Detection of mechanically transmitted bacteria by *Musca domestica* (Diptera: Muscidae) in Malabe Medical Officer of Health (MOH) Division, Sri Lanka

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Abstract House flies (*Musca domestica*) have been identified as a mechanical vector, transmitting a range of infectious diseases, including cholera, shigellosis, salmonellosis, and skin infections. They live closely with human and domestic animals and are frequently found in areas of human activities. The present study was conducted to identify the pathogenic microbes potentially transmitted by houseflies since the related knowledge is not available in Sri Lankan settings. Houseflies were collected from the Malabe Medical Officer of Health (MOH) area from September - December 2022 using a prepared bait trap. Collected houseflies were identified up to the species level using standard identification keys. They were sacrificed using a cold shock and were washed in microcentrifuge tubes to collect the bacteria on their outer surface, in 250 µL sterile PBS. A dilution series (10^0- 10^{-7}) was made from the original wash, and 200 µL from each dilution was plated on Plate Count Agar and pure cultures for each microbe were obtained. The isolated microbes were identified by performing Gram's staining and biochemical tests. *Acinetobacter* spp., *Enterococcus* spp., *Serratia* spp., *Klebsiella* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *Escherichia* spp. were identified and presumed as mechanically transmitted bacteria from houseflies. The most abundant bacterium was *Staphylococcus* sp. 2 (36.303%), followed by *Escherichia* sp. (21.452%) and *Pseudomonas* sp. (17.162%), while other bacteria were present in fewer proportions. The identified genera of the bacteria include some potential pathogenic bacterial species, which could cause mild to severe illnesses. Hence, the findings of the present study emphasized the importance of control measures for houseflies and community awareness programs to reduce the transmission of pathogenic bacteria.

Keywords: bacteria, housefly, infectious, pathogenic, vector

Introduction

Musca domestica L. (Diptera: Muscidae), is recognized as the housefly and is a usually domestic, insect that may act as a vector of some human and animal diseases, mainly food-borne microbial intestinal infections. It is also known as a vector that transmits food spoilage microorganisms. Many microorganisms, including pathogens, can be mechanically transmitted by house flies, from one place to another. These pathogens commonly cause infectious diseases, including infantile diarrhoea, anthrax, cholera, ophthalmia, bacillary dysentery, typhoid, and tuberculosis (Shane et al., 1985; Chohen et al., 1991; Raele et al., 2021). House flies transmit pathogenic organisms from infected materials, including waste, and from sick animals and humans to healthy humans. A number of recent studies have proven that there are different possible modes of bacterial transmission by houseflies (Al-Taee et al., 2011). A study on the mechanical transmission of rotavirus has shown that the virus is carried by the housefly on its wings and legs. Further, the authors found that houseflies could transmit the rotavirus by means of a sticky material secreted by the insect, further supporting the finding that it has a potential for transmission of pathogens (Raele et al., 2021).

Several studies have been conducted investigating the food-borne pathogen transmission by houseflies, including the stomach pathogen *Helicobacter pylori* which is responsible for bacterial gastritis (Grubel et al., 1997). Various studies conducted around the world solidly established the fact that houseflies could cause transmission of gastrointestinal infections, including shigellosis, cholera, yaws, and salmonellosis. Houseflies can transmit pathogens

mechanically from a site of contamination or from a host to a healthy person unwittingly, without expansion or development of the pathogenic organism inside of the vector (Greenberg, et al., 1971). They just unintentionally gather up pathogens on their bodies by means of their hairy proboscis and feet.

Therefore, control of houseflies is crucial to interrupt the potential disease transmission by them. An insight into various aspects, such as their behaviour, morphology, biology, life cycle, and pest status, is required before designing control strategies and launching preventive measures. Integrated Pest Management (IPM) and other related programs, as a combination of several control measures of the housefly population, are recommended for effective and efficient results worldwide (Iqbal et al., 2014). Identification of microbes transmitted by houseflies is paramount in implementing targeted vector control measures.

The common housefly is known to be a mechanical vector of transmission of pathogens, including protozoan parasites, bacteria, fungi, and viruses, that cause various diseases. The diversity of the pathogenic bacteria that are transmitted may vary depending on the geographical location. Even though entomological literature is available for *Musca domestica*, the knowledge of bionomical aspects and disease transmission by the housefly is not available in the Sri Lankan setting so far. The present study was intended to identify the pathogenic bacteria transmitted by the houseflies in a particular urban environment in Sri Lanka, the MOH division Malabe.

Methodology

Field collection of adult houseflies

The study area, Malabe MOH area (N 6° 54' 0", E 79° 57' 0"), Sri Lanka, lies in close proximity to both Colombo, the financial capital of Sri Lanka, and Sri Jayawardenepura Kotte, the administrative capital; hence, with a large residential population and many educational and commercial facilities.

Sampling was performed from October 2022 to December 2022. Cafeterias were selected as the sampling sites, and particularly the dining tables, and waste food in a general environment were used to collect samples of houseflies. *M. domestica* commonly resides in the locations where the food is consumed and wasted. Around 150 house flies were collected using a baited trap from 25 different cafeterias in the sampling site. The baited trap was prepared using a sterilized plastic container with a hole in the lid. An upper part of a transparent bottle was attached to the hole to avoid the flies flying back to the environment. A sterilized net covering the border of the lid was attached to reserve the flies inside the trap until they were transferred to the laboratory. The bottom of the bait trap was covered with sterile sheets of gauze, on which the flies could land. Before the lid of the baited trap was fixed and sealed, a sugar-mixed syrup was placed in the container. When the houseflies were attracted to the container, the hole was closed with the help of the net, and the collected houseflies were transported to the Research Laboratory of the Faculty of Health Sciences, CINEC Campus, Malabe.

Processing of housefly samples

Collected houseflies were sacrificed using a cold shock, followed by species separation based on key morphological characteristics (Dodge, 1953). The specimens were surface-washed individually for 30 seconds in a microcentrifuge tube containing 250 µL of phosphate-buffer saline (PBS). The wash was taken for the screening of bacteria to confirm the transient flora. The homogenized original wash was serially diluted in PBS (900 µL) to prepare a dilution series from 10^{-1} to 10^{-10}. A minimum sample size of 150 adult houseflies was screened for the identification of mechanically transmitted bacteria.

Culturing and isolation of bacteria

A volume of 100 µL from each dilution was plated on a sterile Plate Count Agar (PCA) medium and incubated at 35^0C for 24-48 hours. All microbiological culturing was performed strictly adhering to aseptic techniques at the Microbiology Laboratory, Faculty of Health Sciences, CINEC Campus. To assess microbial growth, the total number of Colony-Forming Units (CFUs) was determined. Bacterial colonies obtained on plates were differentiated morphologically on the basis of the shape, size, colour, margin, opacity, elevation, etc, in order to determine the identity and the diversity of microbes. All bacterial colonies were selected from primary plates for repeated subculture on nutrient agar plates until pure cultures were

obtained (Figure 1). Isolates were differentiated by Gram staining and the morphological shape of stained bacterial cells. Gram's Staining followed by Biochemical tests namely the KIA test, MR test, VP test, Citrate test, Urease test, Oxidase test, Catalase test, Indole test and Bile Esculin test were performed according to Cowan and Steel's manual (Cowan & Steel, 1961) to identify bacteria up to the genus level.

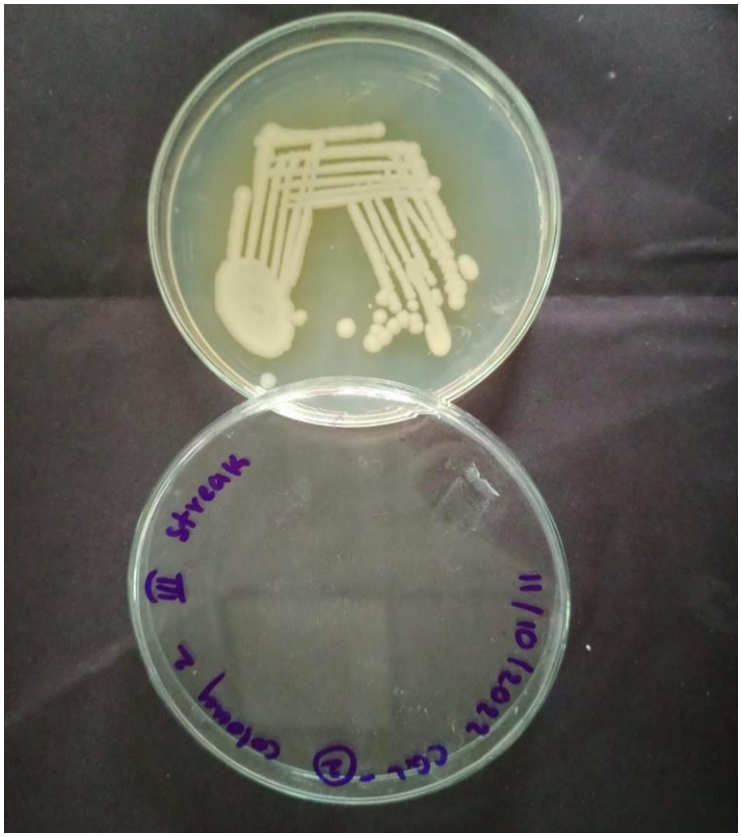

Figure 1: Streak plate technique to isolate the pure colonies of bacteria

Data analysis

The Colony Forming Units (CFU) for each identified bacteria were calculated using the following equation.

$$\text{CFU/ml} = \frac{\text{(No of colonies X Total dilution factor)}}{\text{Volume of culture plated in ml}}$$

The percentage relative abundance of each genus of bacteria was calculated. All the interpretations were done considering 95% significant intervals.

Results and Discussion

Bacterial isolates from the house fly at different locations

A total number of 10 bacterial isolate were identified and labelled as the following; *Acinetobacter* sp., *Enterococcus* sp 1, *Serratia* sp, *Klebsiella* sp 1, *Staphylococcus* sp. 1, *Pseudomonas* sp., *Enterococcus* sp 2, *Staphylococcus* sp.2, *Klebsiella* sp 2 and *Escherichia* sp. They were determined as the bacteria transmitted mechanically on the body surface of houseflies. The highest colony count was recorded from *Staphylococcus* sp 2 and the lowest

colony count was from *Acenetobacter* spp. (Figure 2). *Pseudomonas* sp., *Enterococcus* sp 2, *Klebsiella* sp 1, and *Escherichia* sp. showed a significantly higher capability of mechanical transmission via houseflies than *Klebsiella* sp 1, *Acinetobacter* sp. and *Serratia* sp.

From the isolated bacterial strains, the majority (71%) of the housefly isolates belonged to the Phylum Firmicutes. The rest of the isolated species were from Proteobacteria (18.7%), and Actinobacteria (10.19%) phyla. The findings of the present study, that the field-collected housefly showed the mechanical transmission of the bacteria, confirmed the previous shreds of evidence for the transmission of pathogens via houseflies (Ragga Issa, 2019).

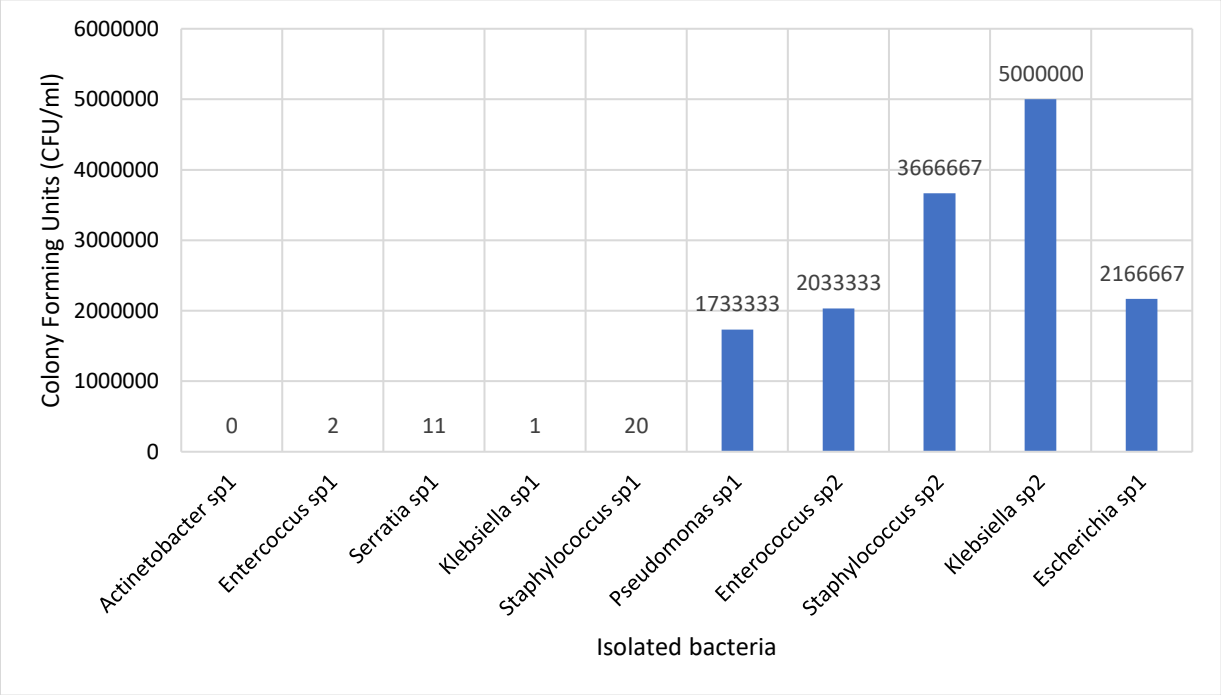

Figure 2. Identified bacteria mechanically transmitted per housefly

The best isolation of the colonies was observed from 10^{-4}-10^{-7} dilutions of the lysate. Biochemical characterization (Figure 3) revealed the isolated bacterial strains only up to the genera level. All the results of biochemical identification, Gram stain, and morphological identifications had shown to be compatible with identified bacteria following the standard keys (Table 1).

The bacteria species identified by the present study, such as *Staphylococcus* sp, *Escherichia* sp, *Enterococcus* sp could transmit mild to severe disease conditions as they have the potential to act as pathogenic bacteria. *Staphylococcus aureus* is a bacterium that causes Staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in the environment (soil, water, and air) and is also found in the nasal cavity, and on the skin of humans. Further, *Enterococcus* could also cause food intoxication through the production of biogenic amines and could also cause opportunistic infections by virulent traits. *E. coli* strains are usually harmless, but some strains could cause serious food poisoning. Shiga toxin-producing *E. coli* (STEC) is a bacterium that could cause a severe foodborne disease. Hence, it is utterly important to follow strategies to control the housefly population in order to reduce the diseases caused as a result of the transmission of pathogens by houseflies (Chohen *et al*., 1991).

Table 1: Biochemical Test results for identified bacteria from housefly

Identified bacteria	**Grams stain**	**Biochemical test**									
		Oxidase	**Catalase**	**Citrate**	**KIA**	**Mannitol**	**BE**	**VP**	**MR**	**Urease**	**Indole**
Actinetobacter sp.	*Gram negative coccoid rods*	-	+	+	Alkaline slant/ No gas	-	-	-	-	-	-
Entercoccus sp.1	*Gram positive cocci*	-	-	-	A/A	+	+	+	-	-	-
Serratia sp.	*Gram negative rods*	-	+	+	Alkaline slant/ No gas	+	-	+	-	-	-
Klebsiella sp.1	*Gram negative rods*	-	+	+	A/A, +Gas	-	-	+	-	+	-
Staphylococcus sp.1	*Gram positive cocci*	-	+	+	A/A,+ Gas	-	-	-	+	+	-
Pseudomonas sp.1	*Gram negative rods*	+	+	+	K/K	+	-	-	-	-	-
Enterococcus sp.2	*Gram positive cocci*	-	-	-	A/A	-	+	+	-	-	-
Staphylococcus sp.2	*Gram positive cocci*	-	+	+	A/A,+ Gas	-	-	-	+	+	-
Klebsiella sp.2	*Gram negative rods*	-	+	+	A/A, - Gas	-	-	+	-	+	+
Escherichia sp.	*Gram negative rods*	-	+	-	A/A, +Gas	+	-	-	+	-	+

+: Positive for the test; -: Negative for the test
A/A: Yellow slant, Yellow butt
K/K: Red slant, Red butt

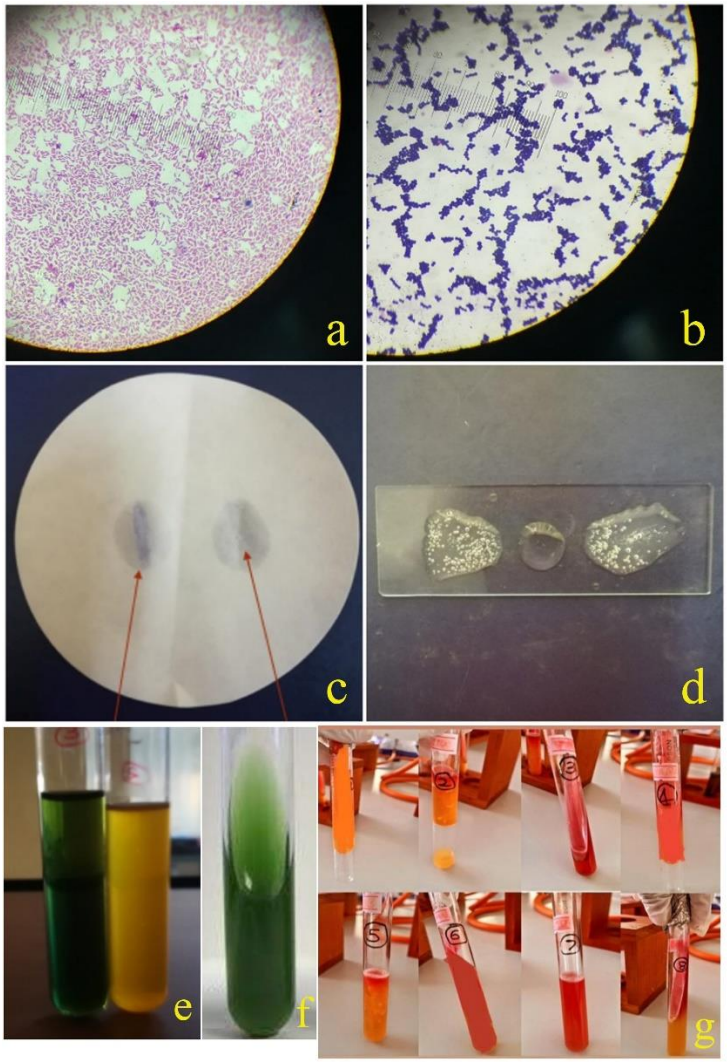

Figure 3: Gram's Statining (a-negative), Gram's Statining (b- positive), c- Oxidase test, d-Catalase test, e- Oxidative/Fermantative Test, f- Citrate test, g- KIA Test

Houseflies in human settings can transmit a wide range of microbes by mechanical transmission. Previous studies conducted elsewhere had proven the pathogens transmitted by house flies could carry dysentery (*Shigella dysenteriae*) and typhoid fever (*Eberthella typhosa)*, and cholera (V*ibrio cholera*) on their feet and mouthparts. Further, these pathogens could cause other disease-causative agents; viruses such as viral hepatitis, Rota virus, and fungi (Miller et al., 1974). The parasites such as enteric protozoa as cysts and helminthic eggs (*Entameona histolytica*, *Isopora* species, *Entamoeba coli*) or trophozoites; Nematodes such as helminthic eggs (*Entamoena histolytica*, *Isospora* species, *Sacrocystis* species, *Entamoeba coli*, *Toxoplasma gondii*, *Giardia* species, *Cryptosporidium parvum*, *Trichomonas* species, *Dipylidium* species, *Hymenolepis* species, and *Diphyllobothrium* species) and larvae of Harbonema are transmitted by *M.domestica* with the aid of their hairy legs and feet (Graczyk et al., 2005; Khan, 2020).

A study conducted by De Jesús et al. (2004) on houseflies captured from various food products revealed that the most prevalent type of bacteria transported by these flies was *E. coli*. That finding was not in line with what we observed in the present study, although *E. coli* was recorded as one of the bacteria which is transmitted mechanically in the study area. The type and number of bacteria that

may be carried by houseflies to a high degree is a function of the place where these flies are captured. Two species of *Staphylococcus* genera were identified by the present study, namely; *Staphylococcus epidermidis*, *Staphylococcus lantos* and *Streptococcus sanguinis* were found to be transmitted by houseflies in a study conducted by Akhtar et al. (2009). This was in agreement with the results of the present study. Bacterial strains from the genus *Campylobacter* were not recorded during the present study, although it was reported as a common bacterial pathogen transmitted mechanically by houseflies (Rosef & Kapperu, 1983).

One of the most important obstacles the world faces in fighting against microorganisms is that they are increasingly becoming resistant to antibiotics currently used. The resistance of various bacteria to antibiotics is encoded by various genes which also are able to transfer between bacteria horizontally (Nazari et al., 2007; Mashouf et al., 2015). House flies can mechanically transport antibiotic-resistant bacteria from hospital environments to non-hospital areas and create serious problems for residents in non-hospital areas.

Since the common housefly is a mechanical vector of transmission of pathogens, including pathogenic bacteria, the combination of different methods for control and prevention or eradication of houseflies should be implemented to stop human or animal diseases (Rockstein et al., 1959; Malik et al., 1978). In high-risk areas, health education, proper environmental sanitation, and personal hygiene are strongly advocated.

Conclusions and Recommendations

A total number of 10 bacteria strains; *Acinetobacter* sp., *Enterococcus* sp. 1, *Serratia* sp., *Klebsiella* sp 1, *Staphylococcus* sp. 1, *Pseudomonas* sp., *Enterococcus* sp 2, *Staphylococcus* sp.2, *Klebsiella* sp 2 and *Escherichia* sp. were identified as bacteria transmitted mechanically on the body surface of houseflies in the study area. Some species in genera, such as *Staphylococcus*, *Escherichia*, and *Enterococcus* could act as pathogenic bacteria to humans. Molecular-based identification of isolated bacteria is recommended to identify them up to the species level. The outcome of this study emphasized the importance of the application of housefly control measures to avoid the diseases transmitted by them. Additionally, community awareness shall be enhanced educating the community on the importance of the elimination of breeding places of houseflies, and the prevention of contamination from disease-causing agents carried by houseflies.

Availability of data and materials

The data supporting the conclusions of this article were included in the article. Data will not be shared in any of the sources.

Conflict of interests

The authors declare that they have no conflict of interest.

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