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MICROBIOLOGICAL ANALYSIS OF NON-BITING FLIES COLLECTED FROM HOSPITALS: NOVEL PATHOGENS AND NEW VECTORS IN THE CLINICAL ENVIRONMENT

[1]MATTHEW PAUL DAVIES, [1]MORAY ANDERSON, AND [2]ANTHONY C. HILTON
[1]Killgerm Chemicals Ltd, Wakefield Road, Ossett, WF5 9AJ.
[2]School of Life & Health Sciences, Aston University, Birmingham, B4 7ET, UK

Abstract The potential for houseflies *Musca domestica* to contribute to transfer of the 'hospital superbug' *Clostridium difficile* in hospitals has been demonstrated, highlighting flies as realistic vectors of this microorganism in clinical areas. Subsequent field studies where houseflies were sampled from hospitals have shown carriage of a variety of pathogenic bacteria. This study continues the previous work and reports on the pathogenic bacteria isolated from other non-biting flies in hospitals. Non-biting flies were collected from pre-existing ultra-violet light flytraps located throughout the hospitals. External washings and macerates of fly samples were prepared and inoculated onto a variety of agars and following incubation bacterial colonies identified by biochemical tests. A clinical serotype of *Escherichia coli* (E1525) was isolated from *Calliphora vicina*. A non-clinical serotype of *E. coli* (O71) was isolated from *Lucilia sericata*. The clinical isolate of *E. coli* from *C. vicina* was likely acquired from the hospital environment, so flies should be considered as a route of spread of clinical isolates of bacteria in hospitals. *L. sericata* likely acquired *E. coli* O71 from calf faeces then by ingress introduced this non-clinical isolate, novel to the hospital environment. Representing a number of novel findings, various bacterial isolates were recovered for the first time ever from a variety of non-biting flies, including *Musca autumnalis*, *Fannia canicularis*, Psychodidae, Phoridae, Sphaeroceridae and *Drosophila* sp. 'Drain flies' / 'small flies' are highlighted as an emerging problem or even new vectors in the hospital environment, due to their described carriage of pathogenic microorganisms in the clinical setting.

Key words Blowflies, drain flies, disease, pest control, infection control, healthcare.

INTRODUCTION

Houseflies *Musca domestica* have been shown to carry and disseminate a variety of bacterial species, including the hospital-associated pathogen *Clostridium difficile* (Davies et al., 2016), while also harbouring Enterobacteriaceae, Bacilli, Clostridia, Staphylococci and Streptococci in hospitals (Davies et al., 2014). Most studies in the literature focus on the bacteria carried by *M. domestica*. Apart from work on houseflies, little research has been done on the bacteria associated with other fly species that are found in hospitals. *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 species of Enterobacteriaceae (Faulde and Spiesberger, 2013). A relative lack of international knowledge is highlighted, regarding the carriage of bacteria by flies other than *M. domestica* in hospitals. This study is of benefit internationally, due to it encompassing the examination of bacterial carriage by non-biting flies aside from *M. domestica*, bridging a gap in current knowledge.

MATERIALS AND METHODS

Flies were collected from pre-existing ultra-violet light flytraps in the form of Electronic Fly Killers (EFK's) and professional sticky traps located throughout 6 health care facility sites from March 2010 to August 2011. The contents of the EFK's were tipped into sterile bags. The glue boards from the sticky traps were removed and covered with a sterile plastic bag. The samples were stored at 4°C in a domestic refrigerator, pending identification and microbiological analysis.

Isolation Of Bacteria

Identified flying insects were washed in phosphate buffered saline (PBS) (Sigma Aldrich, Poole, UK) in a sterile 1.5ml universal micro test tube (Eppendorf, Stevenage, UK) by vortexing for 30 seconds. These external washings were then serially diluted down to 10^{-6} and 0.1ml of each dilution inoculated onto the surfaces of Cycloserine Cefoxitin Fructose Agar + sodium taurocholate (CCFA + Tc), Nutrient agar, Mannitol Salt agar and Violet Red Bile Glucose (VRBG) agar (Oxoid Ltd, Basingstoke, UK). The samples were then washed four further times, (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 and the described inoculation process repeated for the macerates.

Nutrient agar, Mannitol Salt agar and VRBG agar plates were incubated at 37°C for 24 hours in aerobic conditions. CCFA + Tc agar and a set of Nutrient agar plates were incubated in anaerobic conditions at 37°C for 48 and 24 hours respectively.

Identification Of Bacteria

Bacterial colonies were identified by macroscopic morphology, Gram staining, microscopic examination of morphology, oxidase (HPA 2011a) and catalase tests (HPA 2011b) API (analytical profile index) 20E test kits, API Staph test kits, rapid ID (identification) 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 (Oxoid Ltd, Basingstoke, UK) for presumptive identification of Methicillin Resistant *Staphylococcus aureus* (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 2011c). *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.

RESULTS

To our 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* (Table 1). Furthermore, this study provides the first example of *Bacillus subtilis* Group, *Pantoea* spp 2 and *Micrococcus* sp isolation from *F. canicularis* (Table 1). The first example of *Bacillus brevis*, *Escherichia coli* serogroup O71 and *Klebsiella pneumoniae* ssp *pneumoniae* isolation from *L. sericata* is reported (Table 1). *Enterobacter cloacae*, *Escherichia vulneris*, *Klebsiella pneumoniae* ssp *pneumoniae*, *Raoultella terrigena*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* isolation from *M. autumnalis* occurred for the first time (Table 1). Finally, this study shows 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 (Table 1).

DISCUSSION

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. (1992), that 'microbial studies of randomly collected flies from a hospital environment may provide an epidemiological tool for monitoring existing sanitary conditions'.

Enteropathogenic *Escherichia coli* (EPEC) of the serogroup O71 was isolated from *L. sericata* collected from a hospital kitchen. EPEC O71 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 EPEC 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. The significance of EPEC O71 is that it can cause potentially fatal infant diarrhoea (Kaper et al. 2004).

Table 1. Bacteria isolated from flies sampled in UK hospitals

Fly species / family	Bacteria isolated	Hospital location	Medical significance of isolated bacteria
Calliphora vicina	**Enterobacteriaceae**		
	Citrobacter freundii	Live MI	Haemolytic uraemic syndrome
	Enterobacter asburiae	Live MI	Wound infection
	Enterobacter sp (*aerogenes* or *cloacae*)	HS	Neonatal septicaemia
	**Escherichia coli* E1525	HC	Haemolytic uraemic syndrome
	**Klebsiella oxytoca*	HC	Haemorrhagic colitis
	**Klebsiella pneumoniae* ssp *ozaenae*	HS	Chronic rhinitis
	**Leclercia adecarboxylata*	W	Throat tissue abscess
	**Pantoea* species 1	Live MI	Fatal neonatal septicaemia
	**Raoultella terrigena*	M	Resistant neonatal sepsis
	Staphylococci		
	Staphylococcus aureus	HC	Resistant infection of blood, skin, urine, respiratory tract
	Staphylococcus aureus	HC	
	**Staphylococcus hominis*	HC	Oxacillin-resistant sepsis
	Streptococci		
	b-hemolytic *Streptococcus* sp	WN	Endocarditis
	Non-hemolytic streptococci	WN	

Musca autumnalis	**Enterobacteriaceae**		
	**Enterobacter cloacae*	HC	Resistant neonatal bacteraemia
	**Escherichia vulneris*	HC	Soccer wound infection
	**K. pneumoniae* ssp *pneumoniae*	HC	Pneumonia
	**Raoultella terrigena*	HC	Resistant neonatal sepsis
	Staphylococci		
	**Staphylococcus aureus*	HC	Resistant infection of skin
	**Staphylococcus saprophyticus*	HC	Oxacillin-resistant sepsis
Fannia canicularis	***Bacillus* spp**		
	**Bacillus subtilis* Group	HC	Fatal brain and lung infection
	Enterobacteriaceae		
	**Pantoea* spp 2	HC	Fatal neonatal septicaemia
	Staphylococci		
	Staphylococcus aureus	HC	Resistant infection of blood, skin, urine, respiratory tract
	Staphylococcus aureus	WK	
	Other		
	Enterococcus sp	HC	Infection of CNS
	**Micrococcus* sp	HC	Peritonitis
Lucilia sericata	***Bacillus* spp**		
	**Bacillus brevis*	HC	Peritonitis
	Enterobacteriaceae		
	Enterobacter cloacae	HC	Resistant neonatal bacteraemia
	**Escherichia coli* O71	HC	Haemolytic uraemic syndrome
	**K. pneumoniae* ssp *pneumoniae*	HC	Pneumonia
	Staphylococci		
	Staphylococcus aureus	HC	Resistant infection of skin
Psychodidae	***Bacillus* spp**		
	**Bacillus cereus* Group	HC	Neonatal lung and CNS infection
	Staphylococci		
	**Staphylococcus aureus*	HC	Resistant infection of blood, skin, urine, respiratory tract
	Other		
	Micrococcus sp	WN	Peritonitis
Phoridae	***Bacillus* spp**		
	**Bacillus cereus* Group	HC	Neonatal lung+CNS infection
	**Bacillus cereus* Group	HC	
	**Bacillus sphaericus*	HC	Bacteraemia
	Clostridia		
	Clostridium sp	HC	

Sphaeroceridae	***Bacillus* spp**		
	**Bacillus cereus* Group*	WN	Neonatal lung+CNS infection
	Bacillus sphaericus	WN	Bacteraemia
	Clostridia		
	**Clostridium clostridioforme*	WN	Intra-abdominal abscess
	Staphylococci		
	Staphylococcus aureus	WN	Resistant infection of blood, skin, urine, respiratory tract
Trichiaspis sp	***Bacillus* spp**		
(Family Sphaeroceridae)	**Bacillus licheniformis*	HS	Septicaemia
	Staphylococci		
	**Staphylococcus aureus*	HS	
Drosophila sp	***Bacillus* spp**		
	**Bacillus pumilus*	HS	Food poisoning

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 authors.

CONCLUSION

The isolation of the clinical strain of *E. coli* (E1525) from *C. vicina*, likely acquired from the hospital environment, means that flies should be considered as a route of spread of clinical isolates of bacteria in hospitals and not merely a nuisance. The isolation of EPEC O71 (non-clinical *E. coli* typically associated with calf faeces) from *L. sericata* leads to the recommendation that fly-proofing measures should be an essential feature in hospitals, to prevent fly ingress and therefore limit the introduction of non-clinical bacterial isolates into the clinical environment.

Psychodidae, Phoridae, Sphaeroceridae and *Drosophila* i.e. ‘drain flies’ / ‘small flies’ are highlighted as an emerging problem or even new vectors in the hospital environment, due to their described carriage of pathogenic microorganisms in the clinical setting. The collected findings continue to emphasise the importance of pest control as a component of infection control in hospitals.

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