Travel Medicine and Infectious Disease 22 (2018) 8–17

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

The role of 'filth flies' in the spread of antimicrobial resistance

Francis C. Onwugamba[a], J. Ross Fitzgerald[b], Kateryn Rochon[c], Luca Guardabassi[d], Abraham Alabi[e,f], Stefan Kühne[g], Martin P. Grobusch[e,f,h], Frieder Schaumburg[a,*]

[a] Institute of Medical Microbiology, University Hospital Münster, Münster, Germany
[b] The Roslin Institute, University of Edinburgh, Edinburgh, UK
[c] Department of Entomology, University of Manitoba, Winnipeg, Canada
[d] Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark
[e] Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon
[f] Institut für Tropenmedizin, Eberhard Karls Universität Tübingen, Deutsches Zentrum für Infektionsforschung, Tübingen, Deutschland, Germany
[g] Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Kleinmachnow, Germany
[h] Center of Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Division of Internal Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

ARTICLE INFO

Keywords:
Diptera
Antimicrobial resistance
Transmission
Bacteria

ABSTRACT

Background: 'Filth flies' feed and develop in excrement and decaying matter and can transmit enteric pathogens to humans and animals, leading to colonization and infection. Considering these characteristics, 'filth flies' are potential vectors for the spread of antimicrobial resistance (AMR). This review defines the role of flies in the spread of AMR and identifies knowledge gaps.

Methods: The literature search (original articles, reviews indexed for PubMed) was restricted to the English language. References of identified studies were screened for additional sources.

Results: 'Filth flies' are colonized with antimicrobial-resistant bacteria of clinical relevance. This includes extended spectrum beta-lactamase-, carbapenemase-producing and colistin-resistant (*mcr-1* positive) bacteria. Resistant bacteria in flies often share the same genotypes with bacteria from humans and animals when their habitat overlap. The risk of transmission is most likely highest for enteric bacteria as they are shed in high concentration in excrements and are easily picked up by flies. 'Filth flies' can 'bio-enhance' the transmission of AMR as bacteria multiply in the digestive tract, mouthparts and regurgitation spots.

Conclusion: To better understand the medical importance of AMR in flies, quantitative risk assessment models should be refined and fed with additional data (e.g. vectorial capacity, colonization dose). This requires targeted ecological, epidemiological and *in vivo* experimental studies.

1. Introduction

"According to our best sanitarians", Samuel Miller reported to the Massachusetts Association of Boards of Health in 1914 "flies breed disease" and concluded: "The fly is a curse." [1]. So-called 'filth flies' have been linked to faecal-oral transmission of bacteria [2], fungi [3,4], parasites [5,6] and viruses [7,8] (Fig. 1). 'Filth flies' are defined as flies that use excrement and decaying matter for nutrition and oviposition [9]. All medically relevant 'filth flies' have some characteristics in common: they are coprophagic (feeding on animal manure and human faeces) or omnivorous, synanthropic (living in association with humans) and endophilic (preferring in-house dwelling) [10]. Of over 125,000 species belonging to the order Diptera (true flies) at least two main families of 'filth flies' are involved in the transmission of medically important pathogens namely *Muscidae,* and *Calliphoridae* [5,11]. Moreover, these flies have a great potential to contribute to the dissemination of bacteria (e.g. enteric pathogens and commensal bacteria) due to their remarkable ability to move freely between different habitats and overcome long flight distances (5–7 km) [12–16]. Therefore, it is likely that they play a role in the spread of antimicrobial resistance (AMR) between animals and humans. Recent reports have shown that the fly gut provides a suitable environment for carriage of antimicrobial resistant bacteria and horizontal transfer of AMR genes [17,18].

Mathematical models are suitable tools to assess the risk bacterial transmission. The quantitative microbial risk assessment (QMRA) is frequently used in food production processes to evaluate food safety. The four stages of QMRA are hazard identification (e.g. population at

* Corresponding author. Institute of Medical Microbiology, University Hospital Münster, Domagkstr. 10, 48149 Münster, Germany.
E-mail address: frieder.schaumburg@ukmuenster.de (F. Schaumburg).

https://doi.org/10.1016/j.tmaid.2018.02.007
Received 20 December 2017; Received in revised form 12 February 2018; Accepted 19 February 2018
Available online 01 March 2018

Abbreviations

AMR	antimicrobial resistance
CFU	colony forming units
CRE	carbapenem-resistant *Enterobacteriacae*
GCP-ICH	Good Clinical Practice-International Conference of Harmonization
ESBL	extended-spectrum beta-lactamase
MLST	multilocus sequence typing
MRSA	methicillin-resistant *Staphylococcus aureus*
PFGE	pulsed field gel electrophoresis
QMRA	quantitative microbial risk assessment

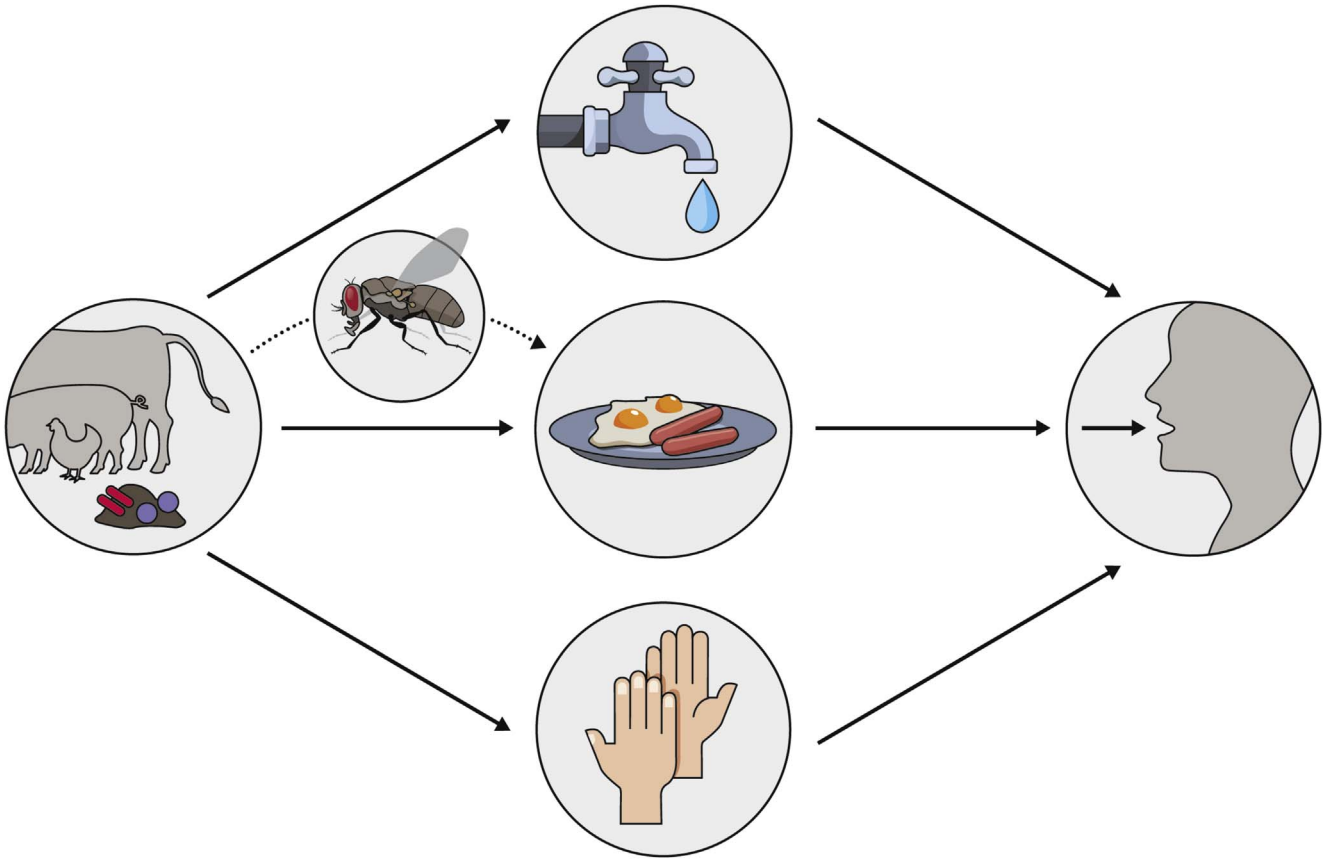

Fig. 1. Pathways of faecal-oral transmission. Pathogens from faeces can be transmitted to humans or animals through drinking water, food or hands (smear infection). Flies can enhance the contamination of food, as no direct contact of food and faeces is necessary.

risk to acquire a certain pathogen), dose-response (e.g. exposure dose to health outcomes), exposure (i.e. pathways of microbes to reach the population) and risk characterization (i.e. probability of an health outcome after exposure) [19]. Such a QMRA has been developed for the transmission of resistant bacteria from poultry to humans through flies [20].

In their review, Zurek and Gosh already described the colonization of insects in general with antimicrobial resistant bacteria and suggested that flies play a role in the spread of antimicrobial resistant bacteria between livestock and urban areas [21]. In our review we built on this work by giving an updated epidemiological picture of AMR with a special focus on 'filth flies' and on emerging antimicrobial resistance phenotypes of clinical relevance (e.g. extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae,* carbapenem- and colistin resistant Gram-negative bacteria, see Supplement and Table A1 for methods). We identified knowledge gaps and suggest topics for future research initiatives.

2. Occurrence and fate of medically relevant bacteria in 'filth flies'

Flies can carry medically relevant bacteria on the surface of their exoskeleton (e.g. legs, mouthparts) and in the alimentary canal (Table A2). Consequently, bacterial transmission can occur through regurgitation, defecation or translocation from the exoskeleton (Figs. 1 and 2) [2]. The ingestion of flies by insectivores could be an additional route of transmission [22]. Finally, degrading flies can contaminate the environment with antimicrobial-resistant bacteria. Numerous studies assessed bacterial colonization in pooled fly samples [12,23–25]. However, this approach is inappropriate to measure the actual prevalence in individual flies.

It is methodically simple to analyze the prevalence of bacteria on the body surface (washing off the microbiota from the exoskeleton) separately from the intestine (dissection of the alimentary canal after surface disinfection) [26]. The available studies applied different pathogen detection methods (e.g. PCR, culture, selective media, and broth enrichment). Therefore, the carrier rates should be compared cautiously.

According to a study analyzing individual flies (*Muscidae*, *Calliphoridae*) collected from urban restaurants in the USA, similar colonization rates were found in the intestine compared to the exoskeleton for *Salmonella enterica* (6 vs. 1%) and *Listeria monocytogenes* (3 vs. 1%) using a PCR-based system [27]. However, the majority of studies analyzed bacterial colonization of the body surface only. Very high rates were found for *Klebsiella* spp. (51.9%), *Escherichia coli* (32.1%) and *Pseudomonas aeruginosa* (26.9%) in flies collected from fresh-food markets, garbage piles, restaurants, school cafeterias and rice paddies in

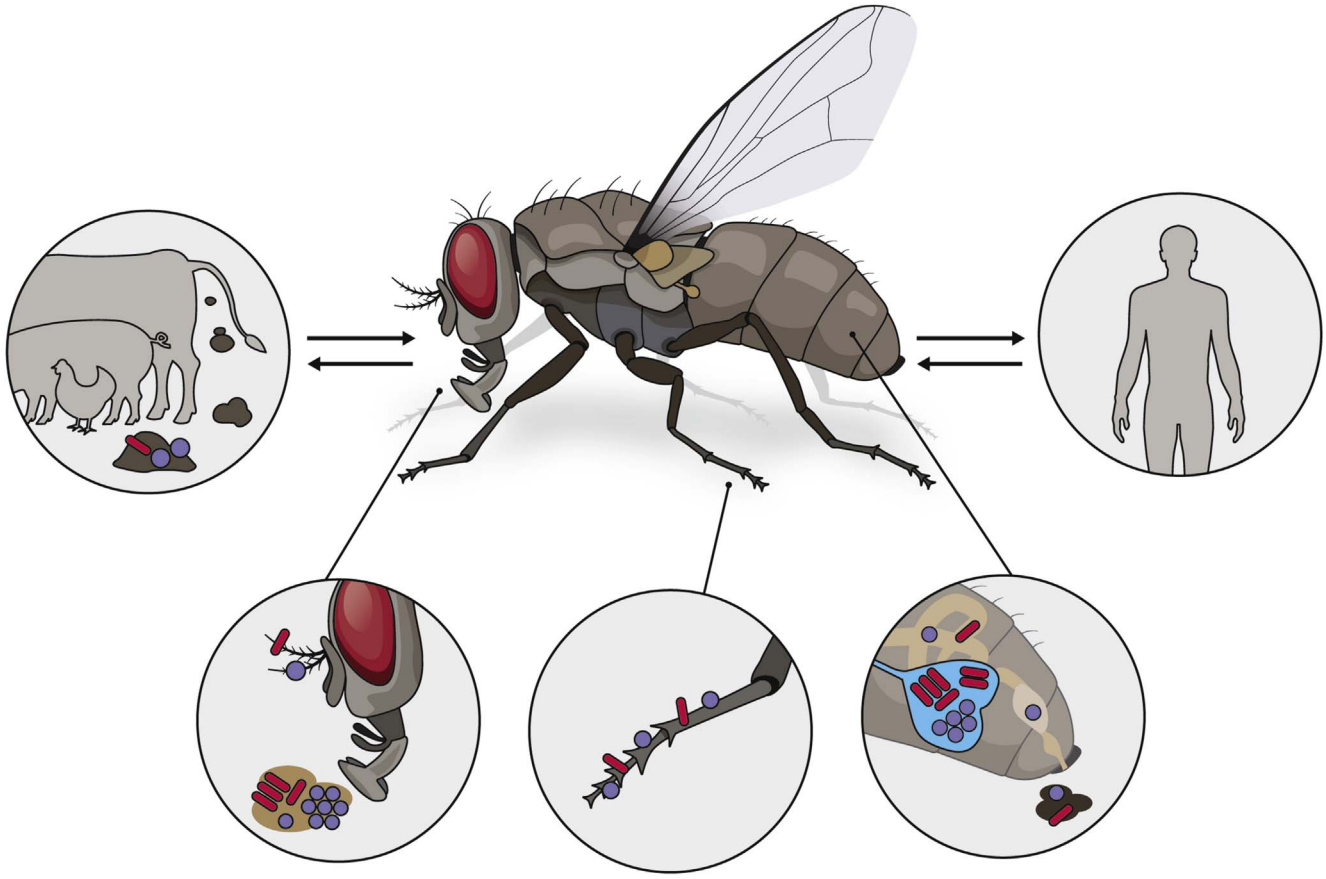

Fig. 2. Modes of bacterial transmission between humans and animals through 'filth flies'. Flies can transmit antimicrobial-resistant bacteria through regurgitation, translocation from the exoskeleton and defeacation. Flies ingest fluids that can be contaminated with bacteria. These bacteria multiply in the crop (a diverticulum of the digestive tract in higher flies, in blue) and are subsequently transferred to the gut or are regurgitated leading to the concept of "bioenhance transmission" [37]. Since flies share their habitat with both animals and humans, transmission of antimicrobial resistance is therefore possible. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Thailand using non-selective solid culture media [28] (Table A2). Colonization rates of flies vary for enteric pathogens such as *Salmonella* (26.4%, Taiwanese swine farms, culture-based detection), *Campylobacter* (up to 25.5%, US broiler farm, PCR detection) or *Shigella* spp. (9.6%, rural Thailand, culture based detection) [23,28,29]. The microbiome of Diptera is dynamic and diverse; its composition depends on the species, life stage, sex and season [30,31].

Adult flies ingest bacteria through contaminated fluids. Bacteria are first stored in the fly's crop where they can multiply (Fig. 2) [32]. From here, bacteria are either regurgitated or transferred to the alimentary canal (proventriculus, midgut, hindgut, and rectum). The midgut epithelium is lined with a peritrophic matrix, which protects the fly from microbes, but allows enzymes and antimicrobial peptides to enter the lumen [33]. Therefore, the alimentary canal of flies seems to be a hostile environment for the majority of bacterial species, resulting in an exponential decline (Fig. 3) [32–37]. The ability to slow down this decrease or even proliferate in the fly's gut (as shown for *Aeromonas caviae* and *Salmonella* typhimurium) seems to depend on the mobility of bacteria, the temperature, and the bacterial exposure dose [33,38,39].

In contrast to the alimentary canal, the fate of bacteria on

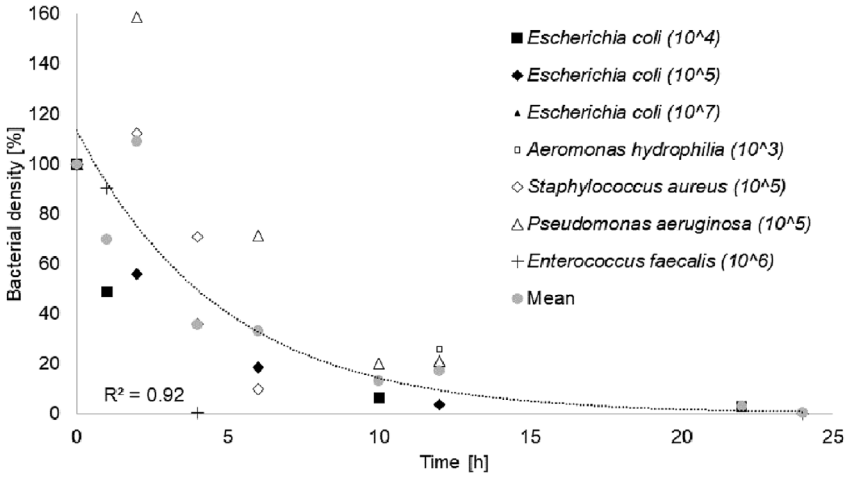

Fig. 3. Fate of bacteria in the alimentary tract of the adult *Musca domestica*. The graph shows the decline of bacterial concentration over 24 h in relation to the feeding concentration (0 h), which was set at 100%. The feeding concentration of the respective bacterial species is given in the legend. Data from experiments with *Escherichia coli* [34,35,37], *Aeromonas hydrophilia* [100], *Staphylococcus aureus* [36], *Pseudomonas aeruginosa* [33] and *Enterococcus faecalis* [32] are included. The trend line was fitted to the mean of all experiments by use of an exponential function (Coefficient of determination $R^2 = 0.92$).

Table 1
Colonization of antimicrobial resistant bacteria of medical relevance in adult 'filth flies'.

Bacterial species (n)	Type of resistance	Colonization rate in flies, % (n of isolates/n of flies)	Country	Sampling period	Site of colonization	Setting	Fly species (n)	Reference
Escherichia coli	ESBL	3.0% (37/1228)[a]	China	April–September 2011	Exoskeleton	Airport	*Chrysomya megacephala* (276), *Aldrichina grahami* (247), *Lucilia sericata* (211), *Boettcherisca peregrima* (107), *Muscina stabulans* (162), *Bercaea cruenta* (225)	[91]
	ESBL	14.3% (13/91)	Japan	August–October 2010	Intestine	Cattle barn	*Musca domestica* (91)	[77]
	ESBL	3.3% (44/1346)	Germany	April–July 2015	Intestine	Urban and rural areas	*Musca domestica* (895), *Calliphora* sp. (447), others (4)	[69]
	ESBL	0.0% (0/240)[a]	Czech Republic	October 2006	Homogenate	Dairy cattle farm	Symbovine flies (240)	[92]
	ESBL	17.2% (72/418)	Zambia	March–June 2015	Exoskeleton	Food markets	Houseflies (418)	[62]
	ESBL	6.2% (42/682)	Spain	May–November 2012	Homogenate	Broiler farms	*Musca domestica* (615), *Ophyra* spp. (33), *Stomoxys calcitrans* (15), *Muscina stabulans* (7), *Fannia canicularis* (6), others (6)	[75]
	ESBL	10.5% (2/19)[b]	The Netherlands	September–October 2011	Homogenate	Poultry farms	*Musca domestica* (54), *Fannia canicularis* (20), *Stomoxys calcitrans* (6), *Lucilia* spp. (7)	[93]
	CRE[c]	25.8% (31/120)	China	November 2014–August 2015	Homogenate	Chicken farm	Not specified (120)	[22]
	CRE[d]	25.0% (1/4)	Germany	January 2011–October 2012	Homogenate	Pig farms	Not specified (4)	[63]
	Colistin[e]	10.0% (12/120)	China	November 2014–August 2015	Homogenate	Chicken farm	Not specified (120)	[22]
	Colistin[e]	33.3% (1/3)	Germany	January 2011–October 2012	Homogenate	Pig farms	Not specified (3)	[68]
	Colistin[e]	5.4% (4/74)	China	July–August 2013	Homogenate	University campus	*Musca domestica* and *Protophormia terraenovae* (74)	[94]
Enterococcus faecium	VRE	0.0% (0/262)	USA	Summer 2006	Intestine and Exoskeleton	Poultry farms	*Muscidae* and *Calliphoridae* (262)	[95]
Klebsiella pneumoniae	ESBL[a]	5.3% (32/600)	Iran	Before 2016	Intestine and Exoskeleton	Kitchens, cattle and chicken farms, animal and human hospitals, slaughterhouses	*Musca domestica* (600)	[64]
	CRE	1.8% (11/600)	Iran	Before 2016	Intestine and Exoskeleton	Kitchens, cattle and chicken farms, animal and human hospitals, slaughterhouses	*Musca domestica* (600)	[64]
Salmonella enterica	ESBL	0.0% (0/220)[a]	Taiwan	2005	Exoskeleton	Swine farms	*Musca domestica* (220)	[29]
Staphylococcus aureus	MRSA	0.1% (1/1346)	Germany	April–July 2015	Intestine	Urban and rural areas	*Musca domestica* (895), *Calliphora* sp. (447), others (4)	[69]
	MRSA	0.2% (1/600)	Morocco	March–October 2006	Exoskeleton	Residential areas	*Musca domestica* (600)	[70]
	MRSA	1.3% (2/150)	Libya	Before 2005	Intestine and Exoskeleton	Urban areas including hospital, abattoir	*Musca domestica* (150)	[71]

Note: CRE, carbapenem-resistant *Enterobacteriacae*; ESBL, extended spectrum beta-lactamases producing *Enterobacteriacae*; MRSA, methicillin-resistant *Staphylococcus aureus*.

[a] Deduced from resistance to third generation cephalosporins.

[b] Pooled fly samples.

[c] bla_{NDM}.

[d] bla_{VIM}.

[e] *mcr-1*.

mouthparts (e.g. labellum, proboscis) was less studied. Two studies suggest a rapid decline in bacterial concentration (*Campylobacter jejuni*, *Enterococcus faecalis*) on mouthparts from 100% of the feeding concentration to < 1% within 2 h [32,38]. Other studies also report this initial decline but showed significantly increased bacterial counts 2–6 days after feeding a defined concentration of *E. faecalis* (3.1×10^6) [32] or *E. coli* (different feeding sources were used, e.g. cow manure with 10^7 cells/ml) [40]. This increase is most likely explained by bacterial proliferation in the crop and subsequent regurgitation, so that bacteria could be recovered from mouthparts (Fig. 2) [32]. The amount and frequency of regurgitation is influenced by a variety of factors, including species [41], fly density and host presence [42], temperature and relative humidity [43], or reproductive status [44].

Flies primarily transmit bacteria through mouthparts and regurgitation and less likely through defeacation. In addition, bacteria continue multiplying in mouthparts and regurgitation spots as shown for *E. coli* O157:H7 [37,40]. Taken together, the fly is not only a mechanical vector that transmits bacteria by translocation from the exoskeleton. "Bio-enhanced transmission", a term initially coined by Kobayashi et al. for *E. coli* O157:H7 [37], best describes the fate of bacteria in flies.

Bacterial contamination of the larval substrate appears to play a role where female flies choose to lay their eggs [45]. Bacteria are essential to the development of house flies [46,47], and stable flies [48,49] (both *Muscidae*). Bacteria remaining in the larval alimentary tract at the time of pupation can proliferate during metamorphosis but are mostly evacuated in the puparium during adult emergence [50,51]. Newly emerged adult flies tend to harbour a surprisingly low amount of bacteria, but puparia contain large amounts and can serve as reservoirs to maintain local contamination (e.g. of a farm) [51].

Nevertheless, how effectively do flies transmit bacteria? This question refers to 'vector competence' (i.e. ability to transmit an infectious agent), a term that is commonly used in mosquitoes. There is good evidence that flies can effectively contaminate food products, animals and the environment. After ingesting a defined concentration of bacteria, flies were exposed to several items from where the bacteria were recovered later on. This has been shown for *E. coli* O157:H7 on spinach leaves and calves [40,52,53], *E. faecalis* on hamburger beef patties [54], *Aeromonas caviae* on chicken meat [55] or *C. jejuni* from chickens [56]. There is only one historic study conducted by Greenberg [57], which assessed the vector competence of house flies to transmit *S.* typhimurium to humans. After flies were fed with *S.* typhimurium and subsequently allowed to contaminate food, *S.* typhimurium was isolated in due course from faeces of 6/10 volunteers, who had eaten the contaminated food. However, none of the volunteers developed symptoms of disease, presumably due to the low inoculum in the house fly feed.

The sole presence of bacteria on food items does not imply transmission or predict subsequent infection in humans or animals. To assess the vector potential of flies more accurately, the transmission of "colony forming units" (CFU) has been quantified under controlled experimental set-ups. Depending on the number of flies, 3.1×10^3 (5 *M. domestica*) – 2.8×10^4 (40 *M. domestica*) CFUs of *E. faecalis* were transmitted within 30 min to 1 g of beef patty [54]. In this study, flies were field collected and were not exposed to a defined bacterial concentration [54]. Similarly, $10^{2.6}$–$10^{3.5}$ CFU of *E. coli* per fly landing were transmitted by *M. domestica* to a sterile surface after exposing the flies to 10^8 CFU/g of either sugar-milk, steak or potato salad for 30 min [58]. A field study from Bangladesh revealed that an average of > 0.6×10^3 CFU of *E. coli* were transmitted to rice per fly landing [59]. *Clostridium difficile* spores were less effectively transmitted by *M. domestica* to cycloserine cefoxitin fructose agar (sodium taurocholate supplement): after exposure to 10^5 *C. difficile* spores for 6 min, 288 CFU were transmitted 1 h later [60].

In conclusion, flies can transmit bacteria to food products, animals and the environment, most likely through mouthparts and regurgitation. It appears plausible that this is also true for antimicrobial-resistant bacteria.

3. Antimicrobial resistance in flies

Numerous studies have shown that flies can carry antimicrobial resistant bacteria (Table 1). Among the AMR phenotypes of clinical interest, ESBL-producing *Enterobacteriaceae* are an increasing public health concern due to their spread in humans and animals [61]. ESBLs are plasmid-borne beta-lactamases conferring resistance to first, second and third generation cephalosporins and monobactams [61]. ESBL-producing *Enterobacteriaceae* have been reported on flies in Asia, Africa and Europe with colonization rates of up to 17% (Table 1) [62].

The occurrence of carbapenemase-producing *E. coli* and *K. pneumoniae* (e.g. NDM, VIM carbapenemases) in flies captured from livestock and farms in China, Germany, and Iran is most worrisome as treatment options for infections with carbapenem-resistant Gram-negative bacteria are limited (Table 1) [22,63,64]. Genes encoding for carbapenemases can be transmitted between bacteria through mobile genetic elements and flies can be a suitable environment for the exchange of resistance genes between different bacterial species [65].

Colistin is often the drug of choice for the treatment of infections caused by carbapenem-resistant *Enterobacteriaceae* in human medicine. However, colistin was used as a growth promotor in poultry production [22] and is nowadays widely used for management of gastroenteritis in pigs and cattle [66]. Chromosomal mutations leading to colistin resistance genes are known for a long time. In 2015, a study from China reported for the first time the occurrence of a gene (*mcr-1*) mediating colistin resistance, which is located on a transferable plasmid [67]. Colistin-resistant (*mcr-1* positive) *E. coli* have now been detected in flies from pig and poultry farms in China and Germany (Table 1) [22,68].

The colonization rates of flies with antimicrobial-resistant Gram-positive bacteria of clinical importance such as vancomycin-resistant *Enterococcus faecium* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) are low (0–1.3%), based on few studies from Africa and Europe (Table 1) [69–71].

In conclusion, the prevalence of AMR in flies is higher in Gram-negative (e.g. *E. coli* and *K. pneumoniae*) than in Gram-positive bacteria (e.g. *S. aureus* and *E. faecium*, Table 1). The risk of AMR transmission might therefore be highest for *Enterobacteriaceae*.

4. Transmission of bacteria and mobile genetic elements

Under optimal living conditions, flies do not tend to roam far. However, they can have a flight range of 5–7 km and therefore disperse bacteria between different regions [15,16]. This is of importance when assessing the spread of AMR through flies.

Many of the studies examining antimicrobial-resistant bacteria in flies have focused on the prevalence of bacterial pathogens and the associated types of phenotypic resistance or resistance genes. By inference, it can be predicted that flies act as vectors of AMR but studies examining either the direct spread of antimicrobial-resistant bacteria by flies or the transfer of resistance genes in flies are relatively uncommon. In some cases, bacterial typing techniques such as pulsed field gel electrophoresis (PFGE) or multilocus sequence typing (MLST) have been used to examine the distribution of bacterial genotypes in flies, humans, and livestock in different settings to provide evidence for transmission of bacteria. Such studies have been carried out in a variety of environments including chicken, pig and cattle farms, as well as hospitals and restaurants in urban areas providing information on which environmental settings support the spread of AMR by flies [2,29,69,71–79]. In one study, ESBL-producing *E. coli* was detected on flies on land areas surrounding broiler chicken farms [75]. Typing analysis of the isolates, resistance genes and plasmids demonstrated persistence of the same clones in the farm environment over several months suggesting that flies can carry antimicrobial-resistant bacteria within defined areas for long periods of time [75]. However, evidence

Table 2
Methods to collect flies for bacteriological analyses in field studies.

Method	Investigated fly body parts	Description	Advantage	Concern	Reference
Aspirator (pooter)	Intestine and exoskeleton	A tube is placed over an insect which is drawn into a (sterile) collecting chamber by sucking.	Individual fly collection, gentle treatment of flies	Trapping method does not kill the fly, labor intensive, if individual flies are collected, because collecting jar and tubes have to be replaced after each fly collection.	[96]
Gaze trap over a bait	Intestine	Multiple flies are collected in one gaze trap and are killed in ethanol, which also sanitizes the exoskeleton.	No contamination from other flies and the trap, simple, low effort	Ethanol might impair bacterial growth.	[69,97]
Individual sweep/aerial net	Intestine and exoskeleton	Flies are caught individually in sweep nets and can be individually analyzed. Nets are sterilized by 70% ethanol after use.	Simultaneous analysis of Intestinal and exoskeleton colonization of individual flies	Surface disinfection might impair the culture of the intestinal flora, labor intensive.	[26,85]
Fly trap paper, fly tape	Intestine and exoskeleton	Flies stick to the surface of the paper and can be collected individually using sterile forceps.	Individual analysis of flies, cheap, simple, low effort	The glue might impair the bacterial culture from the exoskeleton. Body parts (e.g. mouthparts, extremities) could become disconnected from the body when removing the fly from the sticky surface.	[24,29]
Jug traps (i.e. Victor Fly Magnet® Trap, pheromone-baited jug traps)	Intestine and exoskeleton	The system consists of a reservoir for the attractant and a plastic cap that covers the reservoir. Flies enter the trap though slits in the plastic cap.	Cheap, simple, low effort	The bait can contaminate the flies; only pooled samples can be analyzed.	[95,98]
QuikStrike® Abatement strip	Intestine and exoskeleton	Flies are attracted and killed with neonicotinoids. Dead flies can be collected individually or in pools.	Cheap, simple, low effort	The trap can contaminate the exoskeleton.	[99]

for the direction of transmission of antimicrobial-resistant bacteria was not provided. Most likely, bacteria are transmitted from resident animals to flies since flies feed on animal faeces and antibiotic selective pressure is directly exerted on the faecal microbiota of treated animals. However, flies can potentially enhance dissemination of antimicrobial-resistant bacteria to other farms or human households in the vicinity, as well as through different production cycles within the same farm.

When von Salviati et al. examined the spread of ESBL/AmpC-producing bacteria in pig farms, PFGE analysis suggested that flies were vectors of these bacteria as identical PFGE types were identified in samples from flies, and pooled faeces from pigs [78]. This is consistent with a study from China where flies and farmers shared the same genotypes of colistin-resistant (*mcr-1*) and carbapenem-resistant (bla_{NDM}) *E. coli* [22]. In addition, Ahmad et al. identified *Enterococcus* spp. containing the resistance genes *tetM* and *ermB* among samples from house flies, cockroaches and pig faeces on commercial swine farms [72]. The indistinguishable PFGE types suggested that flies were involved in transmitting antimicrobial-resistant bacteria between pigs and the environment [72]. In another study, Wang et al. isolated *S. enterica* resistant to multiple antibiotics that had identical PFGE types in flies and swine faeces suggesting flies could act as vectors for transmission [29]. A study on a cattle farm in Japan revealed that flies carried several different clones of ESBL-producing *E. coli* that were also found in cattle faeces, and suggested that plasmids harboring the $bla_{CTX\text{-}M\text{-}15}$ gene were potentially transferable [76]. Finally, hospitals in Africa can be infested with house flies carrying antimicrobial-resistant *Pseudomonas* spp., enterococci and staphylococci. In some cases, these bacteria display the same susceptibility profiles that were observed in clinical isolates from patients in the same hospital [71,73]. A relatively small number of studies have investigated the capacity of flies to act as biological vectors for the spread of AMR genes among bacteria occupying the fly gut niche. Although there is strong evidence for the mechanical transfer of antimicrobial-resistant bacteria by flies, there is a lack of substantial evidence supporting transfer of AMR genes in the midgut of flies. Wei et al. demonstrated that *Proteus mirabilis* ($bla_{TEM\text{-}}$, and *aphA1*-positive) could persist in the digestive tract of flies for several days and compete favorably with antimicrobial-susceptible isolates [80]. This suggests two important points: the conditions in flies could theoretically support the potential horizontal transfer of AMR genes and antimicrobial-resistant bacteria can persistently (not only transiently) colonize flies. For instance, $bla_{CTX\text{-}M}$ can be transferred on conjugative plasmids from *E. coli* to other bacteria (e.g. *Achromobacter* and *Pseudomonas*) in the intestine of house flies providing direct evidence for the emergence of antimicrobial-resistant bacteria through horizontal transfer in the fly gut [17]. Similarly, the *tetM* gene on conjugative plasmid pCF10 can be transferred among *E. faecalis* strains [18] and Petridis et al. reported a transfer of plasmid-encoded chloramphenicol resistance genes and bacteriophage-encoded Shiga-toxin gene *stx*1 in the midgut of house flies [81]. This horizontal gene transfer might not only happen under laboratory conditions but also occur in real-life settings. For instance, *E. coli* was collected from humans, flies, horses and surfaces in an equine clinic. Although the majority of the isolates belonged to different PFGE types, they shared identical plasmids, highlighting the relevance of horizontal gene transfer [82].

Of note, 'filth fly' larvae can also be involved in reducing the spread of AMR: the larvae can attenuate antimicrobial agents and reduce AMR genes during vermicomposting of manure [83,84].

Overall, these studies stress the potential of flies to act as vectors for the spread of resistant bacteria and for the transfer of AMR genes across bacterial species and hosts.

5. Sampling methods

Surveillance of AMR in flies necessitates regular field studies to assess the geographical spread and colonization rates of the antimicrobial-resistant pathogens of interest. The microbiological analysis

of individual flies should always be preferred over analyses of pooled flies as the latter necessarily overestimates the burden of AMR in flies [69]. GPS data of sampling points would be beneficial when reporting AMR colonization rates in flies. This will help to identify geographical patterns and key regions for potential interventions.

Numerous sampling methods are available and the selection depends on the objective of the study (Table 2). Major challenges are to avoid artificial contamination of the exoskeleton during sampling. One workaround is to sanitize individual flies in 70% ethanol or 0.26% sodium hypochlorite, which will disinfect the surface and not the intestinal microbiota [26,46,85].

6. Medical relevance of antimicrobial resistance in flies

'Filth flies' have since long been considered as playing a role in the spread and transmission of gastrointestinal pathogens, providing one possible link in the interface between faeces, or contaminated soil, and food (Fig. 1) [40,52]. Numerous studies reported colonization of house flies with enteropathogenic bacteria. Whilst it is plausible, there is only limited evidence that house flies transmit these bacteria, causing not only colonization but also disease in humans and animals.

Seasonal peaks of house fly infestation and cases of shigellosis have been described to coincide [86]. Indirect evidence (given that confounding and some methodical limitations cannot be ruled out) for a role of *M. domestica* as vector of *Shigella* spp., leading to a surge in shigellosis stems from a study in Bangladesh [13]. Modelling suggests that about 40% of cases might have been prevented by improved household fly infestation control. There is good evidence that control of flies is protective against shigellosis and campylobacteriosis in animals and humans [87–89].

Definite proof of bacterial transmission from contaminants via flies to people leading to documented disease and tying disease to a contamination chain is scarce mainly due to methodological and ethical challenges. Current evidence suggests that bacteria are transmitted from flies to humans and animals in sufficient doses to cause disease. Therefore, it is likely that this is also true for antimicrobial-resistant bacteria. However, the evidence for this assumption is weak and no transmission studies between flies and humans/animals have been performed under controlled conditions. Nevertheless, several studies from North Africa [71], sub-Saharan Africa [73], and India [14] demonstrated carriage, vector and transmission potential of (often) antimicrobial-resistant bacteria (e.g. MRSA, ESBL-producing *Enterobacteriacae*) by *M. domestica* in hospital settings.

The Mediterranean moth fly *Clogmia albipunctata* (*Diptera*: *Psychodinae*), generally not considered as 'filth flies' in the strict sense, has spread towards Northern Europe and can be a pest in healthcare facilities. In a study amongst four infested German hospitals [90], medically relevant bacteria (e.g. *Acinetobacter baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*) were isolated from the intestinal tract and exoskeleton of *C. albipunctata*; however, no link was established between carriage/infestation and human infection. Some studies revealed that antimicrobial-resistant bacteria from flies, animals and humans shared the same genetic background [14,22,29,69,72,78], suggesting transmission. Since the design of these studies was cross-sectional, the direction of transmission remains speculative and only possible transmission routes can be highlighted [22]. The only evidence to date is that isolates from flies, animals and humans have a common source, which most likely includes transmission events that could be facilitated by flies. Mathematical modelling suggests, that the exposure of humans to ESBL-producing *E. coli* is higher through flies than through chicken fillet [20]. This QMRA, that used a worst-case approach, is valuable to assess the public health relevance of flies and the spread of AMR. However, results should be taken with caution, as several parameters were not included in this model (e.g. "colonization dose", lifespan of the vector, time between donor (poultry) and recipient (human), Table 3). Looking at the current evidence, we identified several knowledge gaps to finally answer the question to what extent flies contribute to the spread of antimicrobial resistance.

7. Conclusions

'Filth flies' carry antimicrobial resistant bacteria of clinical relevance particularly enteric bacteria, such as ESBL-producing *E. coli*. Laboratory studies suggest a "bio-enhanced" transmission since bacteria can multiply in the intestine, mouthparts and regurgitation spots. Several factors influence this transmission route (e.g. climate, fly density, geography, sanitary systems, food chain, vicinity of livestock farms and households). However, limited, data are available to determine whether flies can transmit antimicrobial-resistant bacteria, eventually leading to infections in animals and humans. Some studies have limitations, but those shortcomings are difficult to overcome (e.g. ethical issues of human or animal infection/colonization models). Assuming

Table 3
Knowledge gaps and research agenda.

Knowledge gaps	Research agenda
Models of risk assessment	To extend or refine existing quantitative microbial risk assessment (QMRA) models for AMR transmission between livestock and humans by 'filth flies' [20]. To develop new models to define the vectorial capacity of 'filth flies' and to identify high-risk areas for AMR transmission by these vectors.
Definition of relevant 'filth fly' species	To evaluate filth flies according to their risk for transmission of AMR. The knowledge about the most relevant 'filth fly' species is the basis for focused future research.
Quantification of bacterial translocation	To quantify the concentration (CFU/g) of clinically relevant antimicrobial-resistant bacteria in vomitus and faeces and to identify the factors that influence such concentrations.
Colonization dose	To estimate the concentration of antimicrobial-resistant bacteria required to establish colonization in humans or animals.
Fitness cost of resistant bacteria in flies	To compare the fate of antimicrobial-resistant and -susceptible bacteria in 'filth flies'. If the fitness-cost of AMR is irrelevant, available data from susceptible bacteria could be used for modelling.
Vector capacity	To measure the risk of transmission of AMR from flies to animals or humans under controlled conditions (ethical considerations and GCP-ICH guidelines apply).
Epidemiology	To perform field studies for a detailed epidemiological picture of the geographical spread and prevalence of antimicrobial resistant bacteria in flies. This should also include fly species introduced for biological control (e.g. *Ophyra* spp./*Hydrotea* spp. for manure management).
Transmission	To apply high-resolution sequence-based approaches in order to understand to what extent flies contribute to the spread of AMR in humans and animals.
Interventions	To identify and develop effective and feasible strategies for intervention (e.g. vector control, pest/manure/slurry management etc.) to contain the spread of AMR through flies.

Note: QMRA, quantitative microbial risk assessment; AMR, antimicrobial resistance; CFU, colony forming units; GCP-ICH, Good Clinical Practice-International Conference of Harmonization.

that antimicrobial-resistant bacteria are transmitted to humans and animals similarly to enteric pathogens, one can conclude that effective vector control might reduce the risk of transmission. To better understand the medical importance of AMR in flies, quantitative microbial risk assessment models should be further refined and fed with additional data (e.g. vectorial capacity of flies, colonization dose). This requires targeted ecological, epidemiological and *in vivo* experimental studies.

Funding

The work was supported by the German Federal Ministry of Education and Research (BMBF; grant no. 01KI1711), under the frame of JPIAMR. The funding source was not involved in the design, analysis and writing of this review.

Conflicts of interest

The authors declare no conflict of interest.

Author contribution

All authors conceptualized the review, extracted and interpreted data; FS drafted the first version of the manuscript; all authors critically revised the draft; all authors approved the final version of the manuscript for submission.

Acknowledgement

Christina Helmer designed Figs. 1 and 2. We thank the Advisory Reference Group (Robin Köck (University of Münster, Germany; European Medical School Oldenburg-Groningen, Germany), Karsten Becker (University Hospital Münster, Germany) and Alexander W. Friedrich (University Medical Center Groningen, the Netherlands)) for the valuable comments.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tmaid.2018.02.007.

References

[1] Miller S. Flies and filth. Am J Publ Health 1914;4:512–4.
[2] Graczyk TK, Knight R, Gilman RH, Cranfield MR. The role of non-biting flies in the epidemiology of human infectious diseases. Microb Infect 2001;3:231–5.
[3] Davari B, Khodavaisy S, Ala F. Isolation of fungi from housefly (*Musca domestica* L.) at slaughter house and hospital in sanandaj. Iran. J Prev Med Hyg 2012;53:172–4.
[4] Kassiri H, Zarrin M, Veys-Behbahani R, Faramarzi S, Kasiri A. Isolation and identification of pathogenic filamentous fungi and yeasts from adult house fly (Diptera: muscidae) captured from the hospital environments in Ahvaz City, southwestern Iran. J Med Entomol 2015;52:1351–6.
[5] Graczyk TK, Knight R, Tamang L. Mechanical transmission of human protozoan parasites by insects. Clin Microbiol Rev 2005;18:128–32.
[6] Adenusi AA, Adewoga TO. Human intestinal parasites in non-biting synanthropic flies in Ogun State, Nigeria. Trav Med Infect Dis 2013;11:181–9.
[7] Nielsen AA, Skovgard H, Stockmarr A, Handberg KJ, Jorgensen PH. Persistence of low-pathogenic avian influenza H5N7 and H7N1 subtypes in house flies (Diptera: muscidae). J Med Entomol 2011;48:608–14.
[8] Haddow AD, Nasar F, Schellhase CW, Moon RD, Padilla SL, Zeng X, Wollen-Roberts SE, Shamblin JD, Grimes EC, Zelko JM, Linthicum KJ, Bavari S, Pitt ML, Trefry JC. Low potential for mechanical transmission of Ebola virus via house flies (*Musca domestica*). Parasites Vectors 2017;10:218.
[9] Stafford KC. Fly management handbook a guide to biology, dispersal, and management of the house fly and related flies for farmers, municipalities, and public health officials. New Haven, Connecticut, USA: The Connecticut Agricultural Experiment Station; 2008.
[10] Olsen AR. Regulatory action criteria for filth and other extraneous materials. III. Review of flies and foodborne enteric disease. Regul Toxicol Pharmacol 1998;28:199–211.
[11] Mayhew PJ. Why are there so many insect species? Perspectives from fossils and phylogenies. Biol Rev Camb Phil Soc 2007;82:425–54.
[12] Fotedar R. Vector potential of houseflies (*Musca domestica*) in the transmission of *Vibrio cholerae* in India. Acta Trop 2001;78:31–4.
[13] Farag TH, Faruque AS, Wu Y, Das SK, Hossain A, Ahmed S, Ahmed D, Nasrin D, Kotloff KL, Panchilangam S, Nataro JP, Cohen D, Blackwelder WC, Levine MM. Housefly population density correlates with shigellosis among children in Mirzapur, Bangladesh: a time series analysis. PLoS Neglected Trop Dis 2013;7:e2280.
[14] Fotedar R, Banerjee U, Samantray JC. Shriniwas. Vector potential of hospital houseflies with special reference to *Klebsiella* species. Epidemiol Infect 1992;109:143–7.
[15] Winpisinger KA, Ferketich AK, Berry RL, Moeschberger ML. Spread of *Musca domestica* (Diptera: muscidae), from two caged layer facilities to neighboring residences in rural Ohio. J Med Entomol 2005;42:732–8.
[16] Nazni WA, Luke H, Wan Rozita WM, Abdullah AG, Sa'diyah I, Azahari AH, Zamree I, Tan SB, Lee HL, Sofian MA. Determination of the flight range and dispersal of the house fly, *Musca domestica* (L.) using mark release recapture technique. Trop Biomed 2005;22:53–61.
[17] Fukuda A, Usui M, Okubo T, Tamura Y. Horizontal transfer of plasmid-mediated cephalosporin resistance genes in the intestine of houseflies *(Musca domestica)*. Microb Drug Resist 2016;22:336–41.
[18] Akhtar M, Hirt H, Zurek L. Horizontal transfer of the tetracycline resistance gene *tetM* mediated by pCF10 among *Enterococcus faecalis* in the house fly (*Musca domestica* L.) alimentary canal. Microb Ecol 2009;58:509–18.
[19] Lammerding AM, Fazil A. Hazard identification and exposure assessment for microbial food safety risk assessment. Int J Food Microbiol 2000;58:147–57.
[20] Evers EG, Blaak H, Hamidjaja RA, de Jonge R, Schets FM. A QMRA for the transmission of ESBL-producing *Escherichia coli* and *Campylobacter* from poultry farms to humans through flies. Risk Anal 2016;36:215–27.
[21] Zurek L, Ghosh A. Insects represent a link between food animal farms and the urban environment for antibiotic resistance traits. Appl Environ Microbiol 2014;80:3562–7.
[22] Wang Y, Zhang R, Li J, Wu Z, Yin W, Schwarz S, Tyrrell JM, Zheng Y, Wang S, Shen Z, Liu Z, Liu J, Lei L, Li M, Zhang Q, Wu C, Zhang Q, Wu Y, Walsh TR, Shen J. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nat Microbiol 2017;2:16260.
[23] Szalanski AL, Owens CB, McKay T, Steelman CD. Detection of *Campylobacter* and *Escherichia coli* O157:H7 from filth flies by polymerase chain reaction. Med Vet Entomol 2004;18:241–6.
[24] Fukushima H, Ito Y, Saito K, Tsubokura M, Otsuki K. Role of the fly in the transport of *Yersinia enterocolitica*. Appl Environ Microbiol 1979;38:1009–10.
[25] Mian LS, Maag H, Tacal JV. Isolation of *Salmonella* from muscoid flies at commercial animal establishments in San Bernardino County, California. J Vector Ecol 2002;27:82–5.
[26] Pava-Ripoll M, Pearson REG, Miller AK, Ziobro GC. Detection of foodborne bacterial pathogens from individual filth flies. Journal of visualized experiments. JoVE 2015:52372.
[27] Pava-Ripoll M, Pearson RE, Miller AK, Ziobro GC. Prevalence and relative risk of *Cronobacter* spp., *Salmonella* spp., and *Listeria monocytogenes* associated with the body surfaces and guts of individual filth flies. Appl Environ Microbiol 2012;78:7891–902.
[28] Chaiwong T, Srivoramas T, Sueabsamran P, Sukontason K, Sanford MR, Sukontason KL. The blow fly, *Chrysomya megacephala*, and the house fly, *Musca domestica*, as mechanical vectors of pathogenic bacteria in Northeast Thailand. Trop Biomed 2014;31:336–46.
[29] Wang YC, Chang YC, Chuang HL, Chiu CC, Yeh KS, Chang CC, Hsuan SL, Lin WH, Chen TH. Transmission of *Salmonella* between swine farms by the housefly (*Musca domestica*). J Food Protect 2011;74:1012–6.
[30] Singh B, Crippen TL, Zheng L, Fields AT, Yu Z, Ma Q, Wood TK, Dowd SE, Flores M, Tomberlin JK, Tarone AM. A metagenomic assessment of the bacteria associated with *Lucilia sericata* and *Lucilia cuprina* (Diptera: *Calliphoridae*). Appl Microbiol Biotechnol 2015;99:869–83.
[31] Wei T, Ishida R, Miyanaga K, Tanji Y. Seasonal variations in bacterial communities and antibiotic-resistant strains associated with green bottle flies (Diptera: Calliphoridae). Appl Microbiol Biotechnol 2014;98:4197–208.
[32] Doud CW, Zurek L. *Enterococcus faecalis* OG1RF:pMV158 survives and proliferates in the house fly digestive tract. J Med Entomol 2012;49:150–5.
[33] Joyner C, Mills MK, Nayduch D. *Pseudomonas aeruginosa* in *Musca domestica* L.: temporospatial examination of bacteria population dynamics and house fly antimicrobial responses. PLoS One 2013;8:e79224.
[34] Kumar NHV, Nayduch D. Dose-dependent fate of GFP-expressing *Escherichia coli* in the alimentary canal of adult house flies. Med Vet Entomol 2016;30:218–28.
[35] Fleming A, Kumar HV, Joyner C, Reynolds A, Nayduch D. Temporospatial fate of bacteria and immune effector expression in house flies fed GFP-*Escherichia coli* O157:H7. Med Vet Entomol 2014;28:364–71.
[36] Nayduch D, Cho H, Joyner C. *Staphylococcus aureus* in the house fly: temporospatial fate of bacteria and expression of the antimicrobial peptide defensin. J Med Entomol 2013;50:171–8.
[37] Kobayashi M, Sasaki T, Saito N, Tamura K, Suzuki K, Watanabe H, Agui N. Houseflies: not simple mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7. Am J Trop Med Hyg 1999;61:625–9.
[38] Skovgard H, Kristensen K, Hald B. Retention of *Campylobacter* (Campylobacterales: Campylobacteraceae) in the house fly (Diptera: muscidae). J Med Entomol 2011;48:1202–9.
[39] Chifanzwa R, Nayduch D. Dose-Dependent effects on replication and persistence of *Salmonella enterica* serovar typhimurium in house flies (Diptera: *muscidae*). J Med Entomol 2018 Jan 10;55(1):225–9.
[40] Wasala L, Talley JL, Desilva U, Fletcher J, Wayadande A. Transfer of *Escherichia coli*

O157:H7 to spinach by house flies, *Musca domestica* (Diptera: muscidae). Phytopathology 2013;103:373–80.
[41] Pace RC, Talley JL, Crippen TL, Wayadande AC. Filth fly transmission of *Escherichia coli* O157:H7 and *Salmonella enterica* to Lettuce, *Lactuca sativa*. Ann Entomol Soc Am 2017;110:83–9.
[42] Coleman REG, R.R.. Factors affecting the deposition of regurgitated materials onto a substrate by *Musca autumnalis*. J. Agr. Entomol 1987;4:185–8.
[43] Coleman RE, Gerhardt RR. Effect of temperature, relative humidity, and amount and concentration of trypticase soy broth on regurgitation by female face flies (Diptera: muscidae). Environ Entomol 1988;17:448–51.
[44] Sasaki T, Kobayashi M, Agui N. Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: muscidae) in the dissemination of *Escherichia coli* O157: H7 to food. J Med Entomol 2000;37:945–9.
[45] Romero A, Broce A, Zurek L. Role of bacteria in the oviposition behaviour and larval development of stable flies. Med Vet Entomol 2006;20:115–21.
[46] Schmidtmann ET, Martin PA. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. J Med Entomol 1992;29:232–5.
[47] Watson DW, Martin PA, Schmidtmann ET. Egg yolk and bacteria growth medium for *Musca domestica* (Diptera: muscidae). J Med Entomol 1993;30:820–3.
[48] Gingrich RE. Development of a synthetic medium for aseptic rearing of larvae of *Stomoxys calcitrans* (L.). J Econ Entomol 1960;53:408–11.
[49] Lysyk TJ, Kalischuk-Tymensen L, Selinger LB, Lancaster RC, Wever L, Cheng KJ. Rearing stable fly larvae (Diptera: muscidae) on an egg yolk medium. J Med Entomol 1999;36:382–8.
[50] Rochon K, Lysyk TJ, Selinger LB. Retention of *Escherichia coli* by house fly and stable fly (Diptera: muscidae) during pupal metamorphosis and eclosion. J Med Entomol 2005;42:397–403.
[51] Zurek K, Nayduch D. Bacterial associations across house fly life history: evidence for transstadial carriage from managed manure. J Insect Sci 2016;16:2.
[52] Talley JL, Wayadande AC, Wasala LP, Gerry AC, Fletcher J, DeSilva U, Gilliland SE. Association of *Escherichia coli* O157:H7 with filth flies (*Muscidae* and *Calliphoridae*) captured in leafy greens fields and experimental transmission of *E. coli* O157:H7 to spinach leaves by house flies (Diptera: *muscidae*). J Food Protect 2009;72:1547–52.
[53] Ahmad A, Nagaraja TG, Zurek L. Transmission of *Escherichia coli* O157:H7 to cattle by house flies. Prev Vet Med 2007;80:74–81.
[54] Macovei L, Miles B, Zurek L. Potential of houseflies to contaminate ready-to-eat food with antibiotic-resistant enterococci. J Food Protect 2008;71:435–9.
[55] Nayduch D, Noblet GP, Stutzenberger FJ. Vector potential of houseflies for the bacterium *Aeromonas caviae*. Med Vet Entomol 2002;16:193–8.
[56] Shane SM, Montrose MS, Harrington KS. Transmission of *Campylobacter jejuni* by the housefly (*Musca domestica*). Avian Dis 1985;29:384–91.
[57] Greenberg B. Experimental transmission of *Salmonella typhimurium* by housflies to man. Am J Hyg 1964;80:149–56.
[58] De Jesus AJ, Olsen AR, Bryce JR, Whiting RC. Quantitative contamination and transfer of *Escherichia coli* from foods by houseflies, *Musca domestica L.* (Diptera: *muscidae*). Int J Food Microbiol 2004;93:259–62.
[59] Lindeberg YL, Egedal K, Hossain ZZ, Phelps M, Tulsiani S, Farhana I, Begum A, Jensen PKM. Can *Escherichia coli* fly? The role of flies as transmitters of *E. coli* to food in an urban slum in Bangladesh. Trop Med Int Health 2018 Jan;23(1):2–9.
[60] Davies MP, Anderson M, Hilton AC. The housefly *Musca domestica* as a mechanical vector of *Clostridium difficile*. J Hosp Infect 2016;94:263–7.
[61] Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infect Dis 2008;8:159–66.
[62] Songe MM, Hang'ombe BM, Knight-Jones TJ, Grace D. Antimicrobial resistant enteropathogenic *Escherichia coli* and *Salmonella* spp. in houseflies infesting fish in food markets in Zambia. Int J Environ Res Publ Health 2016:14.
[63] Fischer J, San Jose M, Roschanski N, Schmoger S, Baumann B, Irrgang A, Friese A, Roesler U, Helmuth R, Guerra B. Spread and persistence of VIM-1 Carbapenemase-producing *Enterobacteriaceae* in three German swine farms in 2011 and 2012. Vet Microbiol 2017;200:118–23.
[64] Ranjbar R, Izadi M, Hafshejani TT, Khamesipour F. Molecular detection and antimicrobial resistance of *Klebsiella pneumoniae* from house flies (*Musca domestica*) in kitchens, farms, hospitals and slaughterhouses. J Infect Public Health 2016;9:499–505.
[65] Cornaglia G, Giamarellou H, Rossolini GM. Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis 2011;11:381–93.
[66] Rhouma M, Beaudry F, Thériault W, Letellier A. Colistin in pig production: chemistry, mechanism of antibacterial action, microbial resistance emergence, and one health perspectives. Front Microbiol 2016;7:1789.
[67] Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016;16:161–8.
[68] Guenther S, Falgenhauer L, Semmler T, Imirzalioglu C, Chakraborty T, Roesler U, Roschanski N. Environmental emission of multiresistant *Escherichia coli* carrying the colistin resistance gene *mcr-1* from German swine farms. J Antimicrob Chemother 2017;72:1289–92.
[69] Schaumburg F, Onwugamba FC, Akulenko R, Peters G, Mellmann A, Köck R, Becker K. A geospatial analysis of flies and the spread of antimicrobial resistant bacteria. Int J Med Microbiol 2016;306:566–71.
[70] Bouamamaa L, Sorlozano A, Laglaoui A, Lebbadi M, Aarab A, Gutierrez J. Antibiotic resistance patterns of bacterial strains isolated from *Periplaneta americana* and *Musca domestica* in Tangier, Morocco. J Infect Dev Ctries 2010;4:194–201.
[71] Rahuma N, Ghenghesh KS, Ben Aissa R, Elamaari A. Carriage by the housefly (*Musca domestica*) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospital and other urban environments in Misurata, Libya. Ann Trop Med Parasitol 2005;99:795–802.
[72] Ahmad A, Ghosh A, Schal C, Zurek L. Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community. BMC Microbiol 2011;11:23.
[73] Boulesteix G, Le Dantec P, Chevalier B, Dieng M, Niang B, Diatta B. Role of *Musca domestica* in the transmission of multiresistant bacteria in the centres of intensive care setting in sub-Saharan Africa. Ann Fr Anesth Reanim 2005;24:361–5.
[74] Literak I, Dolejska M, Rybarikova J, Cizek A, Strejckova P, Vyskocilova M, Friedman M, Klimes J. Highly variable patterns of antimicrobial resistance in commensal *Escherichia coli* isolates from pigs, sympatric rodents, and flies. Microb Drug Resist 2009;15:229–37.
[75] Solà-Ginés M, González-López JJ, Cameron-Veas K, Piedra-Carrasco N, Cerdà-Cuéllar M, Migura-Garcia L. Houseflies (*Musca domestica*) as vectors for extended-spectrum β-lactamase-producing *Escherichia coli* on Spanish broiler farms. Appl Environ Microbiol 2015;81:3604–11.
[76] Usui M, Shirakawa T, Fukuda A, Tamura Y. The role of flies in disseminating plasmids with antimicrobial-resistance genes between farms. Microb Drug Resist 2015;21:562–9.
[77] Usui M, Iwasa T, Fukuda A, Sato T, Okubo T, Tamura Y. The role of flies in spreading the extended-spectrum beta-lactamase gene from cattle. Microb Drug Resist 2013;19:415–20.
[78] von Salviati C, Laube H, Guerra B, Roesler U, Friese A. Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. Vet Microbiol 2015;175:77–84.
[79] Vriesekoop F, Shaw R. The Australian bush fly (*Musca vetustissima*) as a potential vector in the transmission of foodborne pathogens at outdoor eateries. Foodb Pathog Dis 2010;7:275–9.
[80] Wei T, Miyanaga K, Tanji Y. Persistence of antibiotic-resistant and -sensitive *Proteus mirabilis* strains in the digestive tract of the housefly *(Musca domestica)* and green bottle flies (*Calliphoridae*). Appl Microbiol Biotechnol 2014;98:8357–66.
[81] Petridis M, Bagdasarian M, Waldor MK, Walker E. Horizontal transfer of Shiga toxin and antibiotic resistance genes among *Escherichia coli* strains in house fly (Diptera: muscidae) gut. J Med Entomol 2006;43:288–95.
[82] Dolejska M, Duskova E, Rybarikova J, Janoszowska D, Roubalova E, Dibdakova K, Maceckova G, Kohoutova L, Literak I, Smola J, Cizek A. Plasmids carrying $bla_{CTX-M-1}$ and *qnr* genes in *Escherichia coli* isolates from an equine clinic and a horseback riding centre. J Antimicrob Chemother 2011;66:757–64.
[83] Wang H, Li H, Gilbert JA, Li H, Wu L, Liu M, Wang L, Zhou Q, Yuan J, Zhang Z. Housefly Larva vermicomposting efficiently attenuates antibiotic resistance genes in swine manure, with concomitant bacterial population changes. Appl Environ Microbiol 2015;81:7668–79.
[84] Zhang Z, Shen J, Wang H, Liu M, Wu L, Ping F, He Q, Li H, Zheng C, Xu X. Attenuation of veterinary antibiotics in full-scale vermicomposting of swine manure via the housefly larvae (*Musca domestica*). Sci Rep 2014;4:6844.
[85] Sproston EL, Ogden ID, MacRae M, Forbes KJ, Dallas JF, Sheppard SK, Cody A, Colles F, Wilson MJ, Strachan NJ. Multi-locus sequence types of *Campylobacter* carried by flies and slugs acquired from local ruminant faeces. J Appl Microbiol 2010;109:829–38.
[86] Levine OS, Levine MM. Houseflies (*Musca domestica*) as mechanical vectors of shigellosis. Rev Infect Dis 1991;13:688–96.
[87] Cohen D, Green M, Block C, Slepon R, Ambar R, Wasserman SS, Levine MM. Reduction of transmission of shigellosis by control of houseflies (*Musca domestica*). Lancet 1991;337:993–7.
[88] Hald B, Sommer HM, Skovgard H. Use of fly screens to reduce *Campylobacter* spp. introduction in broiler houses. Emerg Infect Dis 2007;13:1951–3.
[89] Bahrndorff S, Rangstrup-Christensen L, Nordentoft S, Hald B. Foodborne disease prevention and broiler chickens with reduced *Campylobacter* infection. Emerg Infect Dis 2013;19:425–30.
[90] Faulde M, Spiesberger M. Role of the moth fly *Clogmia albipunctata* (Diptera: Psychodinae) as a mechanical vector of bacterial pathogens in German hospitals. J Hosp Infect 2013;83:51–60.
[91] Liu Y, Yang Y, Zhao F, Fan X, Zhong W, Qiao D, Cao Y. Multi-drug resistant gram-negative enteric bacteria isolated from flies at Chengdu Airport, China. Southeast Asian J Trop Med Publ Health 2013;44:988–96.
[92] Rybaříková J, Dolejská M, Materna D, Literák I, Čížek A. Phenotypic and genotypic characteristics of antimicrobial resistant *Escherichia coli* isolated from symbovine flies, cattle and sympatric insectivorous house martins from a farm in the Czech Republic. Res Vet Sci 2006–2007;2010(89):179–83.
[93] Blaak H, Hamidjaja RA, van Hoek AH, de Heer L, de Roda Husman AM, Schets FM. Detection of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* on flies at poultry farms. Appl Environ Microbiol 2014;80:239–46.
[94] Zhang J, Wang J, Chen L, Yassin AK, Kelly P, Butaye P, Li J, Gong J, Cattley R, Qi K, Wang C. Housefly (*Musca domestica*) and blow fly (*Protophormia terraenovae*) as vectors of bacteria carrying colistin resistance genes. Appl Environ Microbiol 2018:84.
[95] Graham JP, Price LB, Evans SL, Graczyk TK, Silbergeld EK. Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. Sci Total Environ 2009;407:2701–10.
[96] Barro N, Aly S, Tidiane OC, Sababenedjo TA. Carriage of bacteria by proboscises, legs, and feces of two species of flies in street food vending sites in Ouagadougou, Burkina Faso. J Food Protect 2006;69:2007–10.
[97] Gupta AK, Nayduch D, Verma P, Shah B, Ghate HV, Patole MS, Shouche YS. Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica*

L.). FEMS Microbiol Ecol 2012;79:581–93.
[98] Keen JE, Wittum TE, Dunn JR, Bono JL, Durso LM. Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. Emerg Infect Dis 2006;12:780–6.
[99] Holt PS, Geden CJ, Moore RW, Gast RK. Isolation of *Salmonella enterica* serovar Enteritidis from houseflies (*Musca domestica*) found in rooms containing *Salmonella* serovar Enteritidis-challenged hens. Appl Environ Microbiol 2007;73:6030–5.
[100] McGaughey J, Nayduch D. Temporal and spatial fate of GFP-expressing motile and nonmotile *Aeromonas hydrophila* in the house fly digestive tract. J Med Entomol 2009;46:123–30.