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Bacteria fauna from the house fly, *Musca domestica (L.)*

Nazni, W.A., Seleena, B., Lee, H.L., Jeffery, J.[1], T. Rogayah, T.A.R. and Sofian, M.A.[2]
Medical Entomology Unit, Infectious Disease Research Centre, Institute For Medical Research, Jalan Pahang 50588 Kuala Lumpur.
[1]Department of Parasitology and Medical Entomology, Faculty of Medicine, University Kebangsaan Malaysia, Jalan Raja Muda, Kuala Lumpur.
[2] Institute Biology Science, University Malaya, 50603 Kuala Lumpur, Malaysia.

Abstract. The house fly, *Musca domestica* has long been considered a potential agent for disease transmission ever since its existence. The general truth of this assertion remains undisputed till the present day in spite of increasing awareness toward an improved sanitation and better hygiene. The habitual movement of house fly from filthy substrata such as human faeces, animal excreta, carcasses, garbage, etc. makes them ideal candidates for disease transmission such as cholera, shigellosis, salmonellosis and others when settling on food. Fly as a potential mechanical vector of pathogenic bacteria was elucidated in this study by examining flies from various breeding sites such as food courts, dumping ground, food processing areas and poultry farm in Peninsular Malaysia. The flies were baited with 10% sugar solution on a glass slide in the field. All materials used for collection of samples were sterile. Bacteria from fly sample were isolated using the normal isolation technique. *Bacillus* sp., *Coccobacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Enterobacter* sp., *Proteus* sp., *Escherichia* sp., *Klebsiella* sp. and yeast cells were isolated from feaces, vomitus, external surfaces and internal organs of house fly. Newly emerged house fly did not harbour any bacteria.

INTRODUCTION

The behavioural characteristics of the house fly *Musca domestica*, ensure its contact with food and wastes of man and his animals (Gupta *et al.*, 1972). In this manner the house flies are able to transport pathogenic organisms from infected materials to humam.

Studies by several researchers (Axon, 1995; Hulten *et al.*, 1996) have indicated that there are 3 different possible modes of bacterial transmission by flies. A confirmatory study by Kelly *et al.* (1994) and Thomas *et al.* (1992), have shown the isolation of viable bacteria from feaces, thus, suggesting that the feacal-oral route of transmission seems feasible. Tan *et al.* (1997) in Malaysia conducted a study on mechanical transmission of rotavirus by the legs and wings and stated that house fly can transmit the rotavirus depending on which part of the fly body the virus was found. According to De Jesus *et al.* (2004), flies can contaminate clean surfaces with approximately 0.1mg of food per landing.

Synanthropic flies are major epidemiologic factors responsible for the spread of acute gastroenteritis, trachoma among infants and young children in developing countries and transmission of nosocomial infections with multiple antibiotic-resistant bacteria in hospital environment (Graczyk *et al.*, 2001). The role of the house fly in the transmission of pathogens and gastrointestinal diseases such as shigellosis, salmonellosis, cholera, and yaws has been firmly established (Greenberg, 1971).

Structurally, the fly is well adapted for picking up pathogens. Its proboscis is provided with a profusion of fine hairs that readily collect environmental detritus. Furthermore, each of the six feet of the fly is fitted with hairy structures and pads that

secrete a sticky material, thus adding to its pathogen transmission potential. It is therefore not surprising that as many as 6 x 10^6 bacteria have been found on the exterior surface of a single feeding fly (Esten & Mason, 1908) and more than 100 species of pathogenic organisms have been isolated from the digestive tract of flies (Harwood & James, 1979). Pathogenic bacteria remain alive in house flies for an appreciable time (Richards, 1961).

Fly can swallow liquid food, it usually regurgitates ingested material in order to liquefy solid materials to facilitate digestion. In addition , droplets of faeces may be deposited during the feeding process. This remarkable behaviour of flies in which excreta is deposited may particularly contribute to their ability to spread bacterial infection.

Faichnie (1909) was able to show a correlation between house flies and enteric fever and flies were carrier of *Salmonella typhosa* and *S. paratyphi A* and stated that these micro-organisms remain alive for many days in the flies. Flugge (1893) and Buchanan (1897) have already indicated that flies were transmitters of cholera but Flu (1915) was the first to isolate *Vibrio cholera* from flies.

Grubel *et al.* (1997) has stated that house flies probably can act as vectors in the transmission of *Helicobacter pylori* if they carry the bacterium and contaminate human food. *H. pylori* infection is one of the commonest chronic bacterial infections of humans and affects most populations throughout the world.

Study conducted by Esrey (1991) and Cohen *et al.* (1991) suggested that there was a correlation between fly population and diarrhoea and diarrhoea and shigellosis incidence, respectively. Another study by Emerson *et al.* (1999) showed that fly control could reduce trachoma and diarrhoea among children in Gambia. Pruss & Mariotti (2000) suggested that the basis of trachoma was through person-to-person contact and flies appear to constitute the major transmission pathways.

With such emphasis given to flies as a mechanical vector in the spread of diseases, hence, the objective of this study was to study the microbial fauna found in association with the house fly, *M. domestica* under the tropical environment.

MATERIAL AND METHODS

Collection Sites

Sample were collected from food courts, dumping ground, food processing and poultry farm areas from Langkawi Island (Kedah), Perak, Johor, Terengganu, Kelantan, Selangor and the Cameron Highlands. In this study two different methods of collection were employed in obtaining the samples as mentioned below.

Collection Method Using Sugar Solution

The flies were baited with 10% sugar solution on a glass slide in the field. The slides were placed in areas with high density of flies. On each slide three spots of 100 uL of autoclaved sugar solution were provided. All materials used for collection of samples were autoclaved prior to use in the field. It was observed that the sugar solution was consumed by the flies leaving specks of vomitus or faeces on the slide. These specks of faeces and vomitus were carefully flushed into separate sterile 1 mL eppendorf tubes using 500 uL of sterile saline solution and were brought to laboratory and kept at 4°C until further analysis.

Adult flies caught from the field were directly placed into 1 mL eppendorf tube with 500 uL saline and were shaken for at least 2 min. These were also preserved at 4°C.

Collection Method Using Insect - Net

Adult wild flies *M. domestica* were collected from a poultry farm in Kundang, Selangor to determine the presence of microbes on and in the body of the house flies. The house flies were brought to the laboratory and were killed by placing them

in –20°C and were identified. Ten flies were placed individually in test tubes containing 5 mL peptone water and another 10 flies were dissected with sterile dissecting needle and the gut content of each individual fly was placed in test tubes containing 5 mL sterile peptone water. Similarly, 10 adult flies that newly emerged from pupa were individually placed into 5 mL peptone water. The samples were left at room temperature for 2 to 5 hours before being processed.

Isolation of Bacteria from Samples

Bacteria from fly sample collections were isolated using the normal isolation technique. Three hundred microliter of the faeces and vomitus solution were inoculated on nutrient agar and blood agar plates. The solutions were then spread evenly on the agar plates to ensure even growth of bacteria. The nutrient agar and blood agar plates containing the bacteria were incubated aerobically at 35°C for 72 hours. The cultures were then observed daily for growth and all bacterial colonies were subcultured onto corresponding media and further incubated for 7 days. The colonies were streaked repeatedly on the corresponding growth medium until pure colonies were obtained. Pure isolates were then maintained on the appropriate agar slant and stored at 28 C. The bacteria were identified to genus level by colony morphology, texture and Gram staining.

Adult samples collected from poultry farm from Kundang were isolated as stated below. A loopful of samples in 5 ml peptone water was streaked onto Blood Agar and MacConkey agar plates and incubated at 37°C overnight. Three single isolates were then picked from these culture plates and were identified on the basis of triple sugar iron, motility, urease production and sugar reactions following the methods of Cowan & Steel (1993) and confirmed by using the Enterotube procedures identification guide (Roche Diagnosstics, Nutley, New Jersey).

RESULTS

Bacteria isolated from the house flies were from the faeces, vomitus, the external body surface and the internal gut content. Nutrient agar medium as well as Blood Agar medium gave the same type(s) of bacterial growth, hence the bacteria cultures were plated and maintained on Nutrient Agar media.

Faecal and vomitus samples were obtained from anchovies factory, dumping ground, food restaurant and snack (fish cracker) factory. The bacteria isolated from these sites and poultry farm in Kundang, Selangor are as presented in Table 1 and Table 2, respectively. The bacteria identified to the genus level were *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp. and *Streptococcus* sp. There were a number of gram +ve coccobacilli isolates. Most of the bacteria isolated were gram positive with the exception of few gram negative. In some sites yeast cells were also isolated.

From the total isolates, 41% were *Bacillus* sp. and 37.3% were coccobacillus isolates. The *Staphylococcus* sp. were isolated from vomitus sample of house flies from an anchovies factory in Langkawi, Kedah and also from house flies from fish associated with the snack factory from Kelantan. The *Streptococcus* sp. were isolated from the external body surface of the house flies from the Cameron Highlands, Pahang.

The gram negative bacteria were generally from house flies in food restaurant in the Cameron Highlands. Only one gram negative bacteria was isolated from feaces of house flies from fish in the snack factory in Kelantan.

Isolation of bacteria from house flies in poultry farm in Kundang, Selangor showed that 4 to 5 different genera have been obtained from the external body surface and from the gut contents. The bacteria isolated from the external body surface of the house flies were *Acinetobacter* sp, *Bacillus* sp., *Enterobacter* sp. and *Proteus* sp. The most common bacterial species

Table 1. Bacteria isolated from vomitus, faeces and external body surface of *M. domestica* collected from various sites in Peninsular Malaysia

Collection Sites	Origin of Sample From Fly	*Micro-organisms isolated
1. Langkawi		
(Anchovies Factory)	vomitus	Spore forming gram +ve rods – *Bacillus sp.* (10)
(Anchovies Factory)	vomitus	*Staphylococcus sp.*
(Anchovies Factory)	vomitus	*Micrococcus sp.*
(Anchovies Factory)	vomitus	Gram + ve cocorods (6)
(Anchovies Factory)	vomitus	Yeast Cells
(Anchovies Factory)	vomitus	Unknown sp.
(Anchovies Factory)	faeces	Spore forming gram +ve rods - *Bacillus sp.* (8)
(Food Restaurant)	faeces	Gram + ve cocorods
2. Perak		
(Food Restaurant)	vomitus	Spore forming gram +ve rods *Bacillus sp.* (3)
(Food Restaurant)	vomitus	Gram + ve cocorods (2)
(Food Restaurant)	vomitus	Unknown
(Food Restaurant)	faeces	Yeast Cells
3. Cameron Highland		
(Food Restaurant)	adults	Gram – ve cocorods .(5)
(Food Restaurant)	adults	Gram + ve cocorods
(Food Restaurant)	adults	*Streptococcus sp.*
(Food Restaurant)	adults	Gram + ve cocci
4. Terengganu		
(Keropok Factory)	faeces	Spore forming gram +ve rods *Bacillus sp.*
(Keropok Factory)	faeces	Yeast Cells
5. Kelantan		
(Keropok Factory)	faeces	Gram + ve cocorods (2)
(Keropok Factory)	faeces	*Bacillus sp.*
(Keropok Factory)	faeces	Unknown
(Keropok Factory)	vomitus	Gram + ve cocorod
(Keropok Factory)	vomitus	Gram + ve cocci
(Keropok Factory)	vomitus	*Staphylococcus sp.*
(Keropok Factory)	vomitus	Gram + ve cocorods

*Number in parentheses indicates the number of isolates.

Table 2. Bacteria isolated from the external body surface and gut content of *Musca domestica* collected from poultry farm in Kundang, Selangor

Bacteria Isolated	External body surface of *Musca domestica*	Gut of *Musca domestica*
Acinetobacter sp	1	–
Bacillus sp	2	1
Enterobacter sp.	6	4
Escherichia sp.	–	1
Klebsiella sp.	–	1
Proteus sp.	4	5
Total number of isolates	13	12

found on the external body surface were *Enterobacter* sp. followed by *Proteus* sp. The bacteria isolated from the gut contents were *Bacillus* sp, *Enterobacter* sp. *Klebsiella* sp. and *Proteus* sp. The most common species found in the gut content was *Proteus* sp. Flies that emerge from pupa did not harbour any bacteria.

DISCUSSION

In the recent years much attention has been given to the house fly as a potential

mechanical vector of disease transmitting agent. In Malaysia, Sulaiman *et al.* (1988) had isolated several bacterial species from the house fly *M. domestica* and *Chrysomya megacephala*. His study indicated that more variety of bacteria species were isolated from house flies in the poultry farm compared to the dumping ground. Some of the bacterial species they isolated such as *Acinetobacter* sp, *Bacillus* sp., *Enterobacter* sp., *Proteus* sp, *Escherichia* sp. and *Klebsiella* sp. were also obtained in this present study from the flies from poultry farm. It was also interesting to note that one *Bacillus* sp. obtained from vomitus sample produced inhibition zones in the culture plate.

Sulaiman *et al.* (2000) in another study in Chow Kit area, Kuala Lumpur , isolated eighteen species of bacteria from *M. domestica*, twelve species of bacteria from *M. sorbens*, twelve species from *Chrysomya megacephala* and five species from *Chrysomya rufifacies*. In their study, *Burkholderia pseudomallei*, the organism causing melioidosis, has been isolated from all species of flies. But, in our study *B. pseudomallei* was not found in any of the samples.

Staphylococcus sp., and *Bacillus* sp., are causative agents for diarrhoea and is rampant and common in Malaysia. A number of yeast cells was also been isolated from house-fly. Yeast cells are known to be pathogenic and are usually associated with immunosuppression or malignancy but occasionally is seen in apparently normal hosts. *Klebsiella* sp. is present in the respiratory tract and faeces and at a small proportion causes bacterial pneumonias. It occasionally produces urinary tract infection and bacteremia with focal lesions in debilitated patients. *Klebsiella* sp. also causes hospital acquired infections and is also associated with inflammatory conditions of the upper respiratory tract. *Enterobacter* sp. and *Proteus mirabilis* are also known to cause urinary tract infection. Acinetobacters often are commensals but occasionally cause nosocomial infection (Brooks *et al.* 1995).

Olsen & Hammack (2000) isolated *Salmonella enteritidis* from 2 out of 15 pools of house flies collected at caged - layer poultry houses producing eggs. Other species of *Salmonella* i.e. *Salmonella infantis* and *S. heidelberg* have also been isolated from house flies.

Grubel *et al.* (1997) have isolated viable *H. pylori* from body washings for 12 h, from alimentary tracts for 30 h and from excreta droplets for up to 30 h. Their study was the first to indicate that house flies which ingested *Helicobacter pylori* could release the organisms as viable bacteria in excreta. Thus, this study suggested that bacteria were kept alive in the host for many hours. In another study conducted by Zurek *et al.* (2001), they found that *Yersinia pseudotuberculosis* did not establish a permanent population in the house fly colony, however, viable cells were still detected from the digestive tract of flies for up to 36 h after the initial exposure, and flies contaminated their environment for up to 30 h after exposure.

Sasaki *et al.* (2000) reported that *Escherichia coli* 0157:H7 (EHEC) proliferated in the mouth parts of the house fly, and were excreted for at least 3 d after ingestion. The authors have also shown that EHEC persisted in the crop of house flies for at least 4 d.

In a recent study, Fotedar (2001), showed the vector potential of house flies in the transmission of *Vibrio cholerae* in an outbreak in India. Of the ten fly pools examined, six (60%) were positive for *V. cholerae*. From the six positive pools, three pools were *V. cholerae* Ogawa T2 E1 Tor and one pool was *V. cholerae* non - 01. He suggested that house flies acted as mechanical vectors and helped in the dissemination of *V. cholerae*.

The genera *Staphylococcus* and *Streptococcus* that were isolated from Langkawi and the Cameron Highlands, both are recreational and tourist sites, could pose a danger in the spread of diarrhoea diseases. Control measures must be undertaken urgently in order to suppress the fly population. According to Chavasse *et al.* (1994) thousands of people

had died due to diarrhoeal in diarrhoeal epidemic areas.

In communities with good provision for sewerage and waste disposal, flies should not, and cannot, be a health problem but the presence of flies would indicate sanitary deficiency and unhygienic condition. Possible breeding sites for flies should be eliminated and flies should be prevented from gaining access to contaminate human materials.

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