

Roles of Flies in Bacterial Transmission, Maintenance, and Contamination as Vectors and Reservoirs

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ABSTRACT

Flies, especially non-biting flies, are recognized as vectors of various clinically relevant pathogens, including antimicrobial-resistant bacteria in human and veterinary medicine, and can lead to colonization and infection. Studies have attempted to clarify the role of flies for the dissemination and transmission of bacteria from various perspectives. However, most studies have only detected pathogens and antimicrobial-resistant bacteria from flies, and many studies have not shown concrete proof for the bacterial transmission, contamination, and infection in humans and animals. To clarify the bacterial transmission routes to humans and animals *via* flies, from various sources, the control measure must be considered. Additionally, quantitative analysis of the flies carrying bacteria and their bacterial transmission allows the assessment of the risk factors of fly-mediated infectious disease. In this mini-review, we introduce research about the origin, maintenance, and contamination of bacteria harbored by flies, and the trial strategy to prevent transmission of the bacteria. Furthermore, we suggest an effective way to prevent the bacterial transmission *via* flies, to better understand the important role of flies as vectors and reservoirs of microorganisms. This mini-review will be applicable to flies and other insects and animals, to improve the sanitary environments.

Keywords: Vector; Flies; Bacterial transmission

INTRODUCTION

Flies are frequently found in a wide range of habitats and move freely due to their strong ability to fly, sometimes covering distances as much as 10 km [1]. There are around 30,000 species of flies worldwide; however, non-biting flies, such as house and false flies, have been the focus as vectors of clinically relevant microorganisms in human and veterinary medicines [2]. Many reports have shown presence of pathogens and antimicrobial-resistant bacteria (ARB) in flies and suggested a possible dissemination in surrounding areas of hospital and farm environments; and the possible relationships between bacteria-carrying flies and infections in humans and animals [3-5]. Therefore, quantitative control measures to prevent bacterial dissemination *via* flies were required. However, many reports do not show the derivation, dynamics, quantities, and transmission of microorganisms carried by flies for assessing the risk of the bacterial dissemination. However, recently, some reports have shown the required results and, in this review, we

have introduced these studies and suggest topics for future research initiatives.

DERIVATION AND FATE OF BACTERIA IN FLIES

Flies can obtain many kinds of bacterial species from various sources (feces, water, and foods, among others) by eating or by direct contact [6], which can transfer pathogenic bacteria, critical for public health, on their body surface or lead to colonization of their alimentary canal (by ingestion) [7]. Many studies only reported the prevalence and characterization (susceptibility to antimicrobials and harboring antimicrobial resistance and virulence-related genes) of bacteria carried by flies, while the origin of these bacteria was not sufficiently explored. However, few studies have shown antimicrobial-resistant *Escherichia coli*, based on pulse-field gel electrophoresis-based genotypic analysis, that were isolated from flies and livestock feces/manures, thereby indicating the origin of bacteria where flies were the vectors [8-10]. Furthermore, studies have shown the plasmid-

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mediated horizontal transfer of antimicrobial resistance and bacteriophage-encoded Shiga-toxin genes in the intestine and on the mouth of houseflies using *E. coli* and *Enterococcus faecalis* [11,12]. Besides, this event can occur in the same bacterial species and also across genera [13]. These reports suggest that flies are not only a mechanical vector for pathogens and ARB but also act as a biological vector for their dissemination; thus, they elicit new pathogen transmission and ARB occurrence through acquiring virulence-related and antimicrobial resistance genes in/on the flies.

The determination of fate of bacteria in/on flies was conducted by bacteria ingestion of adult flies, and these studies suggested that the intestines and surfaces of flies were not a good environment for the several bacteria species, because of an exponential decrease of the midgut epithelium, enzymes, and antimicrobial peptides of flies [7]. Whereas, other studies showed the initial decrease of bacteria but a significantly increase thereafter [14,15]. Generally, ARB strains show inferior growth compared to wild types without antimicrobial selective pressure. Wei et al. showed that the conditions in the flies' intestines may allow resistant strains to survive the competition with sensitive strains using *Proteus mirabilis* [16]. The amount and frequency of bacterial survival in/on flies are influenced by a variety of factors, including bacterial species, density, temperature, relative humidity, and reproductive status.

Flies undergo a complete metamorphosis after laying eggs, then mature into the adult flies. During this life cycle, the microbiota of flies is affected and changes due to various factors [17]. Despite going through such a developmental life cycle, enteric pathogens and ARB, and their related antimicrobial resistance genes, persist in each life cycle stage (adult flies, eggs, maggots, and pupae). Further, the bacteria are finally transmitted vertically from adult flies to the next generation of adult flies [18,19]. These studies further support the notion that flies can act not only as a vector for spreading but also as a reservoir for maintaining bacteria including pathogens and ARB.

Compared to the fate of bacteria in the intestine of flies, investigation on the flies' surface (body, mouth, leg, and wing) has been less studied. The surface constantly and directly touches various environments, and bacteria can be bidirectionally transmitted.

DISSEMINATION AND CONTAMINATION OF FLIES CARRYING BACTERIA

Flies have the potential to pose a bacterial contamination risk through direct contact because of carrying bacteria on their surface; thus, through touch, in the crop by vomit, and the alimentary canal by excretion [7]. A scenario for flies' bacterial transmission to humans and animals is by flies contaminating food products with their carried bacteria, which is subsequently eaten by the human or animal. In several studies, laboratory bioassays showed the quantitative analysis of food contaminated with *E. coli*, through fly transmission, on a spinach leaf, rice, steak, potato salad, sugar and milk mixture, apple, and castella (a sponge cake); *E. faecalis* on a beef hamburger patty; and *Aeromonas caviae* on chicken meat [13,20-24]. These studies were conducted by calculating the bacterial colony forming unit

(CFU) on food material which was derived from flies exposed to a defined concentration of bacteria or captured from fields. Bacterial food contamination by flies depended on various factors (number of flies and contacts, bacterial load of flies, contact time, the attractiveness of food material to flies, and bacterial species). Depending on the number of flies, an increase in bacterial contamination was observed; 3.1×10^3 and 2.8×10^4 CFU of *E. faecalis* were transmitted by five and forty flies, respectively, to 1 g of beef hamburger patty within 30 min [20]. The number of flies landing on food material was measured to assess bacterial contamination through direct contact; 103 CFU of *E. coli* was approximately transferred from flies to rice, steak, potato salad, and sugar and milk mixture [21,22]. Additionally, this study calculated that flies had potential to contaminate the clean surfaces with approximately 0.1 mg of food per landing [21]. Degree of bacterial contamination was positively related with a fly's bacterial load, which was assessed using the quantification of bacterial load in flies after ingestion of a defined concentration of bacteria [23]. A higher and rapid concentration of bacterial contamination occurred with higher bacterial load-carrying flies; 106-8 CFU of *E. coli* transferred 103-4 CFU/g of the bacteria to the food material within 5 minutes; further 101-2 CFU of *E. coli*-carrying flies transferred 101 CFU/g of bacteria to the food material within 60 minutes. Additionally, a longer contact time between the flies and food material caused the increase of food contamination (concentration and detection rate of bacteria in food material). Feeding habits of the flies, and the food materials chosen, whether attracting flies or not, were closely linked to flies contact and food material; therefore, this is a considerable risk factor for food contamination [25]. One study showed that 288 CFU of *Clostridioides difficile*, a spore-forming anaerobe in healthcare-associated infection, were transmitted to agar within 1 hour, after flies were exposed to 105 CFU of the spore, thus showing that the transmission from the flies was less effective than other studies [24].

Flies could contaminate the various materials with the bacteria they carry, caused by direct touch (landing on the food and non-food surfaces). To analyze the risk of bacterial contamination by flies, it is not enough to detect contamination, but it is also necessary to quantify the contamination level (concentration, time lapse, and frequency). Quantification analysis may expose risk factors more clearly; this leads to consider the strategy for the prevention and control of bacterial transmission and contamination by flies. In addition, more effort is needed to clarify the related factors of contamination by flies in laboratory and field situations.

CONTROL OF FLIES TO MANAGE MICROORGANISMS

Flies have long been considered as a disease-causing insect and as a vector to disseminate and transmit pathogens, especially in intestinal tract-related infectious diseases [6-15]. Moreover, flies rapidly multiply, especially in comfortable environments, due to the high reproductive capacity of female adult flies and the ability to lay 150 eggs during each gonotrophic cycle, and 500 eggs during each life cycle (approximately 2 weeks) [2,26]. However, there is lacking evidence that infectious diseases in humans and animals are caused by flies transmitting pathogens.

In epidemiological studies, a relationship between the flies' density and the number of the shigellosis patients was suggested that there were peaks of shigellosis incident after that of flies [27,28]. Few practical field studies have shown an attempt to control flies and provide effectual strategy to reduce infections on humans and animals. In farm situations, using fly screens reduced the prevalence of *Campylobacter* spp. among broiler flocks by preventing influx of flies into the broiler houses [29]. Whereas, introducing a bait and trap strategy in human living areas, the number of flies and shigellosis patients dropped by 64% and 85%, respectively [30]. Concomitantly, number of clinical visits, patients of diarrheal disease, and retention of antibody for enterotoxigenic *E. coli* dropped by 42%, 85%, and 57%, respectively. These strategies were effective for flies and particular pathogen-related infections plus other vectors such as insects and animals, such as cockroaches and mice, and microorganisms [3-29]. Additionally, awareness toward hygiene management would be improved through the control of flies. Therefore, a need to clarify the intermediary bacterial contamination sources from flies and the suggestion to prevent the bacterial transmission effectively is needed. Furthermore, the need exists to create an environment less prone to attract insects and animals that act as vectors daily.

CONCLUSION

Flies carry various kinds of bacteria including pathogens and antimicrobial-resistant bacteria (ARB). Field and laboratory studies have shown potential to maintain and disseminate bacteria. However, the proof of the chain of process where flies gain bacteria from sources, maintain the bacteria, contaminate food and non-food surfaces, and transmit and cause infections in humans and animals is limited. Especially, the process of causing infections by flies carrying bacteria was only indicated through circumstantial evidence. Flies almost certainly play a role as vectors and reservoirs of clinically important bacteria.

However, more research is needed to investigate the transmission chain, for effective control to reduce the dissemination, and to quantify microbial risk assessment, to clarify, and to remove risk factors. Lastly, hygiene management, including the control of vectors, is important for public, human, and animal health.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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