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Are Houseflies Still Important Vector of Gastrointestinal Infections?

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Abstract

The housefly, *Musca domestica*, is a common widely distributed insect. This study aims at assessing the role of houseflies as a vector for transmission of bacteria. In this study 100 houseflies were collected and examined. Seventy five percent flies carried no bacteria, 20% carried coliform bacteria, while five percent flies carried more than one type of bacteria. Thirty two isolates (*E. coli* 25%, *Citrobacter* spp 18.75%, *Klebsiella pneumoniae* 15.63%, *Enterococcus* spp 12.5%, *Staphylococcus aureus* 12.5%, Coagulase negative *staphylococcus* 12.5% and *Proteus mirabilis* 3.12%) were recovered. Classical enteric pathogenic bacteria like *Salmonella*, *Shigella* and Vibrio were not isolated from any fly. There is no human open field defecation but a number of cattle, poultry farms and agriculture land where animal manure is used is present within the flying range of the houseflies. Since, the coliforms are normal gastrointestinal flora of human, animals and birds, it can be surmised that the surrogate markers (coliform organisms) have been carried by the houseflies from the excreta of farm animals, poultry or animal manure. The study reveals that the houseflies can play a significant role in transmission of enteric bacterial pathogens. However, transmission can be prevented by maintenance of good hygiene and sanitation.

Keywords: *Musca domestica*; Sanitation; Surrogate markers

Introduction

The housefly, *Musca domestica*, is one of the most common and widely distributed insects found all over the world. Since houseflies as the name suggests, cohabit with humans, poultry, and animals, these can readily contaminate food and utensils. These are considered important mechanical vector for number of pathogenic bacteria, protozoa, metazoan, fungi and viruses [1]. Microorganisms are picked up by the flies from garbage, sewage and other sources of filth on their mouthparts and other body parts, and then transferred to human and animal food. Houseflies also transmit diseases by regurgitates and feces [2].

The major diseases that are associated with the housefly as a vector include Shigellosis, Salmonellosis, Cholera, Hepatitis A and E, Polio, Amoebic dysentery, parasitic worms and eye infections (trachoma and epidemic conjunctivitis) [3]. Most of these microorganisms are excreted in feces of human, animals and birds. The microorganisms that stick to the outside surfaces of the fly may survive only for a few hours, but those that are ingested with the food may survive in the fly's gut for variable period [4]. Transmission takes place when the fly makes contact with people or their food. Most of the diseases can also be contracted through contaminated food, water, air, hands and person-to-person contact. Faecal contamination of food and water is detected by surrogate markers, *E. coli* and other coliforms [5]. This study was conducted to determine the presence of surrogate markers of fecal contamination on individual houseflies.

Material and Methods

This prospective, cross sectional study was conducted from April 2013 to June 2013, in the Department of Microbiology, Manipal College of Medical Sciences (MCOMS), Pokhara, Nepal. Houseflies were collected from the college canteen ground, kitchen and dining hall of the college at Deep campus. There was no open field defecation, open toilets, or garbage waste collection in the vicinity of the collection area. The area is maintained clean and hygienic by periodic daily cleaning and mopping.

A total of 100 houseflies were collected and sampled. The houseflies were collected using the electric pest killer bats and pest killer machine. The electric pest killer bat and pest killer machine tray were sterilized by spirit swab before use. Care was taken not to roast the housefly while trapping them. Each killed housefly was put into separate sterile test tube using sterile tweezers. These test tubes were labeled and 5ml sterile peptone water was added to each test tube. The test tubes were incubated at 37°C for 2 h. After incubation, the samples were inoculated into MacConkey's Agar and incubated at 37°C for 48 h. The growth was identified by standard microbiological procedures such as colony morphology, Gram's staining, growth on differential media, motility and conventional biochemical tests. Antibiotic sensitivity tests were performed by modified Kirby Bauer disc diffusion method.

Results

A total of 100 houseflies were collected and examined. Various species of bacteria were recovered from 25 out of 100 flies Table 1.

Number of houseflies	Bacterial isolates
No. of houseflies from which bacteria were isolated	25
No. of houseflies from which no bacteria were isolated	75
Total no. of houseflies collected	100

Table 1: Number of houseflies and bacterial isolates.

S. No	Bacterial isolates	Number	Percentage
1	*E. coli*	8	25%
2	*Citrobacter* spp	6	18.75%
3	*Klebsiella* spp	5	15.63%
4	*Proteus mirabilis*	1	3.12%
5	*Staphylococcus aureus*	4	12.50%
6	*Enterococcus* spp	4	12.50%
7	Coagulase negative *Staphylococcus* spp	4	12.50%
	Total	32	100%

Table 2: Bacterial isolates and their percentage.

Only 6% (6 out of 100) flies were found to carry more than one type of bacteria. Majority 62.5% (20 out of 32 isolates) were Gram Negative bacilli (*E. coli* 8, *Citrobacter* spp 6, *Klebsiella* spp 5, and *Proteus mirabilis* 1), while 37.5% (12 out of 32 isolates) were gram positive (*Enterococcus* spp 4, *Staphylococcus* aureus 4, and Coagulase negative *staphylococcus* 4). Classical enteric bacterial pathogens like *Salmonella*, *Shigella* and Vibrio were not isolated from any fly. Surrogate markers of fecal contamination were carried by 24% flies (24 out of 100 houseflies). Most of the Gram negative bacilli isolated from the flies were susceptible to majority of commonly prescribed antibiotics Table 2. A small number of Gram negative bacteria showed resistance to different antibiotics Table 3 and Table 4. Ampicillin, Cefazolin and Nitrofurantoin resistance were shown by 2 *E coli* and 1 *Klebsiella* spp and 1 *Citrobacter* spp. Three organisms showed resistance to all antibiotics (*E coli* 2, *Enterococcus* spp 1).

S. No.	Name of the isolate	Number of isolates	Antibiotic Resistance pattern							
			AMP	GEN	CIP	CZ	COT	NIT	NX	OF
1	*E. coli*	8	6	2	2	5	2	5	2	1
2	*Citrobacter* spp	6	5	0	1	5	0	2	0	-
3	*Klebsiella pneumoniae*	5	4	0	0	2	1	5	0	-
4	*Proteus mirabilis*	1	0	0	0	1	1	1	0	-
	Total	20	15	2	3	13	4	13	2	1

Table 3: Antibiogram of gram negative isolates.

S. No	Name of the isolates	Number of isolates	Antibiotic resistance pattern										
			AMP	GEN	CIP	CZ	P	AK	E	CN	CTR	OX	CX
1	*Enterococcus* spp	4	2	1	2	0	2	0	2	2	1	0	0
2	*Staphylococcus aureus*	4	0	0	0	0	1	0	4	0	0	0	0
3	Coagulase negative *Staphylococcus* spp	4	0	0	0	0	1	0	2	0	0	0	0
	Total	12	2	1	2	0	4	0	8	2	1	0	0

Table 4: Antibiogram of gram positive isolates.

Discussion

The housefly, *Musca domestica*, is one of the most common insect found worldwide. These have been considered as vector of microorganisms in the household as well as hospital settings. The houseflies can pick up microorganisms from environment and infect human beings by contaminating wounds, eyes and food. The role of houseflies in spreading gastrointestinal infections is dependent on their habit of visiting fecal material for oviposition. During oviposition, the houseflies' legs, mouthparts, hair and wings get contaminated with enteric pathogens. The housefly then may drop these pathogens on unprotected food and utensils, thereby facilitating the entry of these organisms in human body. Spread of such infectious agents is directly dependent on the number of houseflies, availability of feces, presence of pathogens in the fecal material, microorganism carrying capacity of each housefly, antimicrobial substances present on the surface of houseflies, and access of houseflies to unprotected food and utensils. The pathogen transmission cycle can be broken at various stages. It is important to determine the role of each factor to devise intervention.

Improved sanitation and hygiene has brought changes in the society. Abolition of open field defecation, provision of sanitary toilets has led to dwindling of breeding places of houseflies. Consequently the opportunities for the houseflies to carry human enteric pathogens also have decreased. This study was undertaken to assess the importance of houseflies as vector of intestinal pathogens in view of these environmental improvements in Pokhara.

There have been number of studies on role of house flies in spread of pathogens [1,6-14]. All these studies need to be interpreted in context of the location of collection of houseflies. Most of the studies

on houseflies have collected houseflies from well-established unhygienic breeding places such as public toilets, garbage dumps etc [6,10,11]. In our study the houseflies were collected from hygienic area (mess and canteen). Some amount of food waste and decaying organic garden waste is available near this study area for the flies to feed and oviposit. There are no human open field defecation or open toilets within the fly zone (8 km radius) of the study area [15]. Hence the chances of houseflies visiting human excreta are nil. A number of poultry farms, cattle sheds and farms where animal manure is frequently stored and used exist near the study area. The flies could obtain their bacterial load from these places (the food waste storage places or from nearby farms). The coliforms are normal gastrointestinal flora of human, animals and birds and the surrogate markers (coliform organisms) have perhaps been carried by the houseflies from the excreta of farm animals, poultry or animal manure. The antibiotic resistance among some isolates may have occurred due to the use of antibiotics in the poultry farm feeds.

The protocol was designed to study how many flies carried the bacteria, types of bacteria carried by each fly, whether the house fly carried enteric pathogens or surrogate markers of fecal contamination or not and antibiogram of all isolates. This determined the role of each housefly in carriage of enteric pathogens.

The number of house flies and their activity depend on ambient temperature and humidity. To ensure that the active houseflies are included in the study, 25 houseflies per day were collected and processed on 4 different days when the meteorological conditions were optimum for housefly activity. The profile of organisms isolated on each day from these houseflies was found to be similar. This validates that the bacterial carriage by the houseflies did not vary on different study days.

The bacteria were isolated from only 25 houseflies (25/100). Earlier studies have not dwelled on this aspect. [7,8,10,12] Lamiaa Bouamama et al. and Ugbogu et al. [8,10] pooled the flies and the isolated bacteria were studied. This pooling of data did not reflect which housefly carried the bacteria and which housefly did not. It could not answer the role played by individual housefly in transmission of bacteria. Thus it could not be used to determine the risk associated with increased or decreased number of houseflies. Why 75 houseflies in our study did not carry any bacteria needs consideration. The flies, both male and female, feed on organic matter like food waste and are likely to carry bacteria from these sites. These bacteria could be of wide variety, but unlikely to be human enteric pathogens. Most of the insects have their own flora and produce substances and bacteriophages to eliminate and control population of other microorganisms [16]. An interesting reference to this is found in an ancient writing where a muslim prophet had mentioned "If a house fly falls in the drink of anyone of you, he should dip it (in the drink), for one of its wings has a disease and the other has the cure for the disease" [17].

Non isolation of bacteria from 75 house flies could be because of the effective antibacterial substances present on these houseflies. Twenty five houseflies that were identified as carriers of bacteria perhaps had less effective antibacterial mechanisms or had picked up higher load of bacteria. The higher number of bacteria could have been picked up by these houseflies if these had visited spots with higher bacterial load (decaying organic waste, poultry and cattle waste, manure pits in the surrounding farms). The female houseflies are more likely to visit such spots in search of suitable place for laying the eggs. Hence the female houseflies are likely to carry higher bacterial load. The normal male: female ratio amongst houseflies is 1:1; but the gender ratio of the flies can vary depending on various factors including climatic and seasonal variations [14,18]. Vector competence of insect vectors is also affected directly or indirectly by environmental factors explaining some of the seasonal variation in epidemics of human pathogens [19]. This study however, has only been conducted in a single season. It is possible that the 25 carrier flies were female while remaining 75 flies were male and did not visit decaying organic waste, poultry and cattle waste, manure pits in the surrounding farms. We did not determine gender of the collected houseflies in this study. Whether the antibacterial activity is more effective in male houseflies than female also needs to be considered.

Thirty two bacterial isolates were recovered from 25 houseflies. More than one type of organisms were isolated from only 6 houseflies. This reflects that the houseflies have effective antibacterial mechanism that keeps the bacterial population in check. Only 24 out of 100 (24% houseflies) carried surrogate markers of fecal contamination. The most prevalent organism amongst these was *E. coli*. These 24 out of 100 houseflies could be potential carriers of human pathogens. The probability of transmission of human pathogens by number of houseflies out of these 24 will further decrease as all of them are unlikely to get adequate load of pathogens. The risk of houseflies as vector of enteric pathogens in this location is quite small.

Vazirianzadeh et al. and Hamid Kassiri et al. [1,6] also had encountered *E. coli* as the commonest organism in their study. Though the bacterial profile in our study was very much similar to the organisms isolated in a study conducted in Malaysia [11] the number of bacterial isolates cannot be compared. The geographical and sanitary parameters of these studies differed from present study. In the above quoted studies, the flies were collected from potentially unhygienic places, and bacterial carriage by individual flies was not studied but the data was pooled.

In a study in Libya, multi-drug resistant pseudomonas was found in >50% cases and MRSA was found in 1.3% cases [12]. However, in our study 3 organisms were found to be multidrug resistant (2 *E. coli*, 1 *Enterococcus* spp). No *Pseudomonas* was isolated in our study. Most of the coliforms isolated were resistant to ampicillin but Methicillin resistant *Staphylococcus aureus* was not isolated.

Classical pathogens like *Salmonella*, *Shigella* and Vibrio were not isolated from any fly unlike the other studies [7,9,10,13]. Most of the samples in these studies were collected from more than one place or from declared unhygienic areas like dumping sites and public toilets [7,9,10,13]. In our study, flies were collected only from the hygienic eatery at college. The bacteria were isolated from each housefly separately unlike in other studies where houseflies from a site were pooled and considered as a single sample. In our study individual houseflies were collected with aseptic precautions unlike in other studies.

Conclusion

Gastrointestinal infections are mainly transmitted by faeco-oral route. Presence of the surrogate markers of fecal contamination on housefly is an indicator of their role as potential vector. But, its role in transmission of diseases in a well sanitized hygienic area seems to be minimal. Maintenance of good hygiene and sanitation and fly proofing can decrease the transmission of diseases.

Limitations

1. The samples were collected from a hygienic eatery at Manipal college premises only. Areas other than that were not included.

2. Only bacteriological assessment was carried out.

3. Molecular typing of the isolates was not performed.

4. Quantitative analysis of the bacterial load carried by the housefly was not done.

5. The gender of the carrier flies was not determined.

6. Antibacterial mechanisms in the fly were not studied.

Conflict of Interest:

The authors declare no conflict of interest.

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