' (Gray *et al.*, 1995). *Streptococcus lactis* has been isolated from *H. punctatissima* found in a hospital (Gray *et al.*, 1995). *H. punctatissima* is recognised by its small size, yellowish brown colour and large wedge-shaped single petiole (also known as the 'waist' or node) as well as the fact that stinging winged Queens are often encountered (Bolton, 2014).

Moths (Order Lepidoptera) in general peaked in summer. Night-flying moths of the family Noctuidae were the most commonly encountered family from the Order Lepidoptera, although their public health significance is probably limited due to their relatively low numbers in hospitals and lack of communicative behaviour. As the most common moths in this study, it is still relevant to be informed regarding Noctuidae, even if it is only to separate them out from species of greater public health significance by identifying them correctly. Moths of the family Noctuidae, referred to as the 'night-flying moths' on account of their nocturnal flying activity, are attracted to light and their larvae develop on foliage. *Agrotis exclamationis* of the family Noctuidae, was shown to carry the following species of bacteria in a study at a hospital in Prague; *Citrobacter amalonaticus*, *Pseudomonas cepacia* and an antibiotic resistant strain of *Acinetobacter calcoaceticus* (Sramova *et al.*, 1992).

Noctuid moths are recognised by their dull coloured forewings, occasionally conspicuously coloured hindwings and characteristic wing venation (Chinery, 2012). There are approximately 400 species of British Noctuidae (Chinery, 1993). Furthermore, the presence of Noctuidae in numbers in hospitals is indicative of poor proofing, as they are a group of moths that are found predominantly outdoors. Their presence should trigger investigations into proofing inadequacies, resulting in remedial measures such as fitting flyscreens or repair of current flyscreens, which also have the benefit of keeping out other flying insects. From experience, some hospitals have their UV light professional sticky traps or EFKs on a timer, so they are turned off at night. This practice renders such equipment redundant in capturing the nocturnally active noctuids, a situation which should be resolved in hospitals.

Beetles (Order Coleoptera) in general showed little seasonal variation in numbers, so pest control and infection control measures should focus on a year-round plan. Of the predominantly crawling insects capable of flight that were sampled from hospitals, beetles (Order Coleoptera) provided the greatest number of species. Based on this fact, the education / training regarding crawling insect identification

for those involved in pest control in hospitals should focus on beetles. Skin feeding beetles of the family Dermestidae were the most commonly encountered beetle samples from hospitals.

Beetles of the family Dermestidae, known as the skin-feeding beetles, hide beetles and leather beetles develop in association with carcasses of rodents and birds, bird nests, hides, skins, dead insects, animal products and other stored products (Busvine, 1980). Dermestidae have not been well studied in terms of bacterial carriage and although they can carry *Salmonella* they are not considered to be important in its spread (Wales *et al.*, 2010). Dermestid beetles are recognised by their compact oval shape, dull colour, downy 'hairs' or scales on their body, clubbed antennae and bristly larvae which are referred to as 'woolly bears' (Chinery, 2012). There are approximately 30 species of Dermestidae in the UK (Chinery, 1993) and *Dermestes peruvianus*, the Peruvian leather beetle, is a common species. The family Dermestidae also contains the varied carpet beetle *Anthrenus verbasci* and the two-spotted carpet beetle, *Attagenus pellio*, which are both pests of furs, woollen materials and dead insects, all of which contain the protein keratin, which is a main food source for these species and other dermestids (Busvine, 1980).

The Dermestidae can be described as stored product insects (SPI) due to the damage they cause to stored food products. This study combined stored product insects and 'fungus feeders' into an SPI category and it was found that their numbers in hospitals peaked in winter. This finding is similar to that of the analysis of the KCIIS data, which showed SPI numbers to be highest in October and November, adding further weight to the observations and the same recommendations made in section 5.4 are also relevant here.

Ladybirds of the family Coccinellidae were the next most common beetles and are classed as 'casual intruder' beetles along with some other beetle families and species, as defined in Figure 6.14. Numbers of 'casual intruder' beetles such as ladybirds peaked in summer. *Harmonia axyridis* was the most common species of ladybird, although their public health significance is probably limited, since there appears to be only one reference in the literature regarding bacterial carriage, where *Staphylococcus* spp predominated (Moon *et al.*, 2011).

Harmonia axyridis is a ladybird of the family Coccinellidae, commonly called the Harlequin ladybird or the Asian multi-coloured ladybird and like other ladybirds it is found on plants where it is carnivorous, feeding on aphids (Chinery, 2012). *H. axyridis* is a native of Japan and is an invasive and rapidly spreading pest species, arriving in the UK in 2004 via imported flowers (Roy *et al.*, 2014). Within the UK the Harlequin ladybird causes problems by predating on and outcompeting native species of ladybirds (Roy *et al.*, 2014). *H. axyridis* can also be a nuisance, similar to the cluster fly, in that it forms overwintering clusters in their tens of thousands in buildings, entering buildings in autumn and leaving in spring (Roy *et al.*, 2014). They also damage soft fruits such as grapes and their

'reflex blood' which is released as a defence mechanism taints wine and they can even bite humans causing an irritating bump which stings and some people can even suffer an allergic reaction to the bites (Roy *et al.*, 2014). Recognition features of *H. axyridis* include the fact they are larger than native ladybirds, legs that are typically brown, wing cases (elytra) with a keel at the base (Roy *et al.*, 2014), triangular mark on the head, obvious red border around the base of the abdomen and extremely variable colour patterns of the elytra with a maximum of 21 black spots on an orange background being an example of a common colour variety (Chinery, 2012).

It was possible to compare this study to previous work on insects in hospitals by Sramova *et al.* (1992), by using the synanthropy classification of insects in their paper (see section 6.3.5). Sramova *et al.* (1992) reported that 'eusynanthropic arthropods' were the most common defined group in the Prague hospital that was studied, whereas this work showed that 'occasionally encountered insects' were the insect group most commonly sampled from UK hospitals. This often neglected group, the 'occasionally encountered insects' should actually be recognised as the most numerous in UK hospitals. The preponderance of 'occasionally encountered insects' in UK hospitals probably indicates that proofing standards require improvement, adding weight to prior recommendations regarding flyscreening.

A further comparison with previous work is that the equitability (E_D) of insects sampled in this study was lower than that of the study by Sramova *et al.* (1992), which may be related to the greater number of UK hospitals sampled and length and depth of sampling, compared to the investigation at the single site in Prague. However, a cautious interpretation is that the insect population of UK hospitals was more diverse than that described in the Prague hospital by Sramova *et al.* (1992). This finding further emphasises the need for greater education regarding the significance of the diverse flying insect fauna of UK hospitals.

Examining diversity at a seasonal level in this study, it was highest in spring, followed by summer, autumn and winter, which in itself is useful knowledge for pest control and infection control staff in terms of knowing what to expect when planning control measures. An example of how this information could be used is to inform insect monitoring choices. A wider variety of insect monitors (not just UV light flytraps) with differing lures to attract certain species should be used at the times of year when greatest insect diversity is expected, with lures and other perishable components such as glue boards replaced more frequently at these times. These recommendations are also relevant to the times of year when the greatest numbers of insect individuals are present in hospitals. The greatest numbers of insect individuals per hospital per sampling occasion were sampled in summer, followed by spring, autumn and winter. Appropriate selection, use and replenishment of insect monitoring systems is crucial to provide accurate information regarding insect vector activity in UK hospital, in order to guide targeted pest control and therefore aid effective infection control measures.

Returning to the issue of proofing, which was discussed in relation to the presence of non-biting midges (chironomids), night flying moths (noctuids) and 'occasionally encountered insects', this research provided a key case-study illustrating the importance of proofing. In the old neonatal unit of a particular hospital, 242 individual insects were sampled over three sampling occasions. When the new neonatal unit was built for that particular hospital, it was noted that levels of proofing and general building condition were superior to the old unit. It is known that well-proofed buildings in good condition limit pest access (Killgerm, 2011). The new neonatal unit was sampled over three sampling occasions and 200 individual insects were collected, which is a reduction in numbers compared to the old unit. While a number of factors were no doubt involved, it is suggested that the improved levels of proofing and general building condition had a part to play in the observed reduction in insects.

6.5 CONCLUSION

Regarding the numbers of certain species, interpretation of the entomological study results revealed that true flies (Order Diptera) were the most common insects, Chironomidae were the most common flies by far and are of public health significance, while *C. vicina* were the most common synanthropic flies. 'Drain flies' were surprisingly numerous and represent an emerging problem in hospitals. The family Psychodidae were the most common of the 'drain flies' and were therefore the most important known insect vector of *C. difficile* present in hospitals. Known insect vectors of *C. difficile* were present, which were Psychodidae, *M. domestica*, *F. canicularis* and *Drosophila* sp. Of the known insect vectors of *C. difficile*, *M. domestica* were surprisingly low in numbers. Another perhaps surprising finding was that 'occasionally encountered insects' were actually the group most frequently found in hospitals. It was noted that presence of certain species, specifically some of the 'occasionally encountered insects' is diagnostic of proofing inadequacies in UK hospitals.

Regarding seasonality, many species were present all year round and not all peaks in numbers were in summer, insect diversity was highest in spring and sheer numbers of insects were highest in summer.

Location data showed that insects were found most often in food preparation areas.

Recommendations based on these findings are numerous and are discussed as follows. The numerous 'drain flies', especially those with vector potential for *C. difficile*, should be at the forefront of the education of pest controllers and hospital staff, with control measures being tailored more specifically towards this group of flies. Relating to the presence of 'drain flies' in hospitals, repair of drainage faults and scrupulous hygiene should be a priority in order to limit the activity of this group of flies and therefore minimise the risk to public health.

General recommendations regarding fly control / pest control are that UV light flytraps (professional sticky traps only, due to release of bacteria from flies electrocuted by EFKs) should be used throughout hospitals in order to protect public health and the contents of UV light flytraps should be identified routinely to inform pest control and infection control measures. Therefore, the awareness of pest control and infection control staff needs to be raised regarding fly identification, sources / breeding media, public health significance and control measures. Based on the findings regarding location of flies in hospitals, fly control measures should focus on food preparation areas of hospitals, which is where flies were most frequently reported. A further recommendation is that hospital buildings should be adequately proofed against fly entry, by installing and maintaining flyscreens. Appropriate selection, use and replenishment of insect monitoring systems should be informed by this study i.e. monitors with appropriate lures / attractants (UV light, pheromones, food-based attractants) should be selected that are relevant to the insects recorded in this study. Pest control and infection control staff should use the data on insect seasonality in this study to guide their work in terms of accelerating monitoring and control efforts at key times of year to deal with certain species. Following these recommendations could be complex and expert entomologists should be consulted, especially when assistance is required in identifying insects and designing control strategies in hospitals.

This study updates the knowledge base regarding flies in hospitals and contrasts with the general wisdom that houseflies *M. domestica* are the most numerous in such premises and that flies are mainly a summer problem. Furthermore, this work provides pest control and infection control staff with knowledge of the key flying insect species that are likely to be present in hospitals at certain times of year and in which hospital locations. This knowledge better informs the design of integrated flying insect management programs, in order to minimise the risk of disease transmission by flying insects, with pest control central to infection control. It is recommended that future work should be undertaken regarding field sampling and microbiological analysis of the truly crawling insects that were not covered by this study (e.g. cockroaches) in UK hospitals, to further determine the threat to public health and consider in more detail the role of pest control as infection control.

A final and firm recommendation / conclusion, is that at the very least, flying insects must be included in future editions of the NHS conditions of contract for pest control.

7 CHAPTER 7: MICROBIOLOGICAL ANALYSIS OF FLYING INSECTS COLLECTED FROM HOSPITALS

7.1 INTRODUCTION

Although there are a number of studies relating to bacteria carried by flies in hospitals worldwide, there appears to be only one reference from the UK, which is a study that was undertaken in 1942 by Shooter and Waterworth (1944). Clearly a gap in the knowledge exists, regarding the carriage of bacteria by flying insects in UK hospitals, as there are no recent studies reflecting the current situation.

The Shooter and Waterworth (1944) study reported capturing flies and culturing Group A Beta-haemolytic streptococci (*Streptococcus pyogenes*), which were of the same type (type 4), as those found in the throat of a nurse, as well as wound and throat infections of patients. In the same study, coagulase positive staphylococci, coliform bacilli and *Proteus* spp were also isolated from the flies, which were presumably *Musca domestica*. Although the Shooter and Waterworth (1944) study is clearly useful, the limitations are obvious in that the flies were not actually identified, the findings are not recent at all and the sampling period only provided a snapshot of events. For example, the work was undertaken in September 1942, only two wards were sampled and only 27 flies were collected.

Regarding most of the studies from other countries, their efforts were typically focused on the bacteria carried by just one species of fly, which is *M. domestica* (see Table 1.2). Apart from work on houseflies, little research has been done on the bacteria associated with other fly species that are found in hospitals. Fruit flies, *Drosophila* sp sampled from a hospital in Nigeria were found to harbour *Proteus* sp, *Streptococcus* sp and *Salmonella* sp (Nmorsi *et al.*, 2007). Cluster flies, *Pollenia rudis* sampled from a hospital in Germany were found to harbour *Pseudomonas aeruginosa* and *Erwinia* spp which are also known as *Pantoea* spp (Faulde *et al.*, 2001) and *C. albipunctata* was positive for many species of Enterobacteriaceae (Faulde and Spiesberger, 2013). This highlights a relative lack of knowledge internationally, regarding the carriage of bacteria by flies other than *M. domestica* in hospitals. It follows therefore that the content of this thesis chapter is of benefit internationally, due to it encompassing the examination of bacterial carriage by flying insects in general and not just *M. domestica*.

The aim of this chapter was to fill the described knowledge gaps and isolate and classify bacteria associated with flying insects (including *M. domestica*) collected and identified from UK hospitals (as in Chapter 6), in order to inform pest control measures that are relevant to infection control.

7.2 MATERIALS AND METHODS

7.2.1 Isolation of bacteria

Individual flying insects assigned the same identification and collected from the same flytrap were pooled into PBS and washed / mixed by vortexing for 30 seconds. This method of pooling occurred in most cases – some pooling from different flytraps occurred when numbers of individuals with the same identification at a particular hospital site were low. Larger flies were dealt with in the same way, in that *M. domestica, C. vicina, M. autumnalis, L. sericata, Phaonia* sp, *Helina* sp were pooled into 1ml of PBS per fly i.e. 10 flies of the same identification were pooled into 10ml of PBS. Flies of a medium size such as *F. canicularis* were pooled into 0.5ml PBS per fly. The pooling of smaller flies such as those of the families Psychodidae, Sphaeroceridae, Phoridae and Drosophilidae varied from six flies to eighty per 1ml of PBS. The pooling of flies of the family Dolichopodidae varied from seven to thirteen flies per 1ml of PBS. The pooling of *H. punctatissima* varied from one to fifteen individuals per 1ml of PBS. Chironomidae x 10 were pooled into 1ml of PBS. *C. pipiens, P. rudis, S. carnaria* and *H. axyridis* were pooled into 1ml of PBS per individual. The inconsistency of dilution was compensated for in the results section by converting the bacterial loads to be quoted as 'per ml per flying insect'. These external washings were then serially diluted down to 10^{-6} and 0.1ml of each dilution inoculated onto the surfaces of CCFA plus Tc, Nutrient Agar, Mannitol Salt Agar and VRBG agar. The pooled samples were then washed four further times, with the same amount of PBS as the initial wash (fresh PBS with each wash), in order to remove external bacteria to avoid contamination when examining macerates for bacteria. The flying insects were then macerated with the end of a sterile plate spreader in the same amount of PBS as for the initial external washing and the above process of dilution and inoculation 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 plus Tc agar and a set of Nutrient Agar plates were incubated in anaerobic conditions at 37°C for 48 and 24 hours respectively.

7.2.2 Identification of bacteria

Bacterial colonies were identified by macroscopic morphology, Gram staining, microscopic examination of morphology, oxidase (HPA, 2011e) and catalase tests (HPA, 2011a) 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). Isolates of *Staphylococcus aureus* were cultured on Mannitol Salt Agar with Oxacillin for presumptive identification of MRSA. Isolates of

Bacillus cereus Group were examined under phase contrast microscopy to determine the presence or absence of parasporal crystals in order to confirm or deny identification of *Bacillus thuringiensis* versus *B. cereus* (HPA, 2011b). *Escherichia coli* isolates were cultured on Sorbitol MacConkey Agar (Oxoid Ltd, Basingstoke, UK) for presumptive identification of *E. coli* O157 (HPA, 2011d) and were also sent for serotyping to the Laboratory of Gastrointestinal Pathogens, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London, NW9 5EQ.

Biochemical techniques were chosen to identify bacteria rather than molecular methods. Although molecular methods of identification are seen by many to be the 'gold standard', the use of biochemical techniques was justified based on the fact that such tests are recommended by the HPA for dealing with medically important bacteria (HPA, 2008). Such biochemical techniques are also in routine use in NHS hospitals for the purpose of identifying species of bacteria that are of clinical significance. Of course, species of clinically significant bacteria isolated from flying insects are the focus of this chapter. Furthermore, the use of biochemical techniques for the identification of bacteria isolated from flies in hospitals is still current, relevant and worthy of peer-reviewed publications, as per Faulde and Spiesberger (2013). Another benefit of using biochemical techniques was that they were practical in terms of the project budget, results were produced rapidly (which are consistent between laboratories) and specialist equipment was not required.

7.2.3 Statistical techniques

Statistics used to examine the microbiological associations of flying insects collected from hospitals were; Chi-square, 2 x 2 Chi-square tests, Simpson's diversity, equitability, Analysis of variance (ANOVA) single factor / univariate and Z-test (matched). Equitability was also used as a method of assessing the diversity of species of bacteria associated with flying insects, with different fly species representing different habitats for bacterial colonisation. The measures of diversity (Simpson's diversity and equitability) that were used are detailed in 6.2.6.

7.3 RESULTS

7.3.1 Checklist of bacteria isolated from flying insects sampled from seven UK hospitals

The results of the Microbiological analysis of flying insects collected from seven hospitals from March 2010 to August 2011 are listed in Table 7.1, in the form of a species checklist and are described in the following sections of this chapter.

Table 7.1 A checklist of bacteria isolated from flying insects sampled from seven UK hospitals from March 2010 to August 2011.

Fly species	Bacteria isolated	ID kit code	Estimated CFUs per fly per ml	Location	Medical significance of isolated bacteria	References to medical significance of isolated bacteria
Musca	***Bacillus* spp**					
domestica	*Bacillus lentus*	66260026	930	HC	Resistant neonatal sepsis	(Moodley, 2006)
	**Bacillus licheniformis*	76370437	10	M	Septicaemia	(Matsumoto *et al.*, 2000)
	Bacillus pumilus	76270026	10	HS	Food poisoning	(From *et al.*, 2007)
	Bacillus subtilis Group	76360423	6,000,000	HC	Fatal brain and lung infection	(Ihde and Armstrong, 1973)
	Bacillus subtilis Group	76370423	800	WK		
	Bacillus subtilis Group	76370427	2,000	HC		
	Bacillus subtilis Group	76370427	190	W		
	Clostridia					
	**Clostridium*					
	beijerinckii/butyrricum	4130100000	10	HC	Necrotizing enterocolitis	(Popoff and Dodin, 1985)
	**Clostridium clostridioforme*	4510200000	10	HC	Bacteraemia	(Finegold *et al.*, 2005)
	Clostridium sp	0511000000	10	HC		
	Enterobacteriaceae					
	Citrobacter freundii	1604572	2,110	HC	Haemolytic uraemic syndrome	(Tschape *et al.*, 1995)
	Enterobacter asburiae	3304523	90	W	Wound infection	(Koth *et al.*, 2012)
	Enterobacter cloacae	3305573	10,000,000,000	HC	Resistant neonatal bacteraemia	(Kartali *et al.*, 2002)

	Enterobacter cloacae	3305573	31,100	HC		
	Escherichia coli	5144552 Serotype O unidentifiable	630	HC	Haemolytic uraemic syndrome	(Kaper *et al.*, 2004)
	Escherichia hermannii	1144133	4,300	HC	Catheter-related bacteraemia	(Kaewpoowat *et al.*, 2013)
	Klebsiella oxytoca	5255773	340	HC	Haemorrhagic colitis	(Hogenauer *et al.*, 2006)
	Klebsiella pneumoniae ssp *pneumoniae*	5214763	300	HC	Pneumonia	(Lin *et al.*, 2010)
	Klebsiella pneumoniae ssp *pneumoniae*	5215773	800	HC		
	Klebsiella pneumoniae ssp *pneumoniae*	5215773	10,900	HC		
	Pantoea sp	1005173	54,000	HC	Fatal neonatal septicaemia	(Van Rostenberghe *et al.*, 2006)
	Pantoea species 1	1004123	10,000,000,000	HC		
	Pantoea spp 3	3005133	300,000	HC		
	Pantoea spp 4	0000173	300,000	HC		
	**Raoultella terrigena*	5205773	670	W	Resistant neonatal sepsis	(Elamreen, 2007)
	Staphylococci					
	Staphylococcus aureus		440	W	Resistant infection of blood, skin, urine, respiratory tract	(Kock *et al.*, 2010)
	Staphylococcus aureus		500	HC		
	Streptococci					
	Streptococci		20,000	HC	Endocarditis	(Parker and Ball, 1975)
Calliphora vicina	**Enterobacteriaceae**					
	Citrobacter freundii	1604572	16,000,000	Live MI	Haemolytic uraemic syndrome	(Tschape *et al.*, 1995)

	Enterobacter asburiae	3304523	21,800,000	Live MI	Wound infection	(Koth *et al.*, 2012)
	Enterobacter sp (*aerogenes* or *cloacae*)	7305773	45	HS	Neonatal septicaemia	(Loiwal *et al.*, 1999)
	**Escherichia coli* E1525	5104502 serotype E1525	1,360	HC	Haemolytic uraemic syndrome	(Kaper *et al.*, 2004)
	**Klebsiella oxytoca*	5265673	29,000,000	HC	Haemorrhagic colitis	(Hogenauer *et al.*, 2006)
	**Klebsiella pneumoniae* ssp *ozaenae*	1004553	45	HS	Chronic rhinitis	(Botelho-Nevers *et al.*, 2007)
	**Leclercia adecarboxylata*	1044173	7,100	W	Throat tissue abscess	(Bali *et al.*, 2013)
	**Pantoea* species 1	3004122	2,800,000	Live MI	Fatal neonatal septicaemia	(Van Rostenberghe *et al.*, 2006)
	**Raoultella terrigena*	5204773	17,000	M	Resistant neonatal sepsis	(Elamreen, 2007)
	Staphylococci					
	Staphylococcus aureus		20	HC	Resistant infection of blood, skin, urine, respiratory tract	(Kock *et al.*, 2010)
	Staphylococcus aureus		1,040	HC		
	**Staphylococcus hominis*	6216052	10	HC	Oxacillin-resistant sepsis	(Marshall *et al.*, 1998)
	Streptococci					
	β-hemolytic *Streptococcus* sp		3,520	WN	Endocarditis	(Parker and Ball, 1975)
	Non-hemolytic streptococci		190	WN		
	Other					
	Aerococcus sp		4,200	HC	Bacteraemia/fatal endocarditis	(Rasmussen, 2013)
	Unknown sp		3,500	HC		

	-ve			WN		
Musca	**Enterobacteriaceae**					
autumnalis	**Enterobacter cloacae*	3305573	9,000	HC	Resistant neonatal bacteraemia	(Kartali *et al.*, 2002)
	**Escherichia vulneris*	1004153	290	HC	Soccer wound infection	(Jepsen *et al.*, 1997)
	**Klebsiella pneumoniae* ssp *pneumoniae*	5215773	31,000	HC	Pneumonia	(Lin *et al.*, 2010)
	**Raoultella terrigena*	5204773	25,000	HC	Resistant neonatal sepsis	(Elamreen, 2007)
	Staphylococci					
	**Staphylococcus aureus*		110	HC	Resistant infection of skin	(Kock *et al.*, 2010)
	**Staphylococcus saprophyticus*	6654152	260	HC	Oxacillin-resistant sepsis	(Marshall *et al.*, 1998)
Fannia	***Bacillus* spp**					
canicularis	**Bacillus subtilis* Group	77370627	5	HC	Fatal brain and lung infection	(Ihde and Armstrong, 1973)
	Enterobacteriaceae					
	**Pantoea* spp 2	1205533	1,350	HC	Fatal neonatal septicaemia	(Van Rostenberghe *et al.*, 2006)
	Staphylococci					
	Staphylococcus aureus		1,045	HC	Resistant infection of blood,	(Kock *et al.*, 2010)
	Staphylococcus aureus		10	WK	skin, urine, respiratory tract	
	Other					

	Enterococcus sp		100	HC	Infection of CNS	(Murray, 1990)
	**Micrococcus* sp	0004000	10	HC	Peritonitis	(Kao *et al.*, 2012)
	-ve			WN		
Lucilia sericata	***Bacillus* spp**					
	**Bacillus brevis*	0000030	720,000	HC	Peritonitis	(Parvez *et al.*, 2009)
	Enterobacteriaceae					
	Enterobacter cloacae	3304573	9,000	HC	Resistant neonatal bacteraemia	(Kartali *et al.*, 2002)
	**Escherichia coli* O71	1044572 O71 serotype	31,000	HC	Haemolytic uraemic syndrome	(Kaper *et al.*, 2004)
	**Klebsiella pneumoniae* ssp *pneumoniae*	5214773	80,000	HC	Pneumonia	(Lin *et al.*, 2010)
	Staphylococci					
	Staphylococcus aureus		110	HC	Resistant infection of skin	(Kock *et al.*, 2010)
Psychodidae	***Bacillus* spp**					
	**Bacillus cereus* Group	22220033	7	HC	Neonatal lung & CNS infection	(Hilliard *et al.*, 2003)
	Staphylococci					
	**Staphylococcus aureus*		3	HC	Resistant infection of blood, skin, urine, respiratory tract	(Köck *et al*., 2010)
	Other					
	Micrococcus sp		90	WN	Peritonitis	(Kao *et al*, 2012)
	-ve			HC		

Phoridae	***Bacillus* spp**					
	**Bacillus cereus* Group*	20260033	84	HC	Neonatal lung+CNS infection	(Hilliard *et al*., 2003)
	**Bacillus cereus* Group*	00220037	14	HC		
	**Bacillus sphaericus*	00000014	14	HC	Bacteraemia	(Castagnola *et al.*, 2001)
	Clostridia					
	Clostridium sp		1	HC		
Sphaeroceridae	***Bacillus* spp**					
	**Bacillus cereus* Group*	00020011	32	WN	Neonatal lung+CNS infection	(Hilliard *et al.*, 2003)
	Bacillus sphaericus	00000004	3	WN	Bacteraemia	(Castagnola *et al.*, 2001)
	Clostridia					
	**Clostridium clostridioforme*	4533200000	1	WN	Intra-abdominal abscess	(Finegold *et al.*, 2005)
	Staphylococci					
	Staphylococcus aureus		1	WN	Resistant infection of blood, skin, urine, respiratory tract	(Kock *et al.*, 2010)
Trichiaspis sp (Family Sphaeroceridae)	***Bacillus* spp**					
	**Bacillus licheniformis*	76370423	1	HS	Septicaemia	(Matsumoto *et al.*, 2000)
	Staphylococci					
	**Staphylococcus aureus*		1	HS		
Drosophila sp	***Bacillus* spp**					
	**Bacillus pumilus*	66270024	202	HS	Food poisoning	(From *et al.*, 2007)

Dolichopodidae	***Bacillus* spp** **Bacillus pumilus* **Enterobacteriaceae** **Pantoea* sp **Other** -ve	 66270024 1007173 	 2 3 	 WN WN HC	 Food poisoning Fatal neonatal sepsis 	 (From *et al.*, 2007) (Van Rostenberghe *et al.*, 2006)
Phaonia sp	**Staphylococci** **Staphylococcus aureus*		 10	 HS	 Resistant infection of skin	 (Kock *et al.*, 2010)
Helina sp	***Bacillus* spp** **Bacillus lentus*	 7046065	 110	 HC	 Resistant neonatal sepsis	 (Moodley, 2006)
Hypoponera punctatissima Queens	***Bacillus* spp** **Bacillus megaterium* **Other** -ve -ve -ve	 05262134 	 1 	 WN WN WK HC	 Meningitis 	 (Dib *et al.*, 2003)
Chironomidae	 -ve	 	 	 HC	 	
Culex pipiens	 -ve	 	 	 HC	 	
Pollenia rudis						

	-ve			HC		
Sarcophaga carnaria	-ve			M		
Harmonia axyridis	-ve			HC		

Key: The location in the hospital that the insect carrying that particular isolate was sampled from. Hospital catering areas (HC), ward kitchens (WK), wards (W), Hospital food stores (HS), mortuary (M), neonatal & maternity (WN), Live from Medical illustration department toilet (Live MI). *Isolated from this insect for the first time, to the knowledge of the author.

Bacterial groups isolated from flying insects sampled from UK hospitals

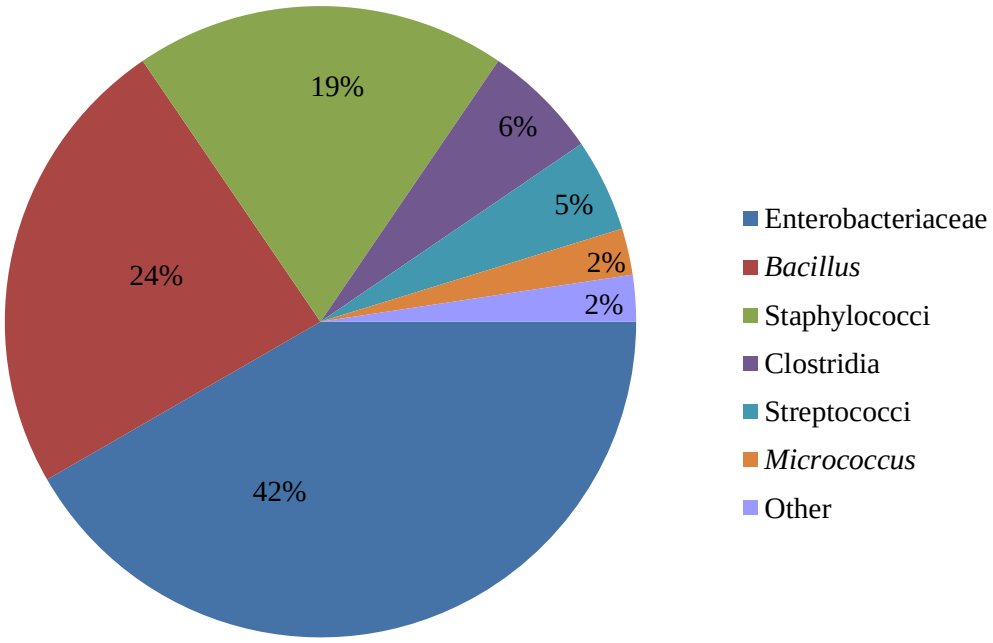

Figure 7.1 Bacterial groups isolated from flying insects sampled from UK hospitals

Figure 7.1 provides a summary of the findings presented in Table 7.1 by showing the bacterial groups isolated from flying insects sampled from UK hospitals. Enterobacteriaceae were the most commonly isolated group of bacteria, accounting for 41% of isolations from flying insects, followed by *Bacillus* spp making up 24% and Staphylococci comprising 19%. Clostridia, Streptococci, *Micrococcus* spp and other species of bacteria accounted for 6%, 5%, 2% and 3% of isolations respectively (Figure 7.1).

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

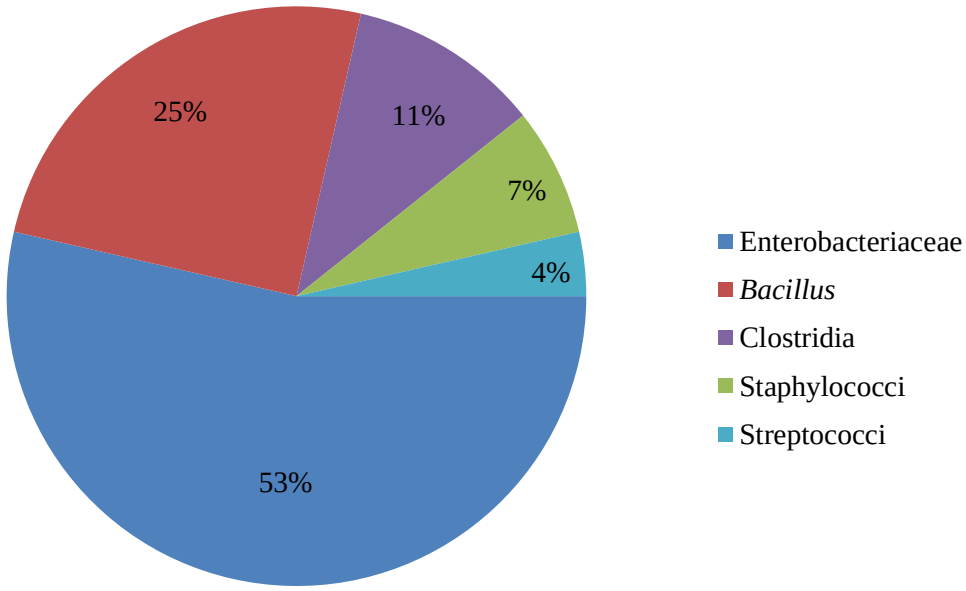

Figure 7.2 Bacterial groups isolated from *M. domestica* sampled from UK hospitals

Fourteen batches of *M. domestica* (n = 67) were sampled microbiologically from six of seven hospitals from March 2010 to August 2011 and 28 bacterial isolates (21 different species) were obtained (Table 7.1). Table 7.1 shows that of the bacteria isolated from *M. domestica*, there were 15 occurrences of Enterobacteriaceae (12 species), seven isolates of *Bacillus* spp (four species), three Clostridia (one to genus level, two other species) two Staphylococci (both *S. aureus*) and one Streptococci. Species of bacteria recovered multiple times were *Bacillus subtilis* Group (four times, with three different identification profiles), *Klebsiella pneumoniae* ssp *pneumoniae* (three times, with two different identification profiles) and *Enterobacter cloacae* (two times, with both identification profiles the same). The estimated CFUs per fly per ml for different species of bacteria varied widely, from 10 up to 10,000,000,000.

Figure 7.2 provides a summary of the findings presented in Table 7.1 by showing the bacterial groups isolated from *M. domestica* sampled from UK hospitals. Figure 7.2 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 7.1.

To the knowledge of the author, this study provides the first example of *B. licheniformis*, *C. beijerinckii* / *C. butyricum*, *C. clostridioforme* and *R. terrigena* isolation from *M. domestica* Table 7.1.

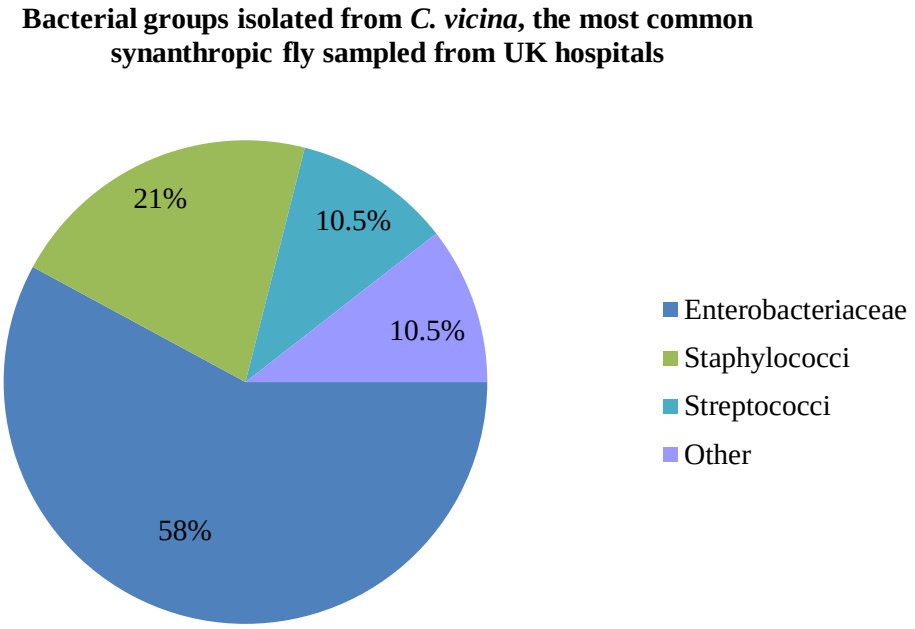

Figure 7.3 Bacterial groups isolated from *C. vicina*, the most common synanthropic fly sampled from UK hospitals

Eleven batches of *Calliphora vicina* (n = 91) were sampled microbiologically from five of seven hospitals from March 2010 to August 2011 and 19 bacterial isolates (15 different species) were obtained (Table 7.1). There were 11 occurrences of Enterobacteriaceae (nine species), zero occurrences of *Bacillus* spp, zero occurrences of Clostridia, four occurrences of Staphylococci (three *S. aureus*, one *S. hominis*), two occurrences of Streptococci (two species), one occurrence of *Aerococcus* sp, one occurrence of unknown sp and one occurrence of no bacteria being isolated. Bacterial species recovered multiple times were *S. aureus* (three times) *Enterobacter asburiae* (twice, with both identification profiles the same), *Raoultella terrigena* (twice, with both identification profiles the same). The most bacterially diverse batch of *C. vicina* was sampled live rather than from fly traps and 3 species were recorded. The live sampled batch of *C. vicina* also exhibited the highest bacterial load, with a total bacterial count of 40.6 million CFUs per fly per ml. The lowest colony counts were estimated as 10 CFUs per fly per ml.

Figure 7.3 provides a summary of the findings presented in Table 7.1 by showing the bacterial groups isolated from *C. vicina* sampled from UK hospitals. Figure 7.3 shows that the majority of bacterial isolates taken from *C. vicina* sampled from hospitals were of the family Enterobacteriaceae (58%),

followed by Staphylococci (21%), Streptococci (10.5%) and other species of bacteria (10.5%). *C. vicina* carrying this variety of microorganisms were sampled from a number of locations, including hospital catering areas, wards, hospital food stores, a mortuary, neonatal & maternity wards and live from medical illustration department toilets (Table 7.1).

To the knowledge of the author, this study provides the first example of *Escherichia coli* serotype E1525, *Klebsiella oxytoca*, *Klebsiella pneumoniae* ssp *ozaenae*, *Leclercia adecarboxylata*, *Pantoea* species 1, *Raoultella terrigena* and *Staphylococcus hominis* isolation from *C. vicina* (Table 7.1).

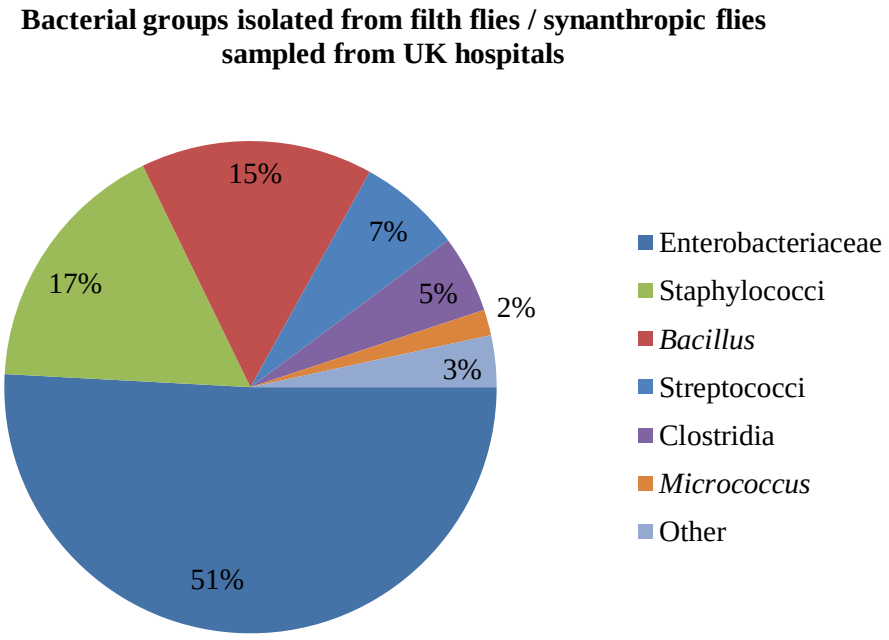

Figure 7.4 Bacterial groups isolated from filth flies / synanthropic flies sampled from UK hospitals

Filth flies / synanthropic flies in this case are defined as *M. domestica*, *C. vicina*, *F. canicularis* and *L. sericata*. The bacterial carriage of *M. domestica* and *C. vicina* has already been described.

Five batches of *F. canicularis* (n = 43) were sampled microbiologically from four of seven hospitals from July 2010 to August 2011 and six bacterial isolates (five different species) were obtained Table 7.1. There was one occurrence of Enterobacteriaceae, one occurrence of *Bacillus* spp, zero occurrences of Clostridia, three occurrences of Staphylococci (two *S. aureus*, one *Micrococcus* sp), one occurrence of *Enterococcus* sp and one occurrence of no bacteria being isolated.

Bacterial species recovered multiple times were *S. aureus*, which was isolated twice. One batch of *Fannia* was bacterially diverse, with three species being recovered. Bacterial loads for different species of bacteria varied from an estimated 5 to 1,350 CFUs per fly per ml.

One batch of *Lucilia sericata* (n = 11) was sampled microbiologically in July 2011 from one of seven hospitals and five bacterial isolates (five different species) were obtained. There were three occurrences of Enterobacteriaceae (three species), one occurrence of *Bacillus* spp, zero occurrences of Clostridia and one occurrence of Staphylococci (*S. aureus*). Of the Enterobacteriaceae, one isolate was *Escherichia. coli*, serotyped to O71. *Escherichia coli* O71 are characteristic enteropathogenic (EPEC) bacteria prevalent in healthy cattle (Orden *et al.*, 2002). Bacterial loads for different species of bacteria varied from an estimated 110 to 720,000 CFUs per fly per ml.

One batch of *Sarcophaga carnaria* (n=1), which are also classed as filth / synanthropic flies was sampled microbiologically from one hospital in June 2010. No bacteria were isolated.

Figure 7.4 provides a summary of the findings presented in Table 7.1 by showing the bacterial groups isolated from filth / synanthropic flies sampled from UK hospitals. Figure 7.4 shows that the majority of bacterial isolates taken from filth / synanthropic flies sampled from hospitals were of the family Enterobacteriaceae (51%), followed by Staphylococci (17%), *Bacillus* spp (15%), Streptococci (7%), Clostridia (5%), *Micrococcus* spp (2%) and other species of bacteria (3%). *F. canicularis* and *L. sericata* carrying this variety of microorganisms were sampled from a number of locations, including hospital catering areas, ward kitchens and neonatal & maternity wards (Table 7.1).

To the knowledge of the author, this study provides the first example of *Bacillus subtilis* Group, *Pantoea* spp 2 and *Micrococcus* sp from *F. canicularis* (Table 7.1).

To the knowledge of the author, this study provides the first example of *Bacillus brevis*, *Escherichia coli* serotype O71 and *Klebsiella pneumoniae* ssp *pneumoniae* isolation from *L. sericata* (Table 7.1).

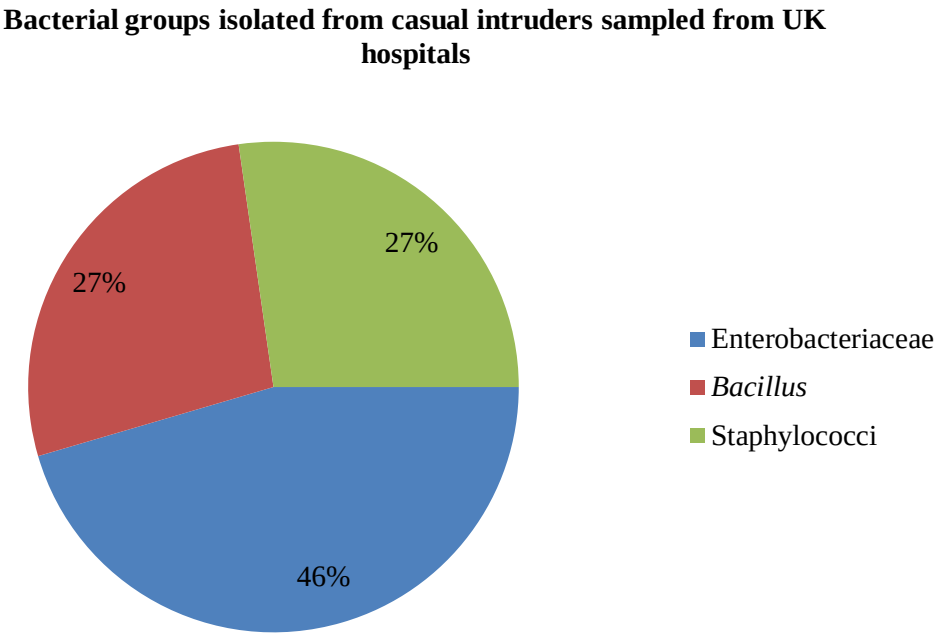

Figure 7.5 Bacterial groups isolated from casual intruders sampled from UK hospitals

Casual intruder flies are defined in this case as *M. autumnalis*, Dolichopodidae, *H. punctatissima* (an ant species frequently found in the flying alate form), *Phaonia* sp and *Helina* sp (Figure 7.5). Two batches of *M. autumnalis* (n = 16) were sampled microbiologically from two of seven hospitals from March 2010 to March 2011 and six bacterial isolates (six different species) were obtained (Table 7.1). There were four occurrences of Enterobacteriaceae (four species), zero occurrences of *Bacillus* spp, zero occurrences of Clostridia and two occurrences of Staphylococci (*S. aureus* and *S. saprophyticus*).

With *M. autumnalis* already dealt with, the remaining casual intruders are described in terms of their bacterial carriage. Thirteen batches of casual intruders (n = 97) were sampled microbiologically from three of seven hospitals from March 2010 to August 2011 and five bacterial isolates (five different species) were obtained. There was one occurrence of Enterobacteriaceae (*Pantoea* spp 3), three occurrences of *Bacillus* spp (*B. pumilus*, *B. megaterium* and *Bacillus* sp), zero occurrences of Clostridia, one occurrence of Staphylococci (*S. aureus*) and eight occurrences of no bacteria being isolated. *M. autumnalis* apart, in cases where bacteria were isolated, there was only ever one species recovered from each batch, so bacterial diversity was low. Bacterial loads for different species of bacteria associated with the casual intruders varied from an estimated 1 to 31,000 CFUs per fly per ml. In addition to the casual intruders defined in Figure 7.5, Chironomidae, *Culex pipiens*, *Pollenia rudis* and *Harmonia axyridis* were all examined microbiologically but no species of bacteria were isolated.

Figure 7.5 provides a summary of the findings presented in Table 7.1 by showing the bacterial groups isolated from casual intruders sampled from UK hospitals. Figure 7.5 shows that the majority of bacterial isolates taken from casual intruders sampled from hospitals were of the family Enterobacteriaceae (46%), followed by *Bacillus* spp (27%) and *Staphylococci* (27%). Casual intruders carrying this variety of microorganisms were sampled from a number of locations, including hospital catering areas, ward kitchens, hospital food stores, a mortuary and neonatal & maternity wards (Table 7.1).

To the knowledge of the author, this study provides the first example of *Enterobacter cloacae*, *Escherichia vulneris*, *Klebsiella pneumoniae* ssp *pneumoniae*, *Raoultella terrigena*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* isolation from *M. autumnalis* (Table 7.1). To the knowledge of the author, this study also provides the first example of the isolation of *Bacillus pumilus* and *Pantoea* sp from Dolichopodidae, *Staphylococcus aureus* from *Phaonia* sp, *Bacillus lentus* from *Helina* sp and *Bacillus megaterium* from *Hypoponera punctatissima* (Table 7.1).

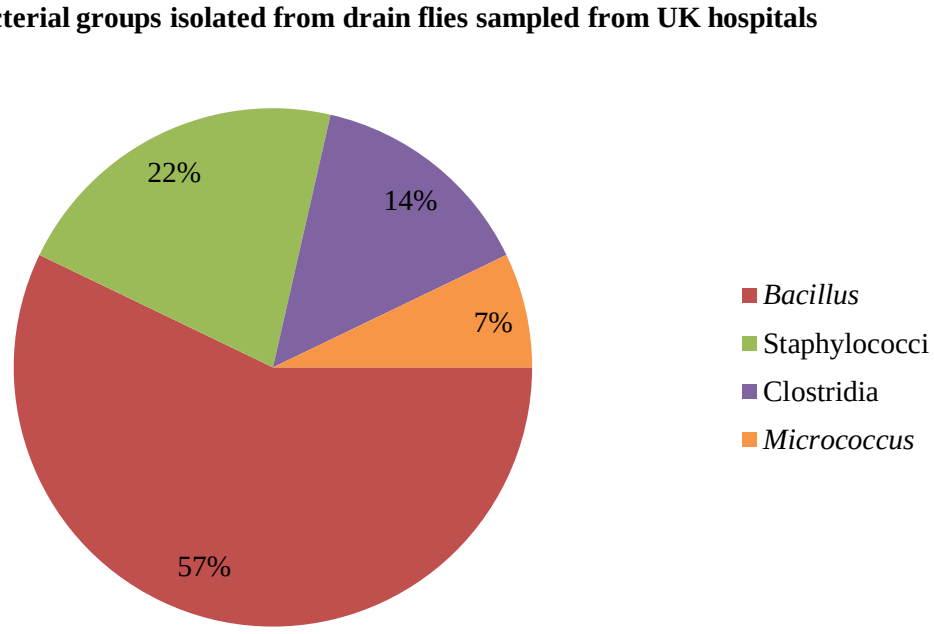

Figure 7.6 Bacterial groups isolated from drain flies sampled from UK hospitals

Drain flies in this case are defined as Psychodidae, Phoridae, Sphaeroceridae, *Trichiaspis* sp (family Sphaeroceridae) and *Drosophila* sp. Nine batches of drain flies (n = 200) were sampled microbiologically from three hospitals from May 2010 to July 2011 and 14 bacterial isolates (eight different species) were obtained (Table 7.1). There were zero occurrences of Enterobacteriaceae, eight occurrences of *Bacillus* spp (four species), two occurrences of Clostridia (*Clostridium clostridioforme*

and *Clostridium* sp), three occurrences of Staphylococci (three *S. aureus*), one occurrence of *Micrococcus* sp and one occurrence of no bacteria being isolated (Table 7.1). Bacterial species recovered multiple times were *Bacillus cereus* Group (four times, with four different identification profiles) and *Bacillus sphaericus* (twice, both with different identification profiles). Of the *B. cereus* Group that were isolated, no isolates were of the *Bacillus thuringiensis* type. A batch of Sphaeroceridae drain flies were bacterially diverse, with 4, 3 and 2 bacterial species being recovered from different batches. Bacterial loads for different species of bacteria isolated from drain flies varied from an estimated 1 to 202 CFUs per fly per ml.

Figure 7.6 provides a summary of the findings presented in Table 7.1 by showing the bacterial groups isolated from drain flies sampled from UK hospitals. Figure 7.6 shows that the majority of bacterial isolates taken from drain flies sampled from hospitals were *Bacillus* spp (57%), followed by Staphylococci (22%), Clostridia (14%) and *Micrococcus* sp (7%). Drain flies carrying this variety of microorganisms were sampled from a number of locations, including hospital catering areas, hospital food stores and neonatal & maternity wards (Table 7.1).

To the knowledge of the author, this study provides the first examples of isolation of *Bacillus cereus* Group and *Staphylococcus aureus* from Psychodidae, *Bacillus cereus* Group and *Bacillus sphaericus* from Phoridae, *Bacillus cereus* Group and *Clostridium clostridioforme* from Sphaeroceridae, *Bacillus licheniformis* and *Staphylococcus aureus* from *Trichiaspis* sp (family Sphaeroceridae) and *Bacillus pumilus* from *Drosophila* sp (Table 7.1).

7.3.2 Fly – bacteria associations

Fly – bacteria associations were examined to assess whether any statistically significant associations exist.

A chi^2 2 x 2 contingency table test was performed to examine the association of certain bacterial groups with *M. domestica* and fly species other than *M. domestica*. The null hypothesis was that there is no association between carriage of certain bacterial groups and particular flying insect species. The alternate hypothesis is that carriage of certain bacterial groups is associated with particular flying insect species. In this example, 'not *Musca domestica*' were all species listed in Table 7.1, apart from *Musca domestica*.

Table 7.2 Chi^2 test for association between carriage of certain bacterial groups and flying insect species

Flying insect Species	**Occurrences of isolation of bacterial groups**			
	Enterobacteriaceae	Spore-formers (Bacilli & Clostridia)	Staphylococci	**Total**
Musca domestica	15	10	2	27
Not *Musca domestica*	20	15	14	49
Total	35	25	16	76
Chi^2	**4.743**			

Not Significant

Based on the results in Table 7.2, there is no association between carriage of certain bacterial groups and particular flying insect species, X^2 (2, N = 76) = 4.743, p >0.05

A chi^2 2 x 2 contingency table test was performed to examine the association of fly synanthropy and carriage of bacterial groups. The null hypothesis was that there is no association between fly synanthropy (synanthropic vs non-synanthropic classifications) and carriage of bacterial groups (Enterobacteriaceae vs non-Enterobacteriaceae). The alternative hypothesis is that fly synanthropy is associated with carriage of certain bacterial groups. In this example, synanthropic fly species were: *Musca domestica*, *Calliphora vicina*, *Fannia canicularis*, *Lucilia sericata* and *Sarcophaga carnaria.* Non-synanthropic flying insects were Psychodidae, Phoridae, Sphaeroceridae, *Drosophila* sp *Musca autumnalis*, Dolichopodidae, *Hypoponera punctatissima*, *Phaonia* sp, *Helina* sp, Chironomidae, *Culex pipiens*, *Pollenia rudis*, *Harmonia axyridis*.

Table 7.3 Chi^2 2x2 contingency table test for association between fly synanthropy and carriage of bacterial groups

Bacterial group	**Flying insect group**		
	Synanthropic (includes *M. domestica, Calliphora vicina, Fannia* sp & *Lucilia* sp)	Non-synanthropic (Includes drain flies & casual intruders)	**Total**
Enterobacteriaceae	30	5	35
Non-Enterobacteriaceae	22	18	40
Total	52	23	75
Chi^2	**6.901**		

*****Statistical significance. p<0.01***

Based on the results in Table 7.3 there is an association between synanthropy and carriage of certain bacterial groups, specifically that there is an association of flying insects being non-synanthropic and carrying non-Enterobacteriaceae, X^2 (1, N = 75) = 6.901, p <0.01.Yates's correction is already included in this calculation.

A chi^2 2 x 2 contingency table test was performed to examine the association of fly synanthropy and an approximation of bacterial diversity. The null hypothesis was that there is no association between fly synanthropy (synanthropic vs non-synanthropic classifications) and an approximation of bacterial diversity (occurrences of >1 bacterial species being isolated per batch of flying insects vs occurrences of ≤1 bacterial species isolated per batch of flying insects). The alternate hypothesis is that fly synanthropy is associated with the number of bacterial species isolated per batch of flying insects. In this example, synanthropic fly species were: *Musca domestica, Calliphora vicina, Fannia canicularis, Lucilia sericata,* Psychodidae, Phoridae, Sphaeroceridae, *Drosophila* sp, *Sarcophaga carnaria*. Non-synanthropic flying insects were: *Musca autumnalis,* Dolichopodidae, *Hypoponera punctatissima, Phaonia* sp, *Helina* sp, Chironomidae, *Culex pipiens, Pollenia rudis* and *Harmonia axyridis*.

Table 7.4 Chi^2 2x2 contingency table test for association between fly synanthropy and an approximation of bacterial diversity

	Occurrences of >1 bacterial species isolated per batch of flying insects	Occurrences of ≤1 bacterial species isolated per batch of flying insects	**Total**
Synanthropic flying insects	20	20	40
Non-synanthropic flying insects	1	14	15
Total	21	34	55
Chi^2	**6.940**		

*****Statistical significance. p<0.01***

Based on the results in Table 7.4, there is an association between synanthropy and bacterial diversity, specifically that carrying a single species type or no bacterial load is associated with non-synanthropic flying insect species, X^2 (1, N = 55) = 6.940, p <0.01. Yates's correction is already included in this calculation.

7.3.3 Diversity of bacterial species associated with their fly habitats

Measures of species diversity were calculated for bacteria isolated from flies (which were seen as the habitat for such bacteria) using species richness and Simpson's diversity index (*D*). However, because there was an unequal sample size, equitability (E_D) was calculated (Begon *et al.*, 1996).

Table 7.5 Measures of biodiversity of bacterial populations associated with fly habitats

	Diversity indices of bacterial populations associated with fly habitats		
Habitat (fly species)	**Species richness**	**Simpson's Diversity index**	**Diversity index (E_D Equitability)**
Musca domestica	21	0.936	0.747
Calliphora vicina	15	0.920	0.830
'Drain fly'	9	0.828	0.646
Musca autumnalis	6	0.813	0.889
Lucilia sericata	5	0.8	1
Fannia canicularis	5	0.750	0.800
'Casual intruder'	5	0.735	0.754

Species richness was highest in the *M. domestica* habitat, with 21 species of bacteria sampled from this habitat, decreasing to 15 in *C. vicina*, nine in drain flies, six in *M. autumnalis* and five in *L. sericata*, *F. canicularis* and casual intruders. However, species richness is purely a simple count of the number of species (bacteria in this case) associated with a particular habitat (fly species are the habitats in this case) and does not take the relative abundance of different species into account. This provides a measure not just of species richness but also of the evenness of individuals' distribution between different species. This evenness is often termed 'equitability'. Although *L. sericata*, *F. canicularis* and casual intruder habitats all have a bacterial species richness of 5, their diversity is not the same, as shown by Simpson's diversity indices (Table 7.5). Of the three habitats, *L. sericata* is the most diverse, followed by decreasing diversity in *F. canicularis* and drain fly habitats. The Simpson's diversity indices of these habitats are 0.8, 0.75 and 0.735 respectively. The measure of evenness or equitability is useful in this analysis because the sample sizes in terms of the number of species sampled were unequal between habitats. Equitability takes this into account as it uses Simpson's *D* to calculate equitability E_D by dividing by the total number of species *S* in the habitat (community). Using E_D compensates for sampling effort, which is a weakness of the other described measures of

diversity, which are always dependent on sampling effort and sample size – the more a habitat is sampled, the more likely that the number of species recorded will be greater. With this in mind, it is no surprise that the *M. domestica* habitat is classed as the most diverse by species richness and Simpson's diversity methods, as this habitat was sampled the most. Using E_D, the *M. domestica* habitat is actually the sixth most equitable habitat, so it is one of the least diverse habitats when using this measure. Using Simpson's diversity indices, the diversity of habitats are ranked (from most diverse to least diverse) as *M. domestica*, *C. vicina*, Drain fly, *M. autumnalis*, *L. sericata*, *F. canicularis* and Casual intruder. This is different to when E_D is used, as the diversity of habitats is ranked (from most diverse to least diverse) as *L. sericata*, *M. autumnalis*, *C. vicina*, *F. canicularis*, Casual intruder, *M. domestica* and drain fly.

7.3.4 Bacterial load and occurrences of isolation of bacteria

Table 7.6 Mean bacterial load (CFUs) per fly per ml for different fly groups

Fly groups	*M. domestica*	Filth/domestic	Drain flies	Casual Intruders
Mean bacterial load (CFUs) per fly per ml	143,337,868	3,931,709	50	4,394
Median bacterial load (CFUs) per fly per ml	970	1,973	36	10

The mean bacterial load (CFUs) per fly per ml were highest in *M. domestica* at 143,337,868, second highest in filth/domestic flies at 3,931,709, third highest in casual intruders at 4,394 and lowest for drain flies at 50. Use of means in this case, although valuable, could however be misleading, as the figures for *M. domestica* are distorted by one large figure. The median figures for CFUs per ml per fly were actually highest for filth/domestic flies at 1,973, then *M. domestica* with 970, drain flies with 36 and lowest for casual intruders at 10.

There was no significant difference between mean bacterial loads (CFUs) per fly per ml for different fly groups, following ANOVA, probably due to the large variance in results that is often a feature of microbiological work due to the great numbers of organisms dealt with.

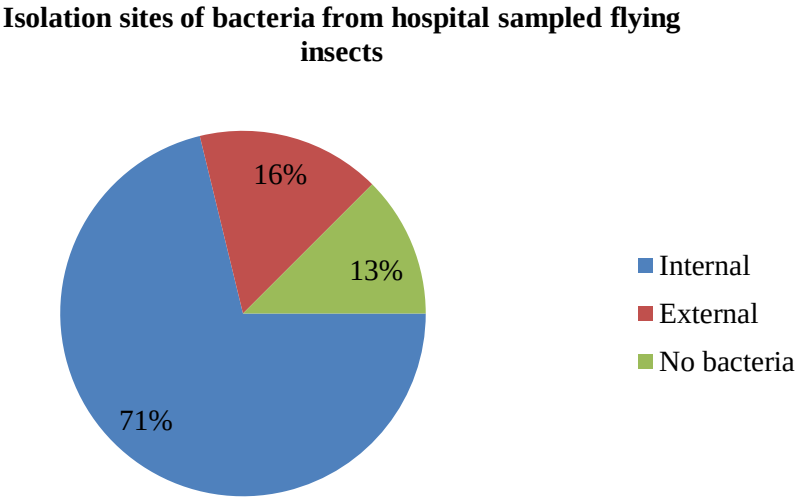

Figure 7.7 Isolation sites of bacteria from hospital sampled flying insects

Of the flying insects that were examined microbiologically, 71% of occurrences of bacterial isolation were from internal structures, 16% from external structures and no bacteria were recovered in 13% of cases (Figure 7.7).

A Z-test for matched samples was used to look for any significant difference between the occurrences of bacteria isolated internally versus occurrences of bacteria isolated externally, for all batches of flying insects that were examined microbiologically. The null hypothesis was that there is no difference between the occurrences of bacteria isolated internally versus occurrences of bacteria isolated externally from flying insects.

There was a statistically significant result observed, with Z (N = 56) = 5.786, $p < 0.001$, meaning there is a significant difference between the occurrences of bacteria isolated internally versus occurrences of bacteria isolated externally from flying insects. This is interpreted as there being significantly more occurrences of bacteria being isolated internally (a mean of 1.321 occurrences per flying insect batch) than occurrences of bacteria isolated externally (a mean of 0.321 occurrences per flying insect batch) from flying insects.

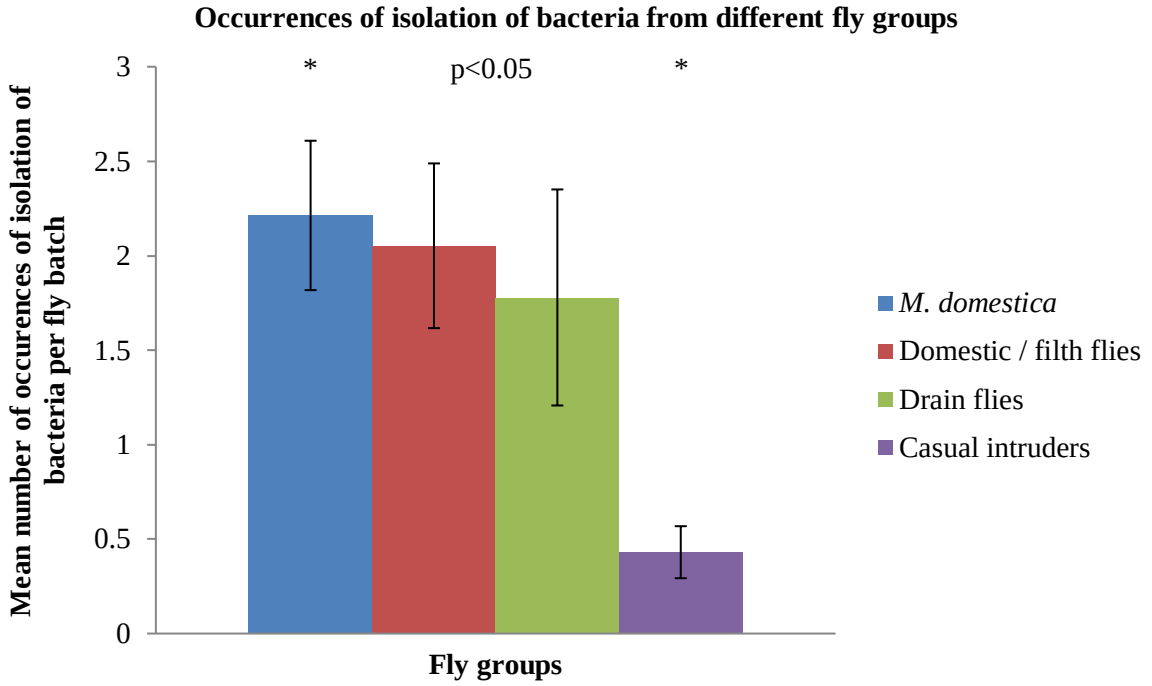

Figure 7.8 Occurrences of isolation of bacteria from different fly groups. The mean number (± Standard Error (SE)) of occurrences of isolation of bacteria per fly batch from different fly groups.

Figure 7.8 shows the occurrences of isolation of bacteria from different fly groups, expressed as mean occurrences per fly batch. The occurrences of isolation of bacteria were highest in *M. domestica* with a mean of 2.21, second highest in domestic / filth flies with 2.05, third highest in drain flies with 1.78 and lowest in casual intruders with 0.43.

A univariate ANOVA was used to look for any significant differences in the occurrences of isolation of bacteria between different fly groups. The null hypothesis was that there was no difference in the occurrences of isolation of bacteria between different fly groups.

There was a statistically significant difference in occurrences of isolation of bacteria between different fly groups, with $F(3, 52) = 4.166$, $p<0.05$, meaning that the null hypothesis can be rejected. A post hoc LSD test revealed that a statistically significant difference lay between *M. domestica* and casual intruders, with occurrences of isolation of bacteria being significantly greater in *M. domestica* compared to casual intruders. Near significant results were found between domestic / filth flies and casual intruders ($p = 0.050$) and between drain flies and casual intruders ($p = 0.099$).

Note that 'Domestic / filth flies' were defined as *C. vicina*, *F. canicularis*, *L. sericata* and *S. carnaria*. Drain flies in this case were defined as Psychodidae, Phoridae, Sphaeroceridae, *Trichiaspis* sp (family

Sphaeroceridae) and *Drosophila* sp. 'Casual intruders' were defined as Dolichopodidae, *Hypoponera punctatissima*, *Phaonia* sp and *Helina* sp Chironomidae, *Culex pipiens*, *Pollenia rudis* and *Harmonia axyridis*.

M. autumnalis were included as 'domestic / filth flies' in this particular test. *M. autumnalis* has not been included in the 'domestic / filth flies' classification in this study previously, instead being classified as a 'casual intruder' which it has traditionally been viewed as. It is this re-classification of *M. autumnalis* that brings about the statistical significance in this test. However, based on the species of bacteria that have been isolated from *M. autumnalis* in this study, it is recommended that it is equally justifiable to classify it as a 'filth fly', due to its development in animal dung and its association with Enterobacteriaceae, as it is to classify it as a casual intruder in that it does not breed or feed indoors and invades properties to overwinter.

7.4 DISCUSSION

Results show that a variety of flying insects, including synanthropic flies (e.g. *M. domestica* and *C. vicina*) collected from UK hospitals do indeed harbour pathogenic bacteria of different species. Enterobacteriaceae were the group of bacteria most commonly isolated from flying insects, followed by *Bacillus* spp Staphylococci, Clostridia, Streptococci and *Micrococcus* spp. The flying insects harbouring said bacteria were collected from a number of locations throughout hospitals, including areas where food for patient, visitor or staff consumption is prepared or stored, such as hospital catering areas, ward kitchens and food stores. The presence of flying insects in such areas presents a risk of contamination of foodstuffs with bacteria and thus a risk of human infection via consumption of the food. Flying insects carrying bacteria were also found in wards, neonatal units and maternity units and the risk of contamination and therefore human infection is different in these areas, as the most likely routes of infection are via fly-contaminated environment such as surfaces and fomites.

Although *C. difficile* was not isolated from flying insects sampled from UK hospitals, many of the identified species of bacteria were pathogenic and therefore of public health significance, with a number of species being recovered for the first ever time from their insect host. It is still expected that flies will be found to carry *C. difficile* in hospitals in future studies and they should be treated as potential vectors, based on evidence from the results of the laboratory studies in section 2.3, isolation of *C. difficile* from flies on farms (Burt *et al.*, 2012) and the fact that bacteria of the same genus were isolated in this study. *C. vicina* and *L. sericata* are likely candidates for *C. difficile* carriage, due to the fact that this bacterium has been isolated from rodents (Himsworth *et al.*, 2014) and birds (Bandelj *et al.*, 2014), the carcasses of which are breeding / development media (Erzinclioglu, 1996) and a source of bacterial contamination for such flies.

The entomological study discussed in chapter 6 provides pest control and infection control staff with knowledge of the key flying insect species that are likely to be present in hospitals at certain times of year and in which hospital locations. This microbiological study adds to the entomological study by providing pest control and infection control staff with knowledge of the species of bacteria which flying insects are likely to be carrying in UK hospitals, giving a clearer picture of the public health significance of such insects. A key general point from this study is that flying insects in UK hospitals are more likely to be carrying Enterobacteriaceae than other groups of bacteria.

M. domestica

The majority of bacterial isolates taken from *M. domestica* sampled from hospitals were of the family Enterobacteriaceae followed by *Bacillus* spp, Clostridia, Staphylococci and Streptococci. This

association of *M. domestica* and Enterobacteriaceae which are commonly isolated from the gut of animals (Cowan *et al.*, 2003) is no surprise, as moist rotting organic matter, ranging from kitchen waste to animal faeces is the preferred breeding media of houseflies (West, 1951) and is a source of such bacteria.

To the author's knowledge, this study provides the first example of *B. licheniformis, C. beijerinckii* / *C. butyricum, C. clostridioforme* and *R. terrigena* isolation from *M. domestica*

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 houseflies 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* and the same principle is adopted when discussing bacteria identified from other fly species.

B. licheniformis was isolated from *M. domestica* sampled from a hospital mortuary. *Bacillus licheniformis* is a Gram-positive, aerobic and facultatively anaerobic, spore-forming (oval shaped spores, centrally positioned), rod-shaped, motile bacterium (Cowan *et al.*, 2003) that is isolated from soil (Hussein and AL-Janabi, 2006). The reported isolation from *M. domestica* is important 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 that causes serious infection in patients with neutropenia (Ozkocaman *et al.*, 2006).

Bacillus pumilus was isolated from *M. domestica* collected from a hospital food store. *Bacillus pumilus* is a Gram-positive, aerobic, spore-forming (oval shaped spores, centrally positioned), rod-shaped, motile bacterium (Cowan *et al.*, 2003). *B. pumilus* is isolated from soil where it can degrade feathers (El-Refai *et al.*, 2005). It is also isolated from marine environments and animals including sediment, oysters, crabs, fish and starfish (Parvathi *et al.*, 2009) and mango plants (Galal *et al.*, 2006). Toxic strains of *B. pumilus* have been isolated from air sampled indoors, paper and wood pulp, Norwegian spruce (Suominen *et al.*, 2001) and even spacecraft can be contaminated with *B. pumilus* (Link *et al.*, 2004). The significance of the reported isolation from *M. domestica* is that catheter infection in children due to *B. pumilus* has been recorded in the literature (Bentur *et al.*, 2007). The *B. pumilus* infection was only eradicated following catheter removal and antibiotic use (Bentur *et al.*, 2007). *B. pumilus* was isolated from *M. domestica* for the first time from flies that were taken from around refuse bins and the rear entrances of restaurants in Florida (Butler *et al.*, 2010).

C. beijerinckii / *C. butyricum* were isolated from *M. domestica* sampled from a hospital catering area. *Clostridium beijerinckii* and *Clostridium butyricum* are Clostridia from the butyricum group, which are Gram-positive in young cultures, anaerobic, spore-forming (oval shaped spores, centrally positioned), rod-shaped, motile organisms (Cowan *et al.*, 2003), isolated from human faeces (Popoff and Dodin, 1985) and soil (Meng *et al.*, 1999). Clinically significant *C. butyricum* strains have been isolated from the faeces of new-born 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). Other cases of *C. butyricum* infection include a neurotoxigenic foodborne botulism outbreak in a residential school in India (Chaudhry *et al.*, 1998), intestinal botulism (Fenicia *et al.*, 1999), intestinal botulism in an infant (Fenicia *et al.*, 2002) and bacteraemia / sepsis in a patient with a catheter, which responded to treatment with broad-spectrum antibiotics (Gardner *et al.*, 2008). Apart from this study, the only known insect associations include *C. beijerinckii* being isolated from the hindgut of the orange head cockroach *Eublaberus posticus* (Cruden and Markovetz, 1987) and a laboratory strain of termites, *Coptotermes formosanus*, from which *C. butyricum* was also cultured (Taguchi *et al.*, 1993).

C. clostridioforme was isolated from *M. domestica* sampled from a hospital catering area. *Clostridium clostridioforme* are Gram-negative (not typical of *Clostridium* spp), anaerobic, spore-forming (although spores are difficult to find), rod-shaped, organisms isolated from human faeces (Finegold *et al.*, 2005) and horse/mule faeces (Derlet and Carlson, 2002). There appear to be no records in the literature of *C. clostridioforme* isolation from insects.

To the author's 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). The likely source of *C. clostridioforme* contamination in *M. domestica* was probably either from contacting human faeces in the hospital or horse faeces external to the hospital, which are both types of faecal matter that they can breed in (West, 1951), especially as this bacterium has been isolated from human faeces (Finegold *et al.*, 2005) and horse/mule faeces (Derlet and Carlson, 2002).

R. terrigena was isolated for the first time from *M. domestica*, which were sampled from a hospital ward. A relatively newly described species, *Raoultella terrigena* (also called *Klebsiella terrigena*) a member of the Enterobacteriaceae, is a Gram-negative, aerobic and facultatively anaerobic, rod-shaped, non-motile bacterium isolated from soil and water samples (Izard *et al.*, 1981). *R. terrigena* is also isolated from healthy human faeces and from 1988 – 1990 was isolated from clinical samples for the first time, with most isolates taken from the respiratory tract and some from urine and wound

infections (Podschun and Ullmann, 1992). 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 infections in neonates (Hurrell *et al.*, 2009). An infection with extended-spectrum *β*-lactamase producing *R. terrigena* caused fatal endocarditis and is thought to be the first case of this kind in a liver transplant patient (Goegele *et al.*, 2007).

Based on 'read-across' from studies on the transmission of bacteria by *M. domestica* (Kobayashi *et al.*, 1999), it follows that houseflies in hospitals may act as a mobile reservoir and vector of clinically significant *B. licheniformis*, *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.

C. vicina

The majority of bacterial isolates taken from *C. vicina* sampled from hospitals were of the family Enterobacteriaceae, followed by Staphylococci. This association of *C. vicina* and Enterobacteriaceae which are commonly isolated from the gut of animals (Cowan *et al.*, 2003) is no surprise, as these flies typically develop on animal carcasses such as birds and rodents and can feed on faeces (Erzinclioglu, 1996), which are a source of such bacteria.

The live sampled batch of *C. vicina* was the most interesting as it was the most bacterially diverse batch of *C. vicina* and also exhibited the highest bacterial load for this species. In terms of bacterial diversity, three species were recorded, which were the Enterobacteriaceae *Citrobacter freundii*, *Enterobacter asburiae* and *Pantoea* sp 1. There was a total bacterial count of 40.6 million CFUs per fly per ml for live sampled *C. vicina*. This is significant as the infectious dose for *Citrobacter* sp is approximately 1 x 10^7 CFU/ml (Tennant *et al.*, 2008) and 1,000 cells for *Enterobacter* sp (Iversen and Forsythe, 2003), meaning that *C. vicina* would be capable of providing an infective dose to humans, should it contaminate foodstuffs or the environment of hospital patients.

In some cases in this study, the bacterial loads for certain species of bacteria on certain species of fly were low and the risk of transferring an infective dose to foodstuffs or the environment of hospital patients e.g. fomites is correspondingly considered to be low. However, inoculation of foodstuffs by a 'seeding' effect could occur following transmission of bacteria by flies. Although only a small amount of bacteria could be deposited initially, subsequent bacterial growth on the fly-contaminated foodstuff could then occur and this proliferation of bacteria then represents an infection risk to humans, in terms

of providing an infective dose. Even in cases where the bacterial load of flies is low, their significance should not therefore be discounted.

To the author's knowledge, this study provides the first example of *Escherichia coli* serotype E1525, *Klebsiella oxytoca*, *Klebsiella pneumoniae* ssp ozaenae, *Leclercia adecarboxylata*, *Pantoea* species 1, *Raoultella terrigena* and *Staphylococcus hominis* isolation from *C. vicina*

E. coli serotype E1525 was isolated from bluebottle flies *C. vicina* sampled from a hospital restaurant. E1525 cultures are generally extraintestinal isolates i.e. from blood cultures and urine (often from surgical cases in hospital) rather than from faeces (personal communication, Dr Tom Cheasty, Health Protection Agency, 2011). E1525 is a clinical serotype, yet it has been isolated from *C. vicina*, which means it is more likely to have been acquired by *C. vicina* from the hospital environment rather than being brought in from an external source. This finding corresponds with the suggestion of Fotedar *et al.* (1992b), that 'microbial studies of randomly collected flies from a hospital environment may provide an epidemiological tool for monitoring existing sanitary conditions'.

E. coli, a member of the Enterobacteriaceae, is a Gram-negative, aerobic and facultatively anaerobic, rod-shaped, motile bacterium (Cowan *et al.*, 2003), typically isolated from the human gastrointestinal tract and items of food (Kaper *et al.*, 2004). Infection with pathogenic *E. coli* causes diarrhoeal disease, urinary tract infection, haemolytic uremic syndrome (HUS), sepsis and meningitis and can prove fatal particularly in infants (Kaper *et al.*, 2004). Multi-drug resistant strains of *E. coli* have been isolated from houseflies *M. domestica* in hospitals (Nmorsi *et al.*, 2007) and another example of *E. coli* isolation from flies in hospitals is from the cuticle of the moth fly *Clogmia albipunctata* collected from shower cubicles, rest rooms and kitchens of a German hospital (Faulde and Spiesberger, 2013). The *C. vicina* that carried *E. coli* E1525 in this study could transfer this pathogen to foodstuffs in hospitals, thereby presenting a risk to the health of patients. This principle has been shown in a study where *E. coli* O157:H7 was experimentally transferred to spinach by houseflies *M. domestica* and was also isolated from field sampled flies of the family Muscidae and Calliphoridae (the family to which *C. vicina* belongs) taken from areas where spinach was being grown (Talley *et al.*, 2009).

K. oxytoca was isolated from *C. vicina* sampled from hospital kitchens. *Klebsiella oxytoca*, a member of the Enterobacteriaceae, is a Gram-negative, aerobic and facultatively anaerobic, rod-shaped, non-motile bacterium (Cowan *et al.*, 2003) found in the clinical setting where it can infect neonates (Berthelot *et al.*, 2001) and contaminate the hospital environment such as sinks (Lowe *et al.*, 2012). *K. oxytoca* has been identified as the causative agent of antibiotic-associated haemorrhagic colitis and should be considered when patients are negative for *C. difficile* (Hogenauer *et al.*, 2006) and has for the first time caused necrotising fasciitis, which resulted in the death of an elderly liver cancer patient whose leg had become infected (Oishi *et al.*, 2008). An outbreak of extended-spectrum β-lactamase

producing *K. oxytoca*, with hand washing sinks identified as the source of contamination, was controlled by the disinfection of the sinks and drains (Lowe *et al.*, 2012). Another study highlighted hospital sinks and drains as the source of a *K. oxytoca* outbreak, which was eliminated following cleaning procedures (Vergara-Lopez *et al.*, 2013). It is recommended that fly control is included as an infection control measure for *K. oxytoca* outbreaks in hospitals, in addition to the aforementioned disinfection and cleaning procedures. Other sources of infection include infant food, as *K. oxytoca* can survive in dehydrated powdered infant formula for over 2 years, presenting an infection risk to new-born babies (Barron and Forsythe, 2007), contaminated sodium chloride solution introduced by venous catheter into the bloodstream (Watson *et al.*, 2005) and contamination by enteral feeding, causing an outbreak in neonates which was dealt with by hospital workers using gloves to stop cross-contamination (Berthelot *et al.*, 2001). Flies such as *C. vicina* could be a cause of cross-contamination without sufficient fly control measures, circumventing the use of gloves by hospital workers. In terms of detection in flies, *K. oxytoca* has been identified from houseflies *M. domestica* and patients sampled at a hospital in India (Fotedar *et al.*, 1992a) and from the moth fly *Clogmia albipunctata* collected from shower cubicles, patient wards, rest rooms and kitchens of a German hospital (Faulde and Spiesberger, 2013).

K. ozaenae was isolated from *C. vicina* sampled from a hospital dry food store, which as well as being the first case of isolation from *C. vicina* is also the first from flying insects in hospitals. *Klebsiella pneumonia* ssp *ozaenae*, a member of the Enterobacteriaceae, is a Gram-negative, aerobic and facultatively anaerobic, rod-shaped, non-motile bacterium (Cowan *et al.*, 2003) isolated from the nasopharynx and a cause of ozaena (chronic atrophic rhinitis), is now also reported in infections of the blood, urinary tract and soft tissue (Goldstein *et al.*, 1978). Infection with *K. ozaenae* accounted for 0.2% of Klebsiellae infections in intensive care units in Europe (Livermore and Yuan, 1996), is rarely isolated in the clinical setting and when this occurs, it is typically found in the pharynx (De Champs *et al.*, 2005) and is a cause of chronic rhinitis (Botelho-Nevers *et al.*, 2007).

Leclercia adecarboxylata was isolated from *C. vicina*, which were sampled from hospital ward kitchens. *L. adecarboxylata*, a member of the Enterobacteriaceae, was first described as a new species in 1986 (formerly known as *Escherichia adecarboxylata*) and is a Gram-negative, aerobic and facultatively anaerobic, rod-shaped, motile bacterium found in food, water, the environment and clinical isolates such as blood, sputum, wounds, urine and faeces (Tamura *et al.*, 1986). *L. adecarboxylata* infection has been reported in the peritoneal fluid of a child suffering from peritonitis who also had kidney disease (Fattal and Deville, 2000), a catheter-related infection in an adult with kidney disease (Marina *et al.*, 2011), infected gallbladder tissue (de Baere *et al.*, 2001), a heart infection in a cancer patient (Lee *et al.*, 2009), the blood of an infant with acute lymphoblastic leukaemia (Longhurst and West, 2001) and an antibiotic-resistant β-lactamase producing strain has been described in the blood of an adult leukaemia patient (Mazzariol *et al.*, 2003). As described in the

previous references, *L. adecarboxylata* can be found causing infection in immunocompromised individuals and/or those with underlying disease, although isolation from immunocompetent patients can occur, which has been the case in a patient with a heel abscess (Hess *et al.*, 2008) and a throat tissue abscess (Bali *et al.*, 2013). Neonatal infection with *L. adecarboxylata* has now been reported for the first time, causing late-onset sepsis (Myers *et al.*, 2012). The source of *L. adecarboxylata* in *C. vicina* could have been environmental and it was not surprising to isolate it, as it is known to be a pathogen of insects and has insecticidal activity (Muratoglu *et al.*, 2009).

Pantoea spp 1 was isolated from live *C. vicina* sampled from the medical illustration department toilet of a hospital. *Pantoea* spp, members of the Enterobacteriaceae are Gram-negative, aerobic and facultatively anaerobic, rod-shaped, motile organisms (Cowan *et al.*, 2003), which are isolated from plants and can cause infection in humans, particularly following 'penetrating trauma by vegetation' (Cruz *et al.*, 2007). *Pantoea* spp have been identified as contaminants in parenteral nutrition solutions, which were a cause of infection in neonates in a neonatal intensive care unit, resulting in septicaemic shock and respiratory failure with a high fatality rate of 87.5% (Van Rostenberghe *et al.*, 2006). In fact, *Pantoea* spp can survive in dehydrated powdered infant formula for over 2 years, presenting an infection risk to new-born babies (Barron and Forsythe, 2007). Other sources of *Pantoea* spp clinical infections are contaminated transference tubes (Bicudo *et al.*, 2007). Further reports of human infection with *Pantoea* spp refer to cases of bacteraemia in an elderly patient (de Baere *et al.*, 2001), preterm neonates (Aly *et al.*, 2008) cancer patients (Liberto *et al.*, 2009) and peritonitis due to rose-thorn injury (Lim *et al.*, 2006). Cluster flies, *Pollenia rudis* sampled from a hospital in Germany were found to harbour opportunistic pathogens described as *Erwinia* spp in the study, which is another name for *Pantoea* spp (Faulde *et al.*, 2001). Other insects in hospitals positive for *Pantoea* spp include flies, wasps *P. vulgaris*, ants *Lasius* sp (*Lasius niger* or *Lasius niger*) collected outside a hospital and cockroaches *B. germanica*, spiders and non-biting midges of the family Chironomidae sampled from dermatology, urology and infectious disease wards (Sramova *et al.*, 1992).

R. terrigena was isolated from *C. vicina* sampled from a hospital mortuary. The significance of *R. terrigena* has already been discussed.

S. hominis was isolated from *C. vicina*, which were sampled from a hospital restaurant. *Staphylococcus hominis*, of the Staphylococcaceae, are Gram-positive, aerobic and facultatively anaerobic, non-motile, size-variable cocci in pairs and clusters (Cowan *et al.*, 2003), isolated from the urinary tract of young women (Marrie *et al.*, 1982). In studies regarding blood infections, *S. hominis* accounted for 6% of all coagulase-negative staphylococci involved and exhibited oxacillin resistance in 71% of isolates (Marshall *et al.*, 1998). *S. hominis* can be transmitted nosocomially and has been isolated in cases of blood infection in neonates (Chaves *et al.*, 2005) and in adults, with most isolates showing multi-drug resistance (Palazzo *et al.*, 2008). References to *S. hominis* isolation from insects

are relatively rare. A Methicillin-resistant strain of *S. hominis* has been taken from the body surfaces of German cockroaches *B. germanica*, sampled in a hospital surgical ward (Gliniewicz *et al.*, 2003). Other examples of *S. hominis* isolation from insects include from flies (species unknown) collected outside a hospital (Sramova *et al.*, 1992), bark beetles of the subfamily Scolytinae (Cardoza *et al.*, 2009) and the external surface of the eye fly *Siphunculina funicola* sampled from resting sites and following feeding on human wounds (Chansang *et al.*, 2010).

The fact that *C. vicina* were the most common synanthropic fly in hospitals and harboured many species of pathogenic bacteria sometimes with extremely high bacterial loads, means that the seasonal prevalence (their described peak in numbers in autumn) and location of this species within hospitals (found most often in food preparation areas) should be a priority consideration in terms of informing pest control measures to aid infection control.

F. canicularis

To the author's knowledge, this study provides the first example of *Bacillus subtilis* Group, *Pantoea* spp 2 and *Micrococcus* sp isolation from *F. canicularis*.

B. subtilis Group bacteria were recovered from lesser houseflies *Fannia canicularis* collected from a hospital coffee shop. The *Bacillus subtilis* Group are Gram-positive, aerobic, spore-forming (oval shaped spores, centrally positioned), rod-shaped, motile organisms (Cowan *et al.*, 2003) which are isolated from soil (Dhas and Hena, 2012) and include *B. subtilis, B. amyloliquefaciens* and *B. licheniformis* (Wipat and Harwood, 1999). Infection with *B. subtilis* can be of clinical significance and resulted in the death of an 11 year old girl with leukaemia, due to infection in the lung and brain abscesses (Ihde and Armstrong, 1973). Surgical wounds can also become infected with *B. subtilis* following liver surgery (Ihde and Armstrong, 1973) and *B. subtilis* has been detected in blood cultures of cancer patients (Banerjee *et al.*, 1988). The human gastrointestinal tract is a site of colonisation for *B. subtilis*, which has led to recommendations that such bacteria should not be viewed only as a soil organism but also a gut commensal (Hong *et al.*, 2009). Surfactin, a biosurfactant produced by *B. subtilis* shows potential for use a pupicidal compound for mosquito control (Geetha and Manonmani, 2010) and *B. subtilis* also shows promise for use as a mosquito larvicide against the yellow fever mosquito *Aedes aegypti* (Radhika *et al.*, 2011).

Pantoea spp 2 was isolated from lesser houseflies *F. canicularis* sampled from a hospital kitchen / restaurant. The significance of *Pantoea* spp have already been discussed.

Micrococcus sp was isolated from lesser houseflies *F. canicularis* sampled from the main kitchen of a hospital. *Micrococcus* spp, members of the Micrococcaceae are aerobic Gram-positive cocci of

uniform size arranged in pairs, fours and small clusters and are typically non-motile (Cowan *et al.*, 2003). *Micrococcus* spp are isolated commonly from human skin (Kloos *et al.*, 1974). *Micrococcus* spp have been isolated in clinical cases, including urinary infection in young women (Kerr, 1973) and rarely in males (Meers *et al.*, 1975) and have caused fatal pneumonia in an immunocompromised patient (Salar *et al.*, 1997). Cases of catheter-related *Micrococcus* spp infection also occur (Yap and Mermel, 2003), with specific examples including isolation in 27% of blood cultures from patients with pulmonary arterial hypertension (Oudiz *et al.*, 2004) and from the blood of cancer patients (Ramos *et al.*, 2009). Endocarditis has also been caused by *Micrococcus*, specifically by *M. luteus* and typically when a prosthetic heart valve has been fitted (Miltiadous and Elisaf, 2011). Peritonitis is another condition caused by *Micrococcus* spp, usually in patients undergoing dialysis (Kao *et al.*, 2012). *Micrococcus* spp have been isolated from the moth fly *Clogmia albipunctata* collected from shower cubicles, patient wards, rest rooms and kitchens of a German hospital (Faulde and Spiesberger, 2013).

L. sericata

To the author's knowledge, this study provides the first example of *Bacillus brevis*, *Escherichia coli* serotype O71 and *Klebsiella pneumoniae* ssp *pneumoniae* isolation from *L. sericata*.

B. brevis was isolated from *L. sericata* collected from a hospital kitchen. *B. brevis* is a Gram-positive / Gram-variable, aerobic, spore-forming (oval shaped spores, with variable position), rod-shaped, motile bacterium (Cowan *et al.*, 2003) and has been isolated from soil (Dubos and Hotchkiss, 1941). The type strain of *B. brevis* has been reclassified as *Bacillus migulanus* (Takagi *et al.*, 1993) and then more recently as *Aneurinibacillus migulanus* (Shida *et al.*, 1996) although most texts still refer to *B. brevis*. *B. brevis* has been linked with peritonitis in a liver cancer patient (Parvez *et al.*, 2009). The *B. brevis* infection in this case was thought to be a result of consumption of fermented food contaminated with *B. brevis* spores and was treated with antibiotics (Parvez *et al.*, 2009). Despite this record of infection, there are beneficial uses of *B. brevis*, the most notable being the production of two antibiotics, gramicidin and tyrocidine (Dubos and Hotchkiss, 1941). The only *B. brevis* insect associations recorded in the literature are *Culex* spp and *Aedes* spp mosquito larvae *(Araujo-Coutinho et al., 2011)* and oriental cockroaches *Blatta orientalis* sampled from various sites of a hospital such as kitchens and a boiler room (Burgess *et al.*, 1973).

E. coli serotype O71 was isolated from *L. sericata* collected from a hospital kitchen. *E. coli* O71 serogroup (EPEC pathotype) has been detected in samples from healthy calves (Orden *et al.*, 2002) and is not known as a clinical isolate. It is likely therefore that *L. sericata* had acquired *E. coli* O71 from calf faeces and then entered the hospital, illustrating perfectly the dangers of fly ingress and capacity for introduction of non-clinical isolates into the hospital environment where they may prove

pathogenic in humans. *E. coli* O71 is described as being of the EPEC pathotype, which means it is 'enteropathogenic' *E. coli* and can cause potentially fatal infant diarrhoea (Kaper *et al.*, 2004).

K. pneumoniae ssp *pneumoniae* was isolated from *L. sericata* sampled from the main kitchen of a hospital. *Klebsiella pneumoniae* ssp *pneumoniae*, a member of the Enterobacteriaceae, is a Gram-negative, aerobic and facultatively anaerobic, rod-shaped, non-motile bacterium (Cowan *et al.*, 2003) isolated frequently in the clinical setting and is the main cause of pneumonia (Lin *et al.*, 2010). Pneumonia is not the only illness due to infection with *K. pneumonia*, which has caused blood infections in children (Kim *et al.*, 2002) and meningitis in adults (Chang *et al.*, 2012). Infection with *K. pneumoniae* accounted for 74% of Klebsiellae infections in intensive care units in Europe and 29% of *K. pneumoniae* isolates showed resistance to antibiotics due to extended-spectrum β-lactamase production (Livermore and Yuan, 1996) and hospital outbreaks of have been controlled by restricted use of cephalosporins and routine use of disposable gloves, aprons and hand washing procedures (Pena *et al.*, 1998). The length of time that catheters (of the central venous type) are used has been identified as a risk factor in infection with carbapenem resistant *K. pneumoniae* (Correa *et al.*, 2013). Neonatal enteral feeding tubes have been colonised by *K. pneumoniae*, which represent another important risk factor for infection in neonates (Hurrell *et al.*, 2009) and this bacterium can survive in dehydrated powdered infant formula for up to 15 months, presenting another risk to new-born babies (Barron and Forsythe, 2007). There exists extremely convincing evidence of the role played by German cockroaches (*Blattella germanica*) in an outbreak of a bacterial infection caused by *Klebsiella pneumoniae* in a neonatal unit (Cotton *et al.*, 2000) and flies could also be implicated. The study showed that the 'strain' isolated from the cockroaches was indistinguishable from that colonizing and causing invasive disease in the newly born infants. In terms of detection in flies in hospitals, *K. pneumoniae* has been identified from 90% of houseflies *M. domestica* and 85.1% of patients sampled at a hospital in India, as well as 84.7 % of the same species of fly taken from a residential area (Fotedar *et al.*, 1992a). Further examples of *K. pneumoniae* isolation from insects include wasps *Paravespula vulgaris* and ants *Lasius* sp (*Lasius niger* or *Lasius emarginatus*) collected outside a hospital (Sramova *et al.*, 1992). In the same study, a number of species of flies pooled together in the analysis were positive for *K. pneumoniae* and these flies were collected from dermatology, urology and infectious disease wards (Sramova *et al.*, 1992). *K. pneumoniae* has also been isolated from the cuticle of the moth fly *Clogmia albipunctata* collected from shower cubicles, rest rooms and kitchens of a German hospital (Faulde and Spiesberger, 2013).

Casual intruders

The majority of bacterial isolates taken from casual intruders sampled from hospitals were of the family Enterobacteriaceae (46%), followed by *Bacillus* spp (27%) and Staphylococci (27%).

There was a significant association between synanthropy and bacterial diversity, that carrying a single species type or no bacterial load is associated with casual intruder (a.k.a. non-synanthropic) flying insect species. This can be explained by the general biology of this group of flying insects, in that unlike synanthropic flies, they do not often frequent unsanitary areas such as drains, carcasses or animal faeces that provide a rich and diverse source of bacterial contamination.

Not all casual intruder insect samples yielded bacteria though. Chironomidae, *Culex pipiens* (mosquito), *Pollenia rudis* and *Harmonia axyridis* were classed as casual intruders and were all examined microbiologically but no species of bacteria were isolated. This finding raises questions, particularly regarding the significance of Chironomidae in hospitals. Chironomids were the most abundant insect in hospitals in the entomological study and it was recommended that this and details of their public health significance should be communicated to pest control and hospital staff as well as recommendations regarding their control. However, this microbiological study may be interpreted as providing evidence that Chironomidae in UK hospitals are of little public health significance, seeing as they didn't harbour any bacteria. This is probably a risky stance to take, as evidence exists in the literature regarding *Clostridium* spp (Rouf and Rigney, 1993) and *Vibrio cholerae* isolation from Chironomidae (Broza *et al.*, 2005) and only one batch of these flies were analysed microbiologically in this study and further study of other batches may have yielded bacteria. Entomological and microbiological studies were run concurrently, which explains the reason that only one batch of Chironomidae was examined. The sheer numbers of Chironomidae and therefore their significance had not been apparent at the time of microbiological analysis while the entomological analysis was ongoing, so the synanthropic flies were concentrated on, as identified by the literature review as being the most likely candidates for carriage of pathogenic bacteria. It is recommended that future microbiological analysis of flies in hospitals should focus on Chironomidae, to bridge this gap in knowledge. A contributing factor to the lack of bacterial isolation from Chironomidae in this study could have been the sampling method of the flies. From experience and observation, Chironomidae are delicate insects that desiccate and fragment readily in EFKs, which were used to sample these flies in this study. As a result of desiccation and fragmentation, many of the Chironomidae sampled from EFKs lacked their legs, wings, mouthparts, even their heads, all of which are likely sites of bacterial harbourage. Therefore, live sampling may be the best method of collecting Chironomidae in future studies when assessing bacterial carriage.

A lack of isolation of bacteria from the mosquito *C. pipiens* is perhaps not surprising, due to a paucity of prior evidence in the literature. Flies of the family Culicidae are the mosquitoes, whose immature stages develop typically in temporary accumulations of stagnant water and adult females take blood meals from vertebrates, including humans (Marshall, 1938). A number of mosquito species are recorded as presenting a biting nuisance to humans in Britain (Medlock *et al.*, 2012). Mosquitoes are well known as vectors of malaria, yellow fever, dengue, chikungunya and other viruses. However,

investigations into their bacterial associations are limited and only *Staphylococcus* spp (coagulase negative), *Enterococcus* spp, *Enterobacter cloacae*, *Enterobacter intermedius* and *Acinetobacter calcoaceticus* are reported in known literature (Sramova *et al.*, 1992). Adult mosquitoes are recognised by their proboscis, plumose antennae in males, scales on their wing veins and wing margins, slender appearance and long legs (Marshall, 1938). There are 34 species in Britain (Medlock and Vaux, 2010) and *Culex pipiens* is probably the most common mosquito in Britain (Snow, 1990).

A lack of isolation of bacteria from *H. axyridis* is perhaps unsurprising, since there appears to be only one reference in the literature regarding bacterial carriage by *H. axyridis* (Moon *et al.*, 2011). A lack of isolation of bacteria from *P. rudis* is also unsurprising, as a previous study reported no bacterial growth detected by direct inoculation, with detection only occurring by enrichment culture technique (Faulde *et al.*, 2001). This leads to a recommendation for future studies on bacterial carriage of flying insects in hospitals, that enrichment culture technique should be used for certain insects, particular for those in this study where no bacterial isolation occurred.

Of the casual intruders that were found to carry bacteria, *M. autumnalis* was important, with many species of bacteria being isolated for the first time from this fly.

M. autumnalis, the autumn fly or face fly, is a symbovine fly of the family Muscidae, which develops on animal dung, the adult feeding on secretions and sweat from horses and cattle. The adult flies overwinter in large numbers within buildings, where they cause nuisance in autumn then again upon emergence in spring (Busvine, 1980). There are few examples of bacteria being isolated from this species (Greenberg, 1971). In appearance it resembles closely the housefly, *M. domestica* but the body is more rounded and the male has more orange on the abdomen (Chinery, 2012). It can be separated from *M. domestica* via an entomological key (Mallis, 1990).

To the author's knowledge, this study provides the first example of *Enterobacter cloacae*, *Escherichia vulneris*, *Klebsiella pneumoniae* ssp *pneumoniae*, *Raoultella terrigena*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* isolation from *M. autumnalis*.

E. cloacae was isolated from *M. autumnalis* sampled from a hospital café / restaurant. *Enterobacter cloacae*, a member of the Enterobacteriaceae, is a Gram-negative, aerobic and facultatively anaerobic, rod-shaped, motile bacterium (Cowan *et al.*, 2003) isolated from clinical samples (Jiang *et al.*, 2005) and sewage (Wang *et al.*, 1989). Records of *E. cloacae* infection in clinical settings are numerous and include isolates from enteral feed contaminated by detergent dispenser which caused septicaemia (Casewell *et al.*, 1981), blood cultures with 78% of *Enterobacter* spp being identified as *E. cloacae* in patients suffering from bacteraemia in Korea (Kang *et al.*, 2004). Other bloodstream infections with *E. cloacae* have been described along with contamination of sputum and urine (Jiang *et al.*, 2005) and

in some cases, the source was thought to be contaminated sodium chloride solution introduced by venous catheter into the bloodstream (Watson *et al.*, 2005). Isolation of *E. cloacae* from neonates is also reported and there have been cases of resistant strains which have caused fatalities (Modi *et al.*, 1987), with subsequent outbreaks being successfully treated with ciprofloxacin (Bannon *et al.*, 1989). Other outbreaks of *E. cloacae* infection in neonates have been attributed to inadequate disinfection of thermometers (van den Berg *et al.*, 2000) and contaminated parenteral feed (Tresoldi *et al.*, 2000), while resistant strains have surfaced in neonates (Kartali *et al.*, 2002). It is interesting to note that neonatal feed can be a source of infection, as *E. cloacae* can survive in dehydrated powdered infant formula for up to 6 months, presenting an infection risk to new-born babies (Barron and Forsythe, 2007). There are few other cases of *E. cloacae* isolation from flying insects in hospitals, only *Culex pipiens molestus* collected from a dermatology and urology ward (Sramova *et al.*, 1992) and the cuticle of the moth fly *C. albipunctata* collected from shower cubicles, patient wards, rest rooms and kitchens of a German hospital (Faulde and Spiesberger, 2013).

E. vulneris was isolated from *M. autumnalis* sampled from a hospital café kitchen. *Escherichia vulneris* (vulneris is 'wound' in Latin), a member of the Enterobacteriaceae, was first described as a new species in 1982 and is a Gram-negative, aerobic and facultatively anaerobic, rod-shaped, motile bacterium and is typically isolated from wounds (Brenner *et al.*, 1982). *E. vulneris* infections have been reported from a wide variety of wounds, sustained from soccer-related soft tissue injury, cellulitis of the leg, abscess of the foot, crush injuries of the foot and boils (Pien *et al.*, 1985) and thirteen cases of soccer-related wound infection have been recorded in Denmark (Jepsen *et al.*, 1997). However, more recent work has shown that *E. vulneris* is not just a wound-infecting organism and can cause a diverse range of infections, as it has been isolated in a case of catheter-related blood infection (Horii *et al.*, 2001), meningitis following infection of a serious head wound and was recovered from cerebrospinal fluid (Mohanty *et al.*, 2005) and peritoneal fluid in a case of peritonitis that was related to dialysis (Senanayake *et al.*, 2006). Neonatal enteral feeding tubes have been colonised by *E. vulneris*, representing an important risk factor for infection in neonates (Hurrell *et al.*, 2009). There appears to be only one reference in the literature to isolation of *E. vulneris* from flying insects and this was a case involving the lesser dung fly *Coproica hirtula* (Kobayashi *et al.*, 1990). In this case, *C. hirtula* which were positive for *E. vulneris* dropped into the raw material used to make a latex eyelid cosmetic and contaminated it with *E. vulneris*, while the other eyelid cosmetics that did not come into contact with these flies remained sterile (Kobayashi *et al.*, 1990). It was postulated that the flies were emerging from an open sink-hole in the floor of the cosmetic production area (Kobayashi *et al.*, 1990). Based on the evidence in Kobayashi *et al.* (1990), it is feasible therefore that *E. vulneris* contaminated *M. autumnalis* in hospitals could contaminate sterile hospital equipment by contact, thus presenting a significant risk to health.

Isolation of Enterobacteriaceae from *M. autumnalis* is to be expected, due to the association of this species with animal dung, which is likely to be the original source of bacterial contamination. Horse/mule manure has been identified as a source of *E. vulneris* (Derlet and Carlson, 2002) and this could be a site of bacterial acquisition by *M. autumnalis* as the adult flies feed on eye secretions of horses (Krafsur and Moon, 1997) so it is possible that they may contact horse manure.

The significance of *Klebsiella pneumoniae* ssp *pneumoniae* and *Raoultella terrigena* has already been discussed.

Staphylococcus aureus was isolated from *M. autumnalis* sampled from a hospital café and restaurant kitchens. *Staphylococcus aureus*, of the Staphylococcaceae, are Gram-positive, aerobic and facultatively anaerobic, non-motile, size-variable cocci in pairs and clusters (Cowan *et al.*, 2003), isolated extensively from both the hospital and community settings, where Methicillin-resistant *S. aureus* (MRSA) infection affects approximately 150,000 people per year in the European Union (Kock *et al.*, 2010). The literature regarding MRSA infection in hospital and community settings is extensive and review articles refer to cases of infection in the bloodstream, skin, urinary tract and respiratory tract, which can be spread nosocomially, via contaminated food and even by contact with livestock (Kock *et al.*, 2010). *S. aureus* infection is prevented and controlled by surveillance, antibiotic stewardship, hand-washing and other hygiene measures, patient isolation and other general principles of infection management (Coia *et al.*, 2006). MRSA has been isolated from houseflies *M. domestica* sampled from a hospital in Libya (Rahuma *et al.*, 2005) and a hospital in Senegal, where the isolate had a sensitivity profile and phenotype of resistance identical to patients, suggesting that the flies had a role in the dissemination of this pathogen (Boulesteix *et al.*, 2005). Housefly *M. domestica* larvae have also been shown to carry *S. aureus* in their gut and on their external surfaces (Banjo *et al.*, 2005).

S. saprophyticus was isolated from autumn flies *M. autumnalis* sampled from a hospital café and restaurant kitchens. *Staphylococcus saprophyticus*, of the Staphylococcaceae, are Gram-positive, aerobic and facultatively anaerobic, non-motile, size-variable cocci in pairs and clusters (Cowan *et al.*, 2003), typically isolated from the urinary tract of young women (Gillespie *et al.*, 1978). *S. saprophyticus* infection has also been reported in the bloodstream and all isolates were oxacillin resistant (Marshall *et al.*, 1998). There are no records in the literature of *S. saprophyticus* isolation from insects in hospitals, so this study represents a first case of this. Cases of *S. saprophyticus* isolation from insects sampled from non-hospital sites are; the bedbug *C. lectularius* abdomen (Reinhardt, 2005), larvae of the imported fire ant *Solenopsis invicta* (Peloquin and Greenberg, 2003), harlequin ladybirds *Harmonia axyridis* (Moon *et al.*, 2011), south American fire ants *Solenopsis saevissima*, ghost ants *Tapinoma melanocephalum* (Pesquero *et al.*, 2012) houseflies *M. domestica*

from around restaurant bins (Butler *et al.*, 2010), greenbottle flies *Lucilia cuprina* and flesh flies *Sarcophaga haemorrhoidalis* (Habeeb and Mahdi, 2012).

Following on from discussing isolation of bacteria from the casual intruder *M. autumnalis*, other casual intruder insects in this study were also found to be carrying bacteria.

To the author's knowledge, this study also provides the first example of the isolation of *Bacillus pumilus* and *Pantoea* sp from Dolichopodidae, *Staphylococcus aureus* from *Phaonia* sp, *Bacillus lentus* from *Helina* sp and *Bacillus megaterium* from *Hypoponera punctatissima.*

Bacillus pumilus was isolated from dolichopodid flies (family Dolichopodidae) sampled from a neonatal ward. The significance of *B. pumilus* has already been discussed.

Pantoea sp was isolated from dolichopodid flies of the family Dolichopodidae sampled from a neonatal intensive care unit. The significance of *Pantoea* sp has already been discussed.

Flies of the family Dolichopodidae are associated with damp habitats, grass, herbaceous vegetation, some species rest on floating vegetation and several genera are found at the seashore within seaweed (Chinery, 2012, Richards and Davies, 1977). Dolichopodid larvae have been found developing in rotten wood, the humus component of soil (decayed plant or animal matter constituent of soil) and some in aquatic habitats (Richards and Davies, 1977). The isolation of *Pantoea* sp from Dolichopodidae, as reported in this study, can be explained by the biology and habits of these flies i.e. their aforementioned association with vegetation, a known source of *Pantoea* sp (Cruz *et al.*, 2007). The adult flies predate other insects (Chinery, 1993) and also feed on nectar (Richards and Davies, 1977). Dolichopodid flies are small, bristly, long-legged flies, which typically a metallic green / blue-green or bronze in colour and male genitalia are particular prominent (Chinery, 2012). There are approximately 250 species of Dolichopodidae in Britain, of which *Dolichopus popularis* is the most common.

Staphylococcus aureus was isolated from *Phaonia* sp sampled from a hospital cooked food store. The significance of *S. aureus* has already been discussed.

Phaonia spp of the family Muscidae develop in decaying matter and their larvae can be carnivorous, feeding on other Dipteran larvae (Richards and Davies, 1977). They can also develop in decaying fungi and faeces (Colyer and Hammond, 1951). Pathogenic bacteria have been isolated from these flies (Forster *et al.*, 2007). Adults can be found basking in wooded areas and on fences and these large flies are recognised mainly by the 4th long wing vein being almost straight (Chinery, 2012).

Bacillus lentus was isolated from *Helina* sp sampled from a hospital café. *Helina* spp of the family Muscidae bask in wooded and marshy areas and can be recognised by the 4th long wing vein, which curves gently backwards (Chinery, 2012). A number of varieties of *E. coli* have been isolated from *Helina* sp (Greenberg, 1971).

Bacillus lentus is part of a bacterial series or spectrum with *Bacillus firmus* and it is recommended that it should be assigned to the same species and be referred to as *B. firmus (Gordon et al., 1977)*. *B. lentus* is a Gram-positive, aerobic, spore-forming (oval shaped spores, centrally positioned), rod-shaped, motile bacterium (Cowan *et al.*, 2003). *B. lentus* has primarily been isolated from soil samples and foodstuffs and is not typically classed as a human pathogen although there has been a case where vancomycin-resistant *B. lentus* was detected in a blood sample taken from a neonate with sepsis (Moodley, 2006). *B. lentus* is also known to cause infection in plants, causing lysis of the structural component pectin in the cell walls of bean leaves. The industrial significance of *B. lentus* is great and it is used in the commercial production of an alkaline protease (Jorgensen *et al.*, 2000).
Insect associations with *B. lentus* seem to be rare – there is a report of this microorganism being isolated from the frass of leek moth *Acrolepiopsis assectella* larvae when they were reared on an artificial laboratory diet (Thibout *et al.*, 1995).

B. megaterium was isolated from winged Queen Roger's ants *Hypoponera punctatissima* sampled from a neonatal unit in a hospital. As well as being the first case of *B. megaterium* isolation from *H. punctatissima* this was also the first from flying insects in hospitals. *Bacillus megaterium* is a Gram-positive, aerobic, spore-forming (oval shaped spores, centrally positioned), rod-shaped, motile bacterium (Cowan *et al.*, 2003) that is isolated from soil (Shiva Reddy, 2010) and honey (Lopez and Alippi, 2010). *B. megaterium* has been isolated from a blood culture of a woman who had an ovarian cyst and has also been implicated in cases of meningitis, abscess of the brain and blood infection due to catheter use (Dib *et al.*, 2003). Although it has not previously been isolated from flying insects in hospitals, reports of *B. megaterium* from insects include isolation from the oriental cockroach *Blatta orientalis* sampled from various sites of a hospital such as kitchens and boiler room (Burgess *et al.*, 1973), the greater wax moth *G. mellonella* in 2-4% of sampled larvae (Bucher and Williams, 1967), the gut of the adult worker honey bee, queen and larval faeces of *A. mellifera* (Gilliam, 1997), whitefly *Bemisia argentifolii* (Davidson *et al.*, 2000), from the crop of larvae of the ant lion *Myrmeleon bore* (Nishiwaki *et al.*, 2007), oral secretions of bark beetles of the subfamily Scolytinae (Cardoza *et al.*, 2009) and from the mountain pine beetle *Dendroctonus ponderosae* (Winder *et al.*, 2010).

Drain flies

The majority of bacterial isolates taken from drain flies sampled from hospitals were *Bacillus* spp, followed by Staphylococci, Clostridia and *Micrococcus* sp.

There was a significant association between synanthropy and carriage of certain bacterial groups, specifically that there was an association of flying insects being non-synanthropic (casual intruders and drain flies in this case) and carrying non-Enterobacteriaceae. This can be explained by the general biology of this group of flying insects, in that unlike synanthropic flies, casual intruders do not particularly frequent unsanitary areas such as drains, carcasses or animal faeces that provide a source of Enterobacteriaceae. 'Drain flies', despite their commonly used name in pest control circles, are capable of developing in breeding material other than faecal matter of drainage systems which provides an obvious and typical source of Enterobacteriaceae. Examples of other breeding matter for drain flies include fungi, leaves, fruit, vegetables, fermenting alcoholic beverages, bird nests, all of which are not obvious and typical sources of Enterobacteriaceae but can be sources of bacteria from other groups.

In terms of practical advice arising from this finding, when hospital outbreaks of non-Enterobacteriaceae (e.g. *Bacillus* spp, *Clostridium* spp, *Staphylococcus* spp) occur and flying insects are suspected as a source, control efforts should be focused on drain flies and casual intruders as the most likely fly source of such bacteria, rather than other fly species.

To the author's knowledge, this study provides the first example of isolation of *Bacillus cereus* Group and *Staphylococcus aureus* from Psychodidae, *Bacillus cereus* Group and *Bacillus sphaericus* from Phoridae, *Bacillus cereus* Group and *Clostridium clostridioforme* from Sphaeroceridae, *Bacillus licheniformis* and *Staphylococcus aureus* from *Trichiaspis* sp (family Sphaeroceridae) and *Bacillus pumilus* from *Drosophila* sp.

Bacillus cereus Group (non-*Bacillus thuringiensis*) was isolated from Psychodidae sampled from a hospital restaurant. Bacteria in the *B. cereus* Group are Gram-positive / Gram-variable, aerobic and facultatively anaerobic, spore-forming (oval shaped spores, centrally positioned), rod-shaped, motile organisms (Cowan *et al.*, 2003). Members of the *B. cereus* Group are *Bacillus anthracis* the causative agent of Anthrax, *Bacillus cereus* which is isolated from soil and causes food poisoning, *Bacillus thuringiensis* the insect pathogen used in pest control (which has also been isolated in infected burns and cases of gastroenteritis), *Bacillus mycoides* and *Bacillus weihenstephanensis* (Priest *et al.*, 2004). *B. mycoides* has been isolated from soil, water and plant matter, acts as a fungicide (EPA, 2009) has been cultured from the eye lens in humans in a case of endopthalmitis (Ansell *et al.*, 1980) and been identified in an outbreak of food poisoning (McIntyre *et al.*, 2008). *B. weihenstephanensis* presents a

potential health risk in terms of food poisoning and some strains possess the toxin that causes vomiting (Thorsen *et al.*, 2006). *B cereus* has been reported to cause infection of neontates, particularly the central nervous system, bloodstream and lungs (Hilliard *et al.*, 2003). There is one case of *B. cereus* isolation from flies in hospitals, which is from the moth fly *C. albipunctata* collected from shower cubicles, patient wards, rest rooms and kitchens of a German hospital (Faulde and Spiesberger, 2013).

B. sphaericus was isolated from phorid flies, family Phoridae, that were collected from the main kitchen of a hospital. As well as being the first case of *B. sphaericus* isolation from Phoridae, this was also the first from flying insects in hospitals. *Bacillus sphaericus* is a Gram-positive (in young cultures - inconstant in older cultures), aerobic, spore-forming (round shaped spores, terminally positioned), rod-shaped, motile bacterium (Cowan *et al.*, 2003) and is isolated from soil (Massie *et al.*, 1985).
B. sphaericus has been reported in blood cultures of cancer patients (Banerjee *et al.*, 1988) and has produced lesions when injected into the brain or eye (Drobniewski, 1993). Other conditions of clinical significance in humans which are associated with *B. sphaericus* infection include pericarditis, pleuritis, peritonitis, bacteraemia, meningitis, endocarditis and pseudotumour of the lung (Drobniewski, 1993). *B. sphaericus* infection has also caused bacteraemia in immunocompromised children with cancer, cases of which were treated successfully with ciprofloxacin (Castagnola *et al.*, 2001). *B. sphaericus* exhibits toxicity to *Culex* spp and *Anopheles* spp mosquito larvae, has shown potential as a mosquito larvicide (Charles *et al.*, 1996) and is now listed as a biopesticide (EPA, 1999). Although it has not previously been isolated from flying insects in hospitals, reports of *B. sphaericus* from insects include isolation from adult blackflies *Simulium damnosum* (Weiser, 1984), frass of feral honey bees *A. mellifera* and frass of greater wax moth larvae, *G. mellonella* (Gilliam, 1985), from the crop of larvae of the ant lion *Myrmeleon bore* (Nishiwaki *et al.*, 2007) and the spruce bark beetle *Ips typographus* (Muratoğlu *et al.*, 2011).

C. clostridioforme was isolated from lesser dung flies of the family Sphaeroceridae collected from a neonatal and maternity wing of a hospital. The significance of this bacterium has already been discussed and is added to here. *C. clostridioforme* infection has been identified in human peritoneal fluid (Appelbaum *et al.*, 1994), blood cultures, intra-abdominal infections, soft tissue infection (Alexander *et al.*, 1995), as well as from cases of osteomyelitis, liver abscess, subgingival area and in diabetic foot infections (Warren *et al.*, 2006). *Clostridium hathewayi*, a newly described species which is a member of the *C. clostridioforme* group and previously referred to as *C. clostridioforme* has caused bacteraemia in a patient with acute appendicitis (Woo *et al.*, 2004). The likely route of contamination of Sphaeroceridae with *C. clostridioforme* is apparent from their common name ‘lesser dung flies’, as they develop in dung (Chinery, 1993) and *C. clostridioforme* has been isolated from human faeces (Finegold *et al.*, 2005), suggesting that these flies had developed in hospital drains.

B. licheniformis was isolated from lesser dung flies *Trichiaspis* sp (family Sphaeroceridae) from a café at the entrance of a hospital. The significance of *B. licheniformis* has already been discussed but it is added to here, specifically in relation to Sphaeroceridae. *B. licheniformis* has been isolated from the plumage of 39% of wild birds (Whitaker *et al.*, 2005) and is capable of degrading bird feathers (Rozs *et al.*, 2001). It is possible that the lesser dung flies *Trichiaspis* sp examined in this study acquired *B. licheniformis* from contaminated bird feathers as flies of the family Sphaeroceridae have been recorded in the nests of birds (Laurence, 1955) .

Colony counts / CFUs of bacterial species isolated from different fly groups

The data on colony counts show that on average, the bacterial load of *M. domestica* was among the highest carried in this study (Table 7.6). This finding shows that although *M. domestica* had only the sixth most diverse bacterial population (based on E_D), was not the most common synanthropic fly in hospitals and only accounted for 0.9% of sampled Diptera, it is clearly still of significance to public health, due its status in this study as a carrier of some of the highest bacterial loads on average and its ability to transfer *C. difficile*.

Occurrences of bacteria isolated internally vs occurrences of bacteria isolated externally, for all hospital flying insects.

There were significantly more occurrences of bacteria isolated internally than occurrences of bacteria isolated externally from flying insects in hospitals. Therefore, risk of bacterial contamination by flies may be lower by direct contact of their external surfaces, compared to dissemination of bacteria from their internal structures via defecation and regurgitation of pathogens when feeding. In contrast with this finding, bacteria were isolated internally and externally from laboratory reared / insectary-supplied adult *M. domestica* in every case and occurrences were equal. The difference in these observations can be explained by a number of factors, including the germicidal action of sunlight, insect flight and grooming behaviour, plus the limitations of the sampling method of using UV light flytraps.

As flies in this study were sampled with UV light flytraps, they could have been dead for a number of weeks and were therefore exposed to environmental conditions such as sunlight, which is known to have a germicidal effect. This exposure could have reduced the numbers of bacteria on external surfaces of flies captured in the UV light flytraps, which is a limitation of this study compared to sampling live flies. For example, a steady decline in numbers of *Serratia marcescens* associated with *M. domestica* captured in EFKs has been reported (Cooke *et al.*, 2003). It should be noted however, that in all of the sampling visits made by the author, only one instance of live flies was noted and if

activity of live flies had been more widespread, the sampling of live flies would have been the technique of choice for this study.

It is also possible that the flies were carrying a reduced amount or no bacteria externally at the point of capture. Flight behaviour of *M. domestica* has been shown to reduce the quantity of *Vibrio cholerae* on fly wings (Yap *et al.*, 2008), which are an external structure and insect grooming is known to be a mechanism of defence against infection (Zhukovskaya *et al.*, 2013).

The survival of bacteria internally can be explained by fly anatomy, which provides a number of sites for bacterial harbourage. The alimentary canal (inside the peritrophic membrane) and the crop of *M. domestica* have been shown to harbour *E. coli* O157: H7 (Kobayashi *et al.*, 1999). Bacteria have been isolated from the internal structures of flies, specifically *Salmonella* serovar Enteritidis from *M. domestica* gut in all cases and the crop seldom (Holt *et al.*, 2007) and *E. coli* O157: H7 from the crop of *M. domestica* (Sasaki *et al.*, 2000). Although survival of bacteria in the internal structures of flies may be aided due to protection from factors affecting external bacteria, such as germicidal sunlight and grooming, the processes of the insect immune system must still be survived.

Occurrences of isolation of bacteria from different fly groups

Occurrences of isolation of bacteria were significantly greater in *M. domestica* compared to casual intruders. Furthermore, occurrences of isolation of bacteria were nearly significantly greater in domestic / filth flies versus casual intruders and between drain flies and casual intruders. These findings are of no surprise and can be explained quite simply by the biology of these insects. To quote a passage of the introduction to this work (section 1.1) regarding *M. domestica*, 'It has a propensity to breed in faecal matter and moves indiscriminately from filth to food. In addition, there are many studies which show that houseflies harbour pathogenic bacteria obtained from various unsanitary sources'. Unlike synanthropic flies, the general biology of the casual intruder group of flying insects is such that they do not often frequent unsanitary areas such as drains, carcasses or animal faeces that provide a rich and diverse source of bacterial contamination.

7.5 CONCLUSION

Interpretation of the results of the microbiological analysis of flying insects collected from hospitals revealed that a variety of flying insects, including synanthropic flies (e.g. *M. domestica* and *C. vicina*) collected from UK hospitals do indeed harbour pathogenic bacteria of different species. It was discovered that flying insects in UK hospitals are more likely to be carrying Enterobacteriaceae than other groups of bacteria and *Bacillus* spp, Staphylococci, Clostridia, Streptococci and *Micrococcus* spp were also isolated. In some cases, the levels of bacteria carried by flying insects were enough to provide an infective dose to humans. Flying insects harbouring bacteria were collected from a number of locations throughout hospitals, including areas where food for patient, visitor or staff consumption is prepared or stored, such as hospital catering areas, ward kitchens and food stores. Flying insects carrying bacteria were also found in wards, neonatal units and maternity units.

C. difficile was not isolated from flying insects sampled from UK hospitals. However, many of the identified species of bacteria were pathogenic and therefore of public health significance, with a number of species being recovered for the first ever time from their insect host. Of particular interest were certain serotypes of *E. coli*, which were isolated from flies. A clinical serotype of *E. coli* was isolated from *C. vicina* and *E. coli* serotype O71 was isolated from *L. sericata* and is not known as a clinical isolate but is found in calf faeces. Recommendations are made in light of the isolation of these serotypes of *E. coli* from flies in hospitals.

Statisitical analysis yielded important findings, one of which was a significant association of flying insects being non-synanthropic (casual intruders and drain flies in this case) and carrying non-Enterobacteriaceae. There was also a significant association between synanthropy and bacterial diversity, that carrying a single species type or no bacterial load is associated with casual intruder flying insect species. There were significantly more occurrences of bacteria isolated internally than occurrences of bacteria isolated externally from flying insects in hospitals. Finally, occurrences of isolation of bacteria were significantly greater in *M. domestica* compared to casual intruders and the average bacterial load of *M. domestica* was among the highest carried in this study.

Recommendations based on these findings are many. Recommendations relevant to the location of insects in hospitals are made first. The presence of flying insects in the described food preparation areas presents a risk of contamination of foodstuffs with bacteria and thus a risk of human infection via consumption of the food, therefore fly control in these areas should be a priority, due to the special risks posed. In wards, neonatal units and maternity units, the risk of contamination and therefore human infection is different in these areas, as the most likely routes of infection are via fly-

contaminated environment such as surfaces and fomites, so fly control should be prioritised in these areas and regular disinfection and cleaning of fly-alighting surfaces is recommended alongside this.

Although *C. difficile* was not isolated from flies in hospitals in this study, this will probably change if further work is undertaken, due to the following reasoning. It is still expected that flies will be found to carry *C. difficile* in hospitals in future studies and they should be treated as potential vectors, based on evidence from the results of the laboratory studies in this work, isolation of *C. difficile* from flies on farms (Burt *et al.*, 2012) and the fact that bacteria of the same genus were isolated in this study. Although this study focused on carriage of *C. difficile* by *M. domestica*, consideration should be given to *C. vicina* and *L. sericata* as likely candidates for *C. difficile* carriage, due to the fact that this bacterium has been isolated from rodents (Himsworth *et al.*, 2014) and birds (Bandelj *et al.*, 2014), the carcasses of which are breeding / development media (Erzinclioglu, 1996) and a source of bacterial contamination for such flies.

This microbiological study adds to the entomological study by providing pest control and infection control staff with knowledge of the species of bacteria which flying insects are likely to be carrying in UK hospitals, giving a clearer picture of the public health significance of such insects. Due to the fact that many species of bacteria were isolated for the first time from their insect host, infection control measures for dealing with outbreaks of these pathogens should consider pest control as an important component, which may not have been the case previously because of a lack of evidence of particular insect-bacteria associations. It is not just the records of species of bacteria isolated from insects that are of interest. The bacterial loads were also analysed and add to the understanding of the public health significance of insect-bacteria associations in hospitals. For example, although found infrequently in hospitals, *M. domestica* still has public health significance, due its status in this study as a carrier of some of the highest bacterial loads on average. In addition to the described identification and enumeration of bacteria isolated from flies, isolates of *E. coli* were identified further, to serotype level, thus providing an extra level of understanding of the public health significance of flies as carriers of bacteria in hospitals. The clinical strain of *E. coli* isolated from *C. vicina* was likely to have been acquired from the hospital environment, so flies should be considered as a route of spread of clinical isolates of bacteria in hospitals. It is likely that *L. sericata* had acquired *E. coli* O71 from calf faeces and then entered the hospital, illustrating perfectly the dangers of fly ingress and capacity for introduction of non-clinical isolates into the hospital environment where they may prove pathogenic in humans, which leads to the recommendation that fly-proofing measures should be an essential feature in hospitals. To add to the information regarding the enumeration, isolation and serotyping of bacteria isolated from flies in hospitals, the external and internal carriage of such microorganisms by flies was also examined. The fact that there were significantly more occurrences of bacteria isolated internally than occurrences of bacteria isolated externally from flying insects in hospitals leads to the interpretation that the risk of bacterial contamination by flies may be

lower by direct contact of their external surfaces, compared to dissemination of bacteria from their internal structures via defecation and regurgitation of pathogens when feeding.

When hospital outbreaks of non-Enterobacteriaceae (e.g. *Bacillus* spp, *Clostridium* spp, *Staphylococcus* spp) occur and flying insects are suspected as a source, control efforts should be focused on drain flies and casual intruders as the most likely fly source of such bacteria, rather than other fly species. It is this knowledge of the occurrences of isolation of bacteria from certain fly species and groups and awareness of associations between bacterial diversity and insects that should inform the prioritisation of pest control measures.

Based on the findings of this study, it is clear that flying insects must be included in future editions of the NHS conditions of contract for pest control.

8 CHAPTER 8: GENERAL DISCUSSION

C. difficile is the leading cause of nosocomial diarrhoea worldwide, with serious implications in that it can result in the isolation of patients, closure of wards and hospitals and even the death of infected individuals (Dawson *et al.*, 2009). *C. difficile* infection (CDI) typically affects elderly patients on antibiotics, causing severe disease such as pseudomembranous colitis (PMC) via toxins that affect intestinal cells (Schroeder, 2005), with infections contributing to deaths in England and Wales that have peaked at over 8,000 per annum (ONS, 2013). Spores are the main transmissible form of *C. difficile* and can persist in the environment for a long period of time (Dawson *et al.*, 2009). The spores are resistant to most disinfectants and alcohol hand gels (HPA, 2009), so sporicidal agents such as bleach are required to eliminate them from the environment (Wheeldon *et al.*, 2008b).

C. difficile can be excreted by a human patient at levels of 1×10^4 to 1×10^7 per gram of faeces (Mulligan *et al.*, 1979) and adult *Musca domestica* are attracted to, land on, feed on and oviposit on human faeces, upon which the resulting larvae feed and develop (West, 1951). It is well known that *M. domestica* visit faeces then become contaminated with bacteria, which they disseminate (Greenberg, 1964) and this process is likely to occur with *C. difficile* and result in mechanical transmission of this pathogen. Indeed, *C. difficile* has been isolated from fly species, which were collected on pig farms (Burt *et al.*, 2012) and this supports the assertion that *M. domestica* could become contaminated with *C. difficile* by interacting with 'infected' faecal matter and that *M. domestica* is an, as yet, unconsidered factor in the spread of *C. difficile* in the hospital setting.

Previous studies investigating *M. domestica* sampled from hospitals have shown that the flies which were collected harboured pathogenic bacteria, including *Bacillus* spp from hospitals in Nigeria, (Adeyemi and Dipeolu, 1984), *Escherichia coli* (Fotedar *et al.*, 1992b) and *Klebsiella pneumoniae* (Fotedar *et al.*, 1992a) from a hospital in New Delhi, India, Methicillin resistant *Staphylococcus aureus* (MRSA) from a hospital in Libya (Rahuma *et al.*, 2005), MRSA from a hospital in Senegal (Boulesteix *et al.*, 2005) and *Salmonella* sp from a hospital in Nigeria (Nmorsi *et al.*, 2007). Apart from work on *M. domestica*, little research has been done on the bacteria associated with other fly species that are found in hospitals. Fruit flies, *Drosophila* sp sampled from a hospital in Nigeria were found to harbour *Proteus* sp, *Streptococcus* sp and *Salmonella* sp (Nmorsi *et al.*, 2007), cluster flies, *Pollenia rudis* sampled from a hospital in Germany were found to harbour opportunistic pathogens such as *Pantoea* spp (Faulde *et al.*, 2001) and *C. albipunctata* was positive for many species of Enterobacteriaceae (Faulde and Spiesberger, 2013).

The main aim of this thesis was to isolate and characterise bacteria associated with flying insects in hospitals, with particular emphasis on *C. difficile*. Initial laboratory experiments were undertaken in order to assess the potential for mechanical transfer of *C. difficile* by the housefly *M. domestica*, including examination of external and internal isolation, ingestion, deposition via excreta and survival through fly life stages, all after experimental exposure to bacterial suspensions. Results confirmed that low numbers of viable *C. difficile* spores (still enough to provide an infective dose to humans) were carried externally and internally by *M. domestica*, were ingested (as proven by isolation from the alimentary canal) and were isolated from excreta. Although carried externally and internally by *M. domestica* larvae, *C. difficile* did not survive through the development of further life stages. Following on from these experiments, *C. difficile* was successfully isolated from *M. domestica* that had been electrocuted in an EFK in a laboratory setting. These laboratory results showed that *M. domestica* have vector potential for *C. difficile* and that it was viable to use UV light flytraps to sample flying insects from hospitals in order to undertake a microbiological study, screening for carriage of *C. difficile* as well as other species of bacteria. A further aim of this thesis was to undertake an entomological study and identify the species of flying insects associated with hospitals, by analysing a pre-existing entomological database and by way of field sampling, in order to better inform pest control and therefore infection control measures.

A main conclusion of this work was that adult *M. domestica* in hospitals should be viewed as vectors of *C. difficile*. Although *C. difficile* was not isolated from hospital sampled *M. domestica*, bacteria of the same genus were, while the ability of *M. domestica* to transfer this organism mechanically has been shown in the laboratory studies in this thesis and they have also been shown to carry *C. difficile* in practical settings (Burt *et al.*, 2012). *M. domestica* also have potential to introduce novel strains of *C. difficile* into the clinical setting, due to their association with strains that are typically associated with livestock, such as O78 (Burt *et al.*, 2012) and their ingress into hospitals from neighbouring farm premises. Although the following example refers to different organisms, the principles are still relevant to the argument for the transfer of *C. difficile* by *M. domestica* in hospitals, in that flying insects can acquire clinical isolates of bacteria from the hospital environment. For example, this study showed that a clinical serotype of *E. coli* was isolated from *C. vicina* and it was likely that this was acquired from the hospital environment, so flies should be considered as a route of spread of clinical isolates of bacteria in hospitals. Adding to the argument that *M. domestica* has potential to introduce novel strains of *C. difficile* into the clinical setting, the principles behind another key finding are important, even though different organisms are involved. The key finding was the isolation of *E. coli* serotype O71 from *L. sericata*, which is not known as a clinical isolate but is found in calf faeces. It is likely that *L. sericata* had acquired *E. coli* O71 from calf faeces and then entered the hospital, illustrating perfectly the dangers of fly ingress and capacity for introduction of non-clinical isolates into the hospital environment where they may prove pathogenic in humans, which leads to the

recommendation that well-maintained fly-proofing measures should be an essential feature in hospitals.

Despite the establishment of *M. domestica* as known vectors of *C. difficile*, the numbers of such flies in hospitals were actually surprisingly low in this study. The microbiological field study showed that even though *M. domestica* were low in numbers, they were important in terms of bacterial carriage when they were encountered. For example, occurrences of isolation of bacteria were significantly greater in *M. domestica* compared to casual intruders and the bacterial load was among the highest carried in this study. *M. domestica* aside, other known insect vectors of *C. difficile* were present, which were Psychodidae, *F. canicularis* and *Drosophila* sp. In contrast with *M. domestica*, 'drain flies' were surprisingly numerous and represent an emerging problem in hospitals. The family Psychodidae were the most common of the 'drain flies' and were therefore the most important known insect vector of *C. difficile* present in hospitals. In fact, interpretation of the KCIIS data regarding flying insects revealed that 'drain flies' were the flying insect group of greatest importance in UK hospitals in terms of abundance, as well as being present throughout the year. The significance of these findings are that the numerous 'drain flies', especially those with vector potential for *C. difficile*, should be at the forefront of the education of pest controllers and hospital staff, with control measures being tailored more specifically towards this group of flies. It follows that repair of drainage faults and scrupulous hygiene should be a priority in order to limit the activity of this group of flies and therefore minimise the risk to public health. Furthermore, UV light flytraps (professional sticky traps only, due to release of bacteria from flies electrocuted by EFKs) should be used throughout hospitals in order to protect public health and the contents of these should be identified routinely to inform pest control and infection control measures. Awareness also needs to be raised regarding fly identification, sources / breeding media, public health significance and control measures. In terms of predicting which other flying insect species will be important in the transfer of *C. difficile* in hospitals, consideration should be given to *C. vicina* (the most common synanthropic fly in this study) and *L. sericata*. They are likely candidates for *C. difficile* carriage, due to the fact that this bacterium has been isolated from rodents (Himsworth *et al.*, 2014) and birds (Bandelj *et al.*, 2014), the carcasses of which are breeding / development media (Erzinclioglu, 1996) and a source of bacterial contamination for such flies.

Regarding the flying insect species found associated with hospitals in this thesis a major finding was that non-biting midges of the family Chironomidae, which are known to carry *V. cholerae* (Broza *et al.*, 2005), were the most numerous flying insect in the field study of hospitals, peaking in August according to the KCIIS data. The presence of Chironomidae highlights proofing deficiencies, as these flies typically breed outdoors before entering buildings. Based on the experience of the author, it is probably a fair approximation to comment that most pest control operators and infection control staff

would not highlight Chironomidae as the most numerous fly in hospitals or 'drain flies' as the main emerging fly problem, so these flies should feature as a key component of education for such staff.

A key finding of the microbiological study was that flying insects were more likely to be carrying Enterobacteriaceae than any other group of bacteria. *Bacillus* spp, Staphylococci, Clostridia, Streptococci and *Micrococcus* spp were also isolated. There are numerous reports in the scientific literature regarding isolation of bacteria from flies. What are important in this study are the practical relevance of these findings to pest control and infection control measures and an appreciation of the risks to public health, which then informs recommendations regarding the control of flying insects. Regarding the risks to public health, many of the identified species of bacteria were pathogenic and therefore of public health significance, with a number of species being recovered for the first ever time from their insect host, presenting novel and previously unconsidered risks to health. As an example scenario, an outbreak of a particular pathogen may not have been linked to the presence of flying insects in the past, as no prior evidence of insect associations with that particular microorganism existed. The findings of this study therefore add to the list of insect-bacteria associations observed. Adding further to the risks to public health, the levels of bacteria carried by flying insects were enough to provide an infective dose to humans in some cases. The findings described in this thesis also act to inform infection control and pest control staff of the insect species which should be controlled when outbreaks of certain pathogens take place, as well as judging levels of risk. For example, there was a significant association of flying insects being non-synanthropic (casual intruders and drain flies in this case) and carrying non-Enterobacteriaceae. When hospital outbreaks of non-Enterobacteriaceae (e.g. *Bacillus* spp, *Clostridium* spp, *Staphylococcus* spp) occur and flying insects are suspected as a source, control efforts should therefore be focused on drain flies and casual intruders as the most likely fly source of such bacteria, rather than other fly species. In terms of judging risks posed by certain flying insect species, there was a significant association between synanthropy and bacterial diversity, that carrying a single species type or no bacterial load is associated with casual intruder flying insect species.

Comparing this work to previous studies, authors tend to report on the bacterial associations of flying insects without making thorough practical recommendations relevant to fly control. This study is probably unique in that it presents entomological and microbiological findings from experimental work and places these results in context of industrial knowledge, therefore being able to make practical recommendations regarding fly control as a component of infection control. In addition to the fly control recommendations already made, fly control measures should focus on food preparation areas of hospitals, which is where flies were most frequently reported. Hospital buildings should be adequately proofed against fly entry, by installing and maintaining flyscreens. Existing proofing deficiencies were highlighted in hospitals by the presence of 'casual intruders' in great numbers, such as Chironomidae, so it is imperative that this is rectified.

It is important to recognise a limitation of this study, which is that the recommendations given are based on findings from a particular period of time from a certain number of hospitals and sites that were not covered in this study may have their own unique problems. Furthermore, flying insect populations and their associated bacterial fauna could be quite different in hospitals in future years. It is for this reason that appropriate selection, use and replenishment of insect monitoring systems such as UV light flytraps should be ongoing in hospitals, in order to inform control measures over time, rather than rely on a snapshot of information. However, pest control and infection control staff should still use the data in this study, to guide their work in the first instance. Expert entomologists should be consulted when assistance is required in identifying insects and designing control strategies in hospitals, as this is often a specialist job. In terms of designing control strategies, it is clear from the findings of this study that flying insects must be included in future editions of the NHS conditions of contract for pest control, due to their threat to public health.

Continuing with points relevant to the design of flying insect control strategies, reports of flies peaked in the summer months but they were also numerous in October and November with some species being present all year round. With this in mind, fly control is clearly not a summertime consideration alone and a year-round programme should be put in place. There are also specific areas within hospitals that are the most vulnerable and fly control measures should focus on 'treatment areas' of hospitals which is where flies were most frequently reported. Of course, these are not the only areas where fly control is important.The presence of flying insects in food preparation areas presents a risk of contamination of foodstuffs with bacteria and thus a risk of human infection via consumption of the food, therefore fly control in these areas should be a priority, due to the special risks posed. In wards, neonatal units and maternity units, the risk of contamination and therefore human infection is different in these areas, as the most likely routes of infection are probably via a fly-contaminated environment such as surfaces and fomites. Fly control should be prioritised in these areas and regular disinfection and cleaning of fly-alighting surfaces is recommended alongside this. Knowledge of the occurrences of isolation of bacteria from certain fly species and groups and awareness of associations between bacterial diversity and insects should inform the prioritisation of pest control measures, as monitoring and control measures can be targeted to the insect vector that is most likely to be harbouring the species of bacteria that could be involved in a current outbreak.

Opportunities for future work are numerous, justified and will prove beneficial. As this study identified 'drain flies' as an unexpected and emerging problem in hospitals, as well as identifying them as carriers of pathogen bacteria, it is recommended that future studies focus specifically on this group. Chironomidae were the most numerous flies sampled in this study, which became apparent after the bulk of the microbiological analysis had taken place, as the majority of the entomological work was completed after this. For these reasons, the importance of flies of the family Chironomidae was not fully appreciated initially and only one batch was examined microbiologically, with no

isolation of bacteria. This may not be the case following microbiological examination of further batches of Chironomidae, something which should be pursued in future research. It was noted that a number of crawling insects were reported in this study and their importance in terms of the carriage of pathogenic bacteria in UK hospitals is poorly understood and requires updating, the study of which could follow the structure of this thesis. Much has been said in this thesis about the problems of insects but they may in some cases prove to be beneficial. An interesting finding of this study was that *C. difficile* did not survive beyond the larval stage of *M. domestica*, perhaps indicating interactions with antimicrobial peptides which are known to exist in insects. The potential antimicrobial action of *M. domestica* larvae and their extracts against *C. difficile* should form the basis of a future study.

It has already been said but it bears repeating that the main practical piece of advice to come out of this study is that flying insects must be included in future editions of the NHS conditions of contract for pest control, due to their importance in hospitals and the special risks they pose to public health.

Even after a long history of the examination of bacterial associations of flies, the role of flies and the carriage of bacteria is an ever-changing and complex story, still to be understood fully. This is illustrated perfectly by the isolation of some species of bacteria from their insect hosts for the first time in this study. There is undoubtedly still a great deal to learn regarding the role of flying insects and their bacterial associations in terms of public health, pest control and infection control, the study of which by urban entomologists and microbiologists should continue.

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CONFERENCES ATTENDED

July 2014	8th International Conference on Urban Pests (ICUP). Zurich, Switzerland
October 2011	Pest Odyssey 2011: Ten years later. London, United Kingdom.
September 2011	Society of Vector Ecology (SOVE) annual conference. Flagstaff, Arizona, United States of America.
August 2011	7th International Conference on Urban Pests (ICUP). Ouro Preto, MG, Brazil.


7th International Conference on Urban Pests
Ouro Preto, Brazil
7th ICUP

CERTIFICATE

This is to certify that

Matthew Davies

attended the 7th International Conference on Urban Pests held in Ouro Preto, Brazil, August, 7-10, 2011.

August 7-10, 2011

Ana Eugênia de Carvalho Campos
Chairperson

8th ICUP
ZURICH 2014

Certificate

Matthew Davies

attended the 8th International Conference on Urban Pests
in Zurich, Switzerland, July 20 – 23, 2014

Dr. Gabi Müller
Chair of the Conference Organizing Committee

PUBLICATIONS

PUBLICATIONS ARISING FROM THIS WORK

Poster presentations and abstracts

Davies, M.P., Hilton, A.C., & Anderson, M., (2011). *Musca domestica* (Diptera: Muscidae) and the transfer of *Clostridium difficile*. IN: Robinson, W.H., & Campos, A.E.C. (ed.) *Proceedings of the Seventh International Conference on Urban Pests.* 7th. Ouro Preto. 7–10 August 2011. Sau Paulo, SP, Brazil: Instituto Biologico, 382.

7th International Conference on Urban Pests
Ouro Preto, Brazil

7th ICUP

CERTIFICATE

Certificate of Poster

This is to certify that the paper Musca domestica (Diptera: Muscidae) and the Transfer of Clostridium difficile , authored by M. Davies, A.C. Hilton, and M. Anderson was presented in an Poster session at the 7th International Conference on Urban Pests held in Ouro Preto, Brazil, August, 7-10, 2011

August 7-10, 2011

Ana Eugênia de Carvalho Campos
Chairperson

Musca domestica (Diptera: Muscidae) and the transfer of *Clostridium difficile*

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Introduction and purpose

C. difficile is a bacterial healthcare associated infection, which houseflies, *M. domestica*, may transfer due to their synanthropic nature. A clearer understanding of the role of flying insect vectors in the transfer of *C. difficile* will help inform infection control measures such as pest control.

Methods

M. domestica were exposed to 1x10^5 CFU (colony forming units) vegetative cell and spore suspensions of *C. difficile*. Flies were sampled onto selective agar plates immediately post-exposure and at 1 hour intervals, to assess mechanical transfer of *C. difficile*. Fly excreta were cultured and alimentary canals dissected to determine internalisation of cells / spores.

Results

M. domestica exposed to vegetative cell and spore suspensions of *C. difficile* were able to mechanically transfer the bacteria for up to 4 hours upon subsequent contact with surfaces. The most CFU's per fly were transferred immediately following exposure (mean CFU's 123.8 +/- 66.9 for vegetative cell suspension and 288.2 +/- 83.2 for spore suspension). After 1 hour this had reduced (21.2 +/- 11.4 for vegetative cell suspension and 19.9 +/- 9 for spore suspension). Mean *C. difficile* CFU's isolated from the *M. domestica* alimentary canal were 35 +/- 6.5; and per faecal spot were 1.04 +/-0.58. *C. difficile* could be recovered from fly excreta for 96 hours.

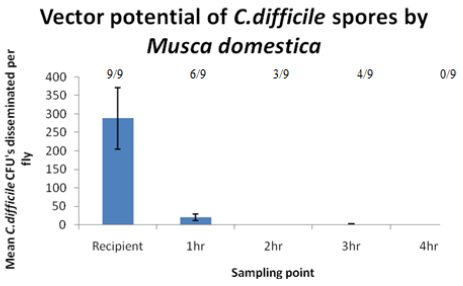

Figure 1. The mean number of *C.difficile* CFU's disseminated per fly (n=9), over time, after exposure to a 1 x 10^5 suspension of spores. Numbers above the columns are numbers of positive flies / number of flies tested.

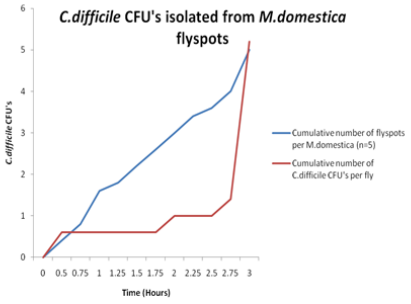

Figure 2. The cumulative number of faecal spots produced per fly (n=5) over a 3hr period and *C.difficile* CFU's isolated from the faecal spots, after flies were exposed to a 1 x 10^5 suspension of spores.

Conclusions

The significance of these data is that *M. domestica* may indeed harbour *C. difficile* for significant periods of time and transfer low numbers of spores in the healthcare environment, presenting an infection risk to susceptible individuals due to the low infective dose. This study highlights the potential for *M. domestica* to contribute to environmental persistence and spread of *C. difficile* and the need to consider pest control as part of infection control strategies.

Peer reviewed publications

Davies, M.P., Hilton, A.C., & Anderson, M., (2014). *Isolation and characterisation of bacteria associated with Musca domestica (Diptera: Muscidae) in hospitals*. IN: Müller, G., Pospischil, R., & Robinson, W.H., (ed.) *Proceedings of the Eighth International Conference on Urban Pests*. 8th. Zurich. 20–23 July 2014. OOK-Press, Veszprem, Hungary, 173-177.


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Conference presentations

'The housefly, *Musca domestica* (Diptera: Muscidae) as a mechanical vector of *Clostridium difficile*-associated diarrhoea in hospitals'.
Presented at: Society of Vector Ecology (SOVE) annual conference. Flagstaff, Arizona, United States of America. 25-29 September 2011.

Articles

'Dirty hands with wings: The importance of flying insect management as infection management in hospitals'. Pest Control News, Issue 85, October 2010. pp 18-19.

'Houseflies, Bacteria and Hospitals: Viewing Pest Control as Infection Control'. Pest Control News, Issue 100, September 2014. pp 18-19.

The importance of flying insect management as infection management in hospitals

DIRTY HANDS

A history lesson

The importance of flies in the transmission of disease should be well known by now, especially as it is backed up by over a hundred years of sound scientific evidence. A line of distinguished researchers, from Gordon-Hewitt and Graham Smith in the early part of the 20th century, through to Luther West in the 1950's, Bernard Greenberg in the 1960's & 70's and many others, have contributed to major advancements in the understanding of the public health significance of flies.

The culmination of this is the publication in 2008 of the World Health Organisation's *'Public Health Significance Of Urban Pests'*, a book that provides an excellent up-to-date coverage of flies and disease, freely available to a worldwide audience via the Internet.

"Even before the scientific evidence began to stack up, it was observed that increases in flies preceded disease outbreaks"

Even before the scientific evidence began to stack up, it was observed that increases in flies preceded disease outbreaks. Muffett wrote 'In the year 1348, great numbers of flies dropping out of the air, did cause in the Eastern Countreys incredible noisomness and putrefaction; upon which followed such a Plague among the people, that scarce the tenth man among them was left alive.' Circa 1577, Mercurialis (an Italian physician) observed famously: 'there can be no doubt that flies, saturated with the juice of the dead or of the diseased, then visit neighbouring houses and infect the food, and persons who eat of it are infected.'

Pest management as infection management in hospitals

Despite the rich history of research, the fact that flies can spread disease is still rarely understood or appreciated. It is possible that this lack of understanding results in fly control in UK hospitals being given a lower priority than it probably deserves. In some UK hospitals, pest control contracts are the responsibility of Facilities Management rather than Infection Control. Would it be more appropriate to view pest management as part of infection management? Furthermore, some authors refer to the problems of pest control in hospitals by using the phrases 'low priority', 'false economy' and even go on to describe how an estimated 'less than one fifth of one per cent of expenditure on domestic services in NHS hospitals is spent on pest control' and 'the priority afforded to pest control is often less than that afforded to window cleaning' (Robinson, 1988). It remains to be seen whether recent reports of proposed multi-million pound spending cuts by the UK government, in response to the global economic crisis, will affect the provision of pest control in the NHS.

The following summary of research regarding the role of flies in the transmission of hospital-associated bacteria may start to shift the mindset towards the view of 'pest management as infection management.'

Flies and disease in hospitals – the research

The majority of research on flies and disease is focused on the housefly *Musca domestica*, a fly that is found often in hospitals, especially during the summer months. Houseflies are associated closely with humans, found often indoors, cycle indiscriminately from filth to food and harbour pathogenic bacteria, therefore presenting a significant threat to public health (West, 1951; Greenberg, 1971; Greenberg, 1973). Houseflies can transmit such pathogenic bacteria mechanically, which is simple transfer from place to place, via external surfaces, regurgitation and defaecation (Lane and Crosskey, 1993). An enhanced form of mechanical transmission has been described, where bacteria replicate and persist within the fly, a process that is termed 'bioenhanced transmission' (Kobayashi *et al*., 1999), which may evoke images of flies as 'flying disease factories'.

Houseflies sampled from hospitals have been shown to harbour pathogenic bacteria, including *E.coli* (Fotedar et al., 1992), the 'superbug' Methicillin Resistant *Staphylococcus aureus* or 'MRSA' (Rahuma *et al*., 2005 and Boulesteix et al., 2005) and *Salmonella* sp (Nmorsi *et*

WITH WINGS

al., 2007). It has been suggested that flies probably obtain some pathogenic bacteria from accessing infected wounds of patients or associated dressings (Fotedar, 1992).

Flies and disease in hospitals – the significance

Of the bacteria isolated from hospital houseflies, the antibiotic resistant forms have the greatest medical significance in terms of threat to public health. Numerous authors have isolated antibiotic resistant bacteria of the super-family Enterobacteriaceae from hospital houseflies (Fotedar, 1992a; Sramova, 1992; Rahuma 2005 and Nmorsi, 2007). Members of the Enterobacteriaceae include 'gut' bacteria typically found in humans and other animals. It is no surprise that houseflies can be contaminated with enteric bacteria, as these flies typically breed and feed in association with animal faeces, unsanitary environments and seem to acquire resistant forms of these bacteria from the hospital environment. To quote an important review paper, research suggests that 'houseflies in hospital environments are vectors of multiple antibiotic-resistant strains of pathogenic bacteria' (Graczyk, 2001).

Flies other than houseflies

Apart from work on houseflies, little research has been done on the bacteria associated with other fly species that are found in hospitals. Fruit flies, *Drosophila* sp sampled from a hospital in Nigeria were found to harbour *Proteus* sp, *Streptococcus* sp and *Salmonella* sp (Nmorsi, 2007), all of which may cause infection in compromised patients. Cluster flies, *Pollenia rudis* sampled from a hospital in Germany were found to harbour opportunistic pathogens such as *Pseudomonas aeruginosa* and *Enwinia* spp. Cluster flies form aggregations of thousands of individuals and their presence in hospitals in such great numbers may present a risk to health when considering the opportunistic pathogens that these flies carry.

Do flies really cause infection?

While there is a lack of direct evidence of the role of flies in disease transmission to humans, there is a wealth of indirect evidence in the form of intervention studies. The lack of direct evidence is understandable – the final experiment of infecting a human volunteer via contaminated flies is unlikely to be viable on ethical grounds, although a classic study by Greenberg in 1964 did show experimental transmission of *Salmonella* Typhimurium from an infected dog to human volunteers via flies. Houseflies were exposed to dog faeces and subsequently to an atole drink. Human volunteers became infected with S. Typhimurium after consuming the drink, lovely!

A number of studies have shown that reducing fly numbers by undertaking fly control measures reduces disease incidence. Levine (1991) reviewed fly control intervention studies relating to *Shigella* spp, showing that fly control measures reduced fly density, *Shigella* incidence, and human mortality in treatment areas. Reduce flies and reduce disease!

Dirty hands with wings?

How important is the presence of flies? Do flies really matter? Based on the evidence, the answer to these questions is likely to be 'Yes'. The view of flies as 'dirty hands with wings' is not too far from the truth and we should all know the importance of hand hygiene in infection management…but do we know the importance of flies and their control in terms of infection management?

To make things clear, it is accepted that thorough hand-washing is an essential component of infection management in hospitals (Health Protection Agency, 2009) and as a point of comparison, a study has shown that the level of recovery of *Vibrio cholerae* from flies can be comparable to the level of recovery from the hands of people that have been in contact with cholera sufferers (Sengupta, 1995). So in terms of bacterial carriage, flies could be just as important as dirty hands and flies are of course much more mobile – they have wings. You would not dream of failing to wash your hands with soap and water or alcohol rub when you enter a hospital ward, something a fly is not capable of considering.

Matthew Davies, the Pest Control News Technical Editor

Houseflies, Bacteria and Hospitals: Viewing Pest Control as Infection Control

Matthew Davies, the Pest Control News Technical Editor and PhD student at Aston University, talks us through an extract of his research on bacteria isolated from flies in UK hospitals. This discussion is based on Matthew's presentation at the International Conference on Urban Pests 2014 in Zurich and reports on species of bacteria that were isolated from houseflies for the first time.

The history

I've always been surprised that people don't seem to take flies seriously when it comes to the transfer of disease-causing microorganisms. It is well established that the housefly *Musca domestica* carries and disseminates a great variety of pathogenic bacteria, many of which can make us severely ill should our foodstuffs or surfaces that we touch become contaminated. The research is out there but there are still sceptics. To be fair, I can understand it when people ask, "Do flies actually infect people with bacteria?" because the majority of research (mine included) shows the deposition of bacteria onto surfaces by flies and lists the bacterial species that were isolated from the flies themselves e.g. from their external surfaces, gut and flyspots. Not unsurprisingly, I didn't gain ethical approval to go and release contaminated flies into hospitals to infect patients with *Clostridium difficile* (the so-called 'hospital superbug'). It just isn't possible to conduct the final experiment these days, which is probably a good thing.

Despite the modern inability to conduct the final experiment, the principles of flies causing infection in humans have already been established, notably by Bernard Greenberg, one of the great researchers in the field of flies and disease. One of his studies showed that houseflies obtained *Salmonella* from infected dog faeces then transferred the same organisms to a drink. Some willing volunteers consumed the drink, becoming contaminated with the same *Salmonella* themselves. Although the volunteers didn't fall ill, they would have done with species of bacteria that have a lower infective dose. Anyway, Greenberg's work is direct evidence of bacterial transfer to humans by flies, pretty convincing as far as I am concerned. Another convincing body of evidence comes from intervention studies. In short, incidence of *Shigella* infection (the causative agent of dysentery) drops significantly in areas where fly control measures are undertaken compared to areas with no fly control. A strong argument for fly control, I would say.

A crash course in microbiology

With the foregoing literature in mind, I began some research of my own with Aston University. I can only report on a very small extract of my study, as the thesis is shaping up to be over 78,000 words, so I shall spare PCN readers some of the detail.

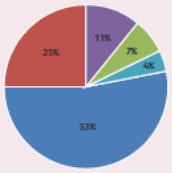
The initial work was with laboratory models, which showed that houseflies *Musca domestica* are able to transfer *Clostridium difficile*, one of the so-called 'hospital superbugs' (Davies et al., 2011). Following on from this, the next aim of the study was to isolate and characterise bacteria associated with flying insects in hospitals, in order to help understand the relevance of pest control as a component of infection control in hospitals. For this article, we'll focus just on the housefly *M. domestica*, rather than attempting to include the other 113 species of arthropod that were sampled from hospitals.

I started off by collecting houseflies from pre-existing ultraviolet light flytraps located throughout a number of hospitals. It's funny how much attention you get when you do this in hospitals – everyone seemingly loves a gruesome story about both types of 'bugs'.

The flies were then subjected to washing and maceration back in the lab, in order to isolate bacteria which were subsequently grown on a variety of agar plates in an incubator. Experience tells me that microbiologists don't appreciate a practical entomologist calling an incubator an 'oven', so I'd advise sticking to the technical terms. The bacterial colonies that grew on the agar plates were then identified by their appearance on the plates, their morphology and colour following staining techniques and finally by a number of biochemical tests.

Results

What we found was certainly interesting because the majority of the species of bacteria isolated from flies were of the family Enterobacteriaceae i.e. faecal / gut bacteria (*E. coli* is in this family). In fact, Figure 1 (below) shows that over half of bacterial isolations from houseflies were Enterobacteriaceae.



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

Enterobacteriaceae, the faecal / gut bacteria isolated from houseflies in hospitals, are shown in the image below (Figure 2), as they appear on a specific type of agar plate.

Figure 2. Typical pink colonies on Violet Red Bile Glucose Agar indicative of Enterobacteriaceae, which were isolated from houseflies

Staphylococcus aureus was also isolated from houseflies taken from hospitals, the antibiotic resistant form of which is known as 'MRSA'. *S. aureus* isolated from houseflies is shown in Figure 3. Note the colour change of the agar caused by the bacterial growth.

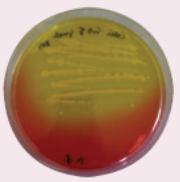


Figure 3. *Staphylococcus aureus* isolated from houseflies sampled from hospitals

Houseflies carrying the described variety of microorganisms were sampled from a number of locations, including hospital catering areas, ward kitchens, wards, hospital food stores and a mortuary. These are clearly sensitive areas where you wouldn't really want to find flies that are carrying bacteria. The UV-light flytraps were clearly doing their job.

The species of bacteria isolated from houseflies on multiple occasions included *Klebsiella pneumoniae* ssp *pneumoniae*, well-known as the causative agent of pneumonia and *Bacillus subtilis* which has been involved in fatal brain and lung infections.

The types of bacteria already mentioned have been isolated from houseflies before, so while this is an important update as far as the situation in UK hospitals in concerned (the last similar UK study was in 1942!), the most interesting findings were arguably the isolation of certain species of bacteria from houseflies for the *first time* (Table 1).

Bacteria isolated from houseflies for the first time	Hospital area	Significance of isolated bacteria
***Bacillus* spp** *Bacillus licheniformis*	M	Septicaemia
Clostridia *Clostridium beijerinckii/butyricum*	HC	Neonatal necrotizing enterocolitis.
Clostridium clostridioforme	HC	Bacteraemia
Enterobacteriaceae *Raoultella terrigena*	W	Resistant neonatal sepsis

Table 1. A list of the bacteria isolated from *M. domestica* for the first time, to the knowledge of the author. Key: The hospital areas that the flies carrying that particular isolate were sampled from are denoted as: mortuary (M), hospital catering areas (HC) and wards (W).

To my knowledge, this study provides the first example of *B. licheniformis, C. beijerinckii / C. butyricum, C. clostridioforme* and *R. terrigena* isolation from houseflies (Table 1). These names don't exactly trip off the tongue and are unlikely to be as familiar as *E. coli, Salmonella* and other more well-known microorganisms but we really need to know something about them. This is of course the really interesting part – we've not heard of these species before, they've been found to be associated with houseflies for the first time ever and what threat do they pose to public health? So, we now need to understand the clinical significance of these species of bacteria.

The clinical significance of bacteria isolated from houseflies for the first time

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; in one case the patient died following infection (Ozkocaman *et al.*, 2006). In the same 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).

Clinically significant *C. butyricum* strains have been isolated from the faeces of new-born babies suffering from Neonatal Necrotizing Enterocolitis (tissue death in the bowel – a common cause of death in premature babies) and those experiencing haemorrhagic colitis (bloody diarrhoea) and an adult with peritonitis (potentially fatal inflammation of the abdomen lining), while *C. beijerinckii* has been detected in dairy products (Popoff and Dodin, 1985).

There appear to be no records in the literature of *C. clostridioforme* isolation from insects. To my 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 (bacteria in the blood), intra-abdominal abscess, peritonitis, wound infection and other infections (Finegold *et al.*, 2005).

Multi-drug resistant strains of *R. terrigena* have been described in over 25% of blood cultures taken from neonates (new-born babies), who were suffering with sepsis (blood poisoning) 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).

So, based on 'read-across' from studies on the transmission of bacteria by *M. domestica* (Kobayashi et al., 1999), it follows that houseflies in hospitals may act as a mobile reservoir and vector of clinically significant *B. licheniformis, 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. Might it be wise then, to include flies in the NHS conditions of contract for pest control?

As a closing comment, we are seeing more and more waste, composting and recycling sites springing up, providing breeding sites for houseflies. In addition to this, housefly populations could increase substantially under likely scenarios of climate change, with increases of up to 244% by 2080 when compared with current levels (Goulson *et al.*, 2005). If this prediction holds true, it is possible that increases in the incidence of fly-borne diseases may occur, which may be of significance in terms of an increased reservoir of flies available to enter hospitals.

Will these factors combine in future to impact on housefly populations available to enter hospitals? What impact will increasing urbanisation and the associated urban heat effect have on housefly populations? Only time will tell. **Perhaps we will start to describe the housefly as an old pest presenting new problems in hospitals and beyond...**

OTHER PUBLICATIONS BY THE AUTHOR

Peer reviewed publications

Davies, M. P., & Anderson, M. (2012). Insecticide Use in Houses. *Outlooks on Pest Management*, 23(5), 199-203.


Aston University
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Davies, M. P., & Anderson, M. (2013). Rodenticide Use in Houses. *Outlooks on Pest Management*, 24(2), 70-75.

RESEARCH PRIZES

Prize for the best Research Talk, School of Life and Health Sciences Postgraduate Research Day, July 2, 2010, Aston University. Presented by Dr Ian Stanford, Associate Dean for Research.

PUBLICATIONS IN PREPARATION

Title: *Musca domestica* and the transfer of *Clostridium difficile*
Target journal: Applied and Environmental Microbiology

Title: Isolation and characterisation of bacteria associated with flying insects in hospitals.
Target journal: Journal of Medical Entomology *or* Medical and Veterinary Entomology *or* Journal of Hospital Infection

Title: An inventory of flying insects sampled from UK hospitals
Target journal: Bulletin of Entomological Research

End.