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Research Article

Identification of Enteric Bacteria in Houseflies (*Musca domestica*) found in Selected Public Eateries in Davao City

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ABSTRACT

Houseflies (*Musca domestica*) are recognized mechanical vectors of various enteric pathogens, particularly in areas with poor sanitation. Their feeding and breeding habits on waste materials make them effective carriers of disease-causing microorganisms. Previous studies have linked houseflies to the transmission of infections such as shigellosis, cholera, and typhoid fever. This study aimed to identify enteric bacteria carried by houseflies collected from selected public eateries in Davao City and assess their potential role in bacterial transmission. A total of 27 samples were examined using conventional microbiological techniques, including microscopy and biochemical testing. Bacteria from the genera *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter* were identified. Statistical analysis using the chi-square test revealed no significant variation in bacterial presence across different collection sites. The findings suggest that houseflies consistently harbor enteric bacteria regardless of location, emphasizing their role as potential vectors in public food environments. This study therefore recommends that local health authorities implement strict preventive measures in waste management, regular inspection, and sanitation permits to reduce the risks of foodborne pathogen transmission.

Keywords: Houseflies (*Musca domestica*), Enteric bacteria, Public eateries, Davao City

Background

Houseflies (*Musca domestica*) pose a considerable threat of exposing humans to infections, including bacteria, for they thrive in various habitats, move erratically, travel long distances, and seek out decaying organic substances (Ansari, 2023). *Musca domestica*, the

most prevalent fly species worldwide, belongs to the order Diptera and family Muscidae (Hasan et al., 2021). They are widely known to spread disease mainly through mechanical transmission, which is considered their primary means of transferring pathogens. Several studies have shown that the species can

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mechanically spread diseases, including those caused by *Aspergillus* species, *Shigella* species, and *Vibrio cholerae*, also known as Cholera. These flies can taint food with eggs and maggots through their saliva and excreta, as well as carry bacteria from their body, hairs, or feet to our meals, which can cause disorders in the digestive tract (Olagunju, 2022). For instance, Davies et al. (2016) showed that houseflies are capable of acquiring *Clostridioides difficile* from contaminated surfaces and transferring it mechanically, demonstrating that pathogens can survive on the external body parts of flies without undergoing any biological development within the insect.

Enteric bacteria were the most commonly isolated from houseflies (Khamesipour et al., 2018). Enterobacteriaceae is a large family of gram-negative, rod-shaped bacteria that grow in the presence or absence of oxygen, with *Escherichia*, *Shigella*, *Enterobacter*, *Klebsiella*, and *Salmonella* as its best-known members. They are usually distinguished by their capacity to ferment lactose. For accurate classification, microbiologists rely on selective media that suppress gram-positive growth (Zainal, 2020). Houseflies can carry these bacteria when they land on one surface—human, animal, or food—to another (Ansari, 2023). In fact, houseflies were shown to be capable of transferring up to 2×10^{12} *E. coli* cells and trace amounts of food between surfaces. They have also been documented to carry *Enterococcus faecium*, *Klebsiella pneumoniae*, and *Salmonella enterica* in kitchens and farms (Yin et al., 2022).

Eateries can also expose patrons to foodborne illnesses, particularly when pests are present, or water supplies are tainted. The public health impact of inadequate food hygiene, reflected in higher rates of gastrointestinal disease, is further amplified by flies, which transmit a range of pathogenic microorganisms. High-density urban environments allow flies to multiply continuously, increasing their contact with both waste and food. Poor cleanliness standards in food-related settings further sustain large housefly populations and heighten contamination risks (Songe et al., 2017). In a diarrhea outbreak in Toril, Davao City, 41% of cases were traced back to food and beverages purchased from street kiosks (Cudis, 2022).

Ejechi et al. (2023) also isolated *Salmonella* spp. from rice meals sold at makeshift eateries located near dumps, open drains, and areas infested with houseflies. In relation to ISO 22000 Food Safety Management System standards, particularly with regard to prerequisite programs (PRPs) on pest control and sanitation, the isolation of enteric bacteria from houseflies suggests potential lapses in food safety practices in public eateries.

In an attempt to mitigate foodborne illnesses, regulations such as the Food Safety Act of 2013 and Integrated Pest Management have been implemented (Hinkle & Hogsette, 2021). Although chemical and biological control strategies have been applied against them, houseflies remain a persistent worldwide problem (Hassan et al., 2021). The involvement of houseflies in spreading disease can be highlighted by evidence that poor management of fly populations corresponds with increased cases of diarrheal illness (Geden, 2021). A study by Al A'Dhami (2023) in Iraq found 126 houseflies contaminated with bacteria, most commonly *Escherichia coli* (24.3 %). Comparable issues occur in the Philippines, where Lubiano (2023) reported fly infestations in Ormoc City barangays lead to typhoid and diarrheal outbreaks. Indeed, houseflies can be significant vectors of diseases, emphasizing the need to study their role in harboring bacteria (Nayduch et al., 2023).

Although numerous existing research have examined the role of houseflies as mechanical vectors of pathogenic microorganisms, most have focused on hospital, slaughterhouses, and agricultural setting (Carhuallanqui et al., 2025; Alusdan & Al, 2022; Ranjbar et al., 2016; Neupane et al., 2023; Cervelin et al., 2018). Relative scarcity of localized studies investigating in public eateries particularly within Philippine settings, still remains. Moreover, limited studies have compared bacterial presence across locations to determine whether contamination varied significantly. This gap in the literature presents the need for targeted study in assessing houseflies as vectors of enteric bacteria in public food service establishments where direct human exposure is most likely. Further, microbial loads and species differ by location and collection environment (Khamesipour et

al., 2019). Thus, investigating the enteric microbes carried by *Musca domestica* from public eateries in Davao City would address this knowledge gap.

The study is conceptually anchored on the One Health Framework, which recognizes the interdependence of human, animal, and environmental health. The presence of enteric bacteria in houseflies collected from public eateries reflects the interaction between environmental sanitation conditions, vector ecology, and potential human health risks. Furthermore, the study contributes to the United Nations' 2030 Agenda for Sustainable Development, specifically Sustainable Development Goal 3—Good Health and Well-being—by generating knowledge that fosters healthier lives. Determining which enteric bacteria houseflies carry in public eateries clarifies the insects' vector potential and offers evidence for upgrading food safety and hygiene measures. These insights can enable local governments and health agencies to craft more focused public health strategies. Guided by this aim, the study set out to address the following questions:

1. What is the frequency of the collected houseflies (*Musca domestica*) per locale?
2. What enteric bacteria are present in the collected houseflies (*Musca domestica*)?
3. What is the frequency of all the identified enteric bacteria in the collected houseflies (*Musca domestica*)?
4. Is there a significant difference in the frequency of bacteria in houseflies (*Musca domestica*) among the selected locales of the public eateries in Davao City

Methods

Research Design

The study employed a quantitative descriptive research design. The research design enabled the researchers to identify and measure the enteric bacteria present in houseflies through systematic data collection and statistical analysis of the variables without manipulating or establishing causal relationships. In line with the research goal, the use of this approach ensured the collection of reliable and objective data.

Research Locale

The houseflies were procured from various public eateries in Davao City, Philippines, near public markets, specifically in Agdao, Bankerohan, and Piapi. These areas attract flies due to poor waste management, drainage issues, and open food displays, creating ideal breeding grounds for the pests. Collectively, Agdao (north), Piapi (central-coastal), and Bankerohan (south) form a rough north-to-south transect. Sampling all three ensures that findings are not biased toward a single micro-environment. The study employed purposive sampling in selecting three eateries from each location. Purposive sampling is an "intentional selection" of samples relevant to the study. The eateries were chosen based on their location being within the 150-meter radius of the markets. These conditions reflect what local studies in Davao City have also observed—public markets tend to be high-risk areas for insect-related contamination because sanitation issues are common and insects, food, and people are in close and constant contact (Martinez et al., 2024 & Aming-Hayudini et al., 2022)

Materials and Instruments

In collecting the samples and identifying the bacterial pathogens in houseflies (*Musca domestica*), the essential materials were as follows:

Fly swatter, forceps, and sterile containers were used to ensure the collection was free from contamination. An icebox was used to store the collected samples throughout transportation. Culture media, such as EMB, TSI, SIM, and MR-VP Broth, were used for bacterial growth and confirmation, which is necessary for the identification of bacterial pathogens isolated from houseflies. To maintain optimal temperature conditions for bacterial propagation, an incubator was used. Since contamination control was crucial for the study, a class 2 biosafety cabinet was used to maintain a sterile environment and protect researchers from any pathogens present in the samples. To identify the shape and structure of the bacteria, a Gram staining technique involving crystal violet, Gram's iodine solution, ethanol, and basic fuchsin solution was employed. According to laboratory safety guidelines, the researchers wore

personal protective equipment (PPE) to minimize the risks associated with the study's procedures

Data Collection

Using fly swatter in the daytime when houseflies are active, a total of fifty-nine (59) houseflies were collected within the premises of the chosen eateries, particularly in areas where they are most concentrated. However, only 27 houseflies (9 from each locale) were analyzed, following the adopted method (Ibrahim et al., 2020). The samples were placed in a sterile container and stored at cold temperatures to immobilize and preserve the specimens' integrity (Issa, 2019). Thereafter, the samples were sent to an accomplished agriculturist for accurate identification of the housefly species before further processes. Only houseflies of the species *Musca domestica* were used in the study.

Houseflies were centrifuged for five minutes in a test tube with 2.0 ml of 0.9% normal saline. For primary isolation of enteric bacteria, each tube's saline was cultured aseptically in the Eosin Methylene Blue (EMB) agar and incubated for 24 hours at 37 °C. To get a pure culture for identification, the presumed colonies were further cultured on EMB.

The identification of pure cultures up to the genus level was performed using conventional identification methods, including microscopy and biochemical tests. Initially, a Gram stain was performed to determine the bacteria's cellular morphology. Thereafter, biochemical assays were conducted, including the sulfide-indole-motility (SIM), triple-sugar iron (TSI), and methyl red-Voges-Proskauer (MR-VP) tests.

Statistical analysis

The results of these tests were interpreted using standard reference charts and identification keys found in authoritative microbiology textbook of Mahon, Lehman, and Manuselis' *Textbook of Diagnostic Microbiology (Fifth Edition)*. These texts provided detailed explanations of expected reactions for various enteric bacteria, including color changes, gas formation, and precipitate development—each of which served as diagnostic indicators. Throughout this process, a licensed medical technologist supervised the interpretation and validation of results. Their clinical expertise ensured that test outcomes were correctly read and matched to textbook standard.

Result and Discussion

Table 1. Frequency of houseflies per locale.

Location of the Public Eateries	Number of Houseflies Collected
Agdao	19
Bankerohan	24
Piapi	16
TOTAL	59

Table 1 shows the frequency of houseflies collected from selected public eateries near Bankerohan, Agdao, and Piapi Public Markets. A total of 59 houseflies were collected: 19 from Agdao, 24 from Bankerohan, and 16 from Piapi. Of these, only 27 were subjected to further analysis, as per the adopted methodology. Dipterans, including houseflies, are regarded as important environmental health pests, as their hairy bodies and feet allow them to spread pathogens mechanically (Lin et al., 2024). It is also known that houseflies flourish in environments rich in organic waste, manure, and fecal

matter, where they acquire pathogens that can be easily transferred to food and public eating areas (Grimaldi, 2025).

Food markets and dining establishments are among the most common locations targeted for housefly sampling (Monyama, 2021). The presence of houseflies in public eateries highlights a significant public health concern. Public markets and eateries lacking efficient waste management systems often become breeding grounds for houseflies, amplifying the potential for disease transmission (Kumar, Gupta, & Malik, 2021).

Table 2. Enteric Bacteria found on the external surfaces of each housefly.

Agdao	
Sample No.	Enteric Bacteria Present
AD-1	<i>E. coli</i>
AD-2a	<i>E. coli</i>
AD-2b	<i>Klebsiella sp.</i>
AD-3a	<i>E. coli</i>
AD-3b	<i>Enterobacter sp.</i>
AD-4	<i>Klebsiella sp.</i>
AD-5a	<i>Klebsiella sp.</i>
AD-5b	<i>E. coli</i>
AD-6a	<i>E. coli</i>
AD-6b	<i>Klebsiella sp.</i>
AD-7	<i>E. coli</i>
AD-8	<i>Enterobacter sp.</i>
AD-9	<i>E. coli</i>
Bankerohan	
BK-1	<i>Klebsiella sp.</i>
BK-2a	<i>E. coli</i>
BK-2b	<i>Citrobacter sp.</i>
BK-3	<i>Enterobacter sp.</i>
BK-4	<i>E. coli</i>
BK-5	<i>E. coli</i>
BK-6	<i>E. coli</i>
BK-7	<i>Enterobacter sp.</i>
BK-8a	<i>Klebsiella sp.</i>
BK-8b	<i>Enterobacter sp.</i>
BK-9a	<i>E. coli</i>
BK-9b	<i>Klebsiella sp.</i>
Piapi	
PP-1	<i>E. coli</i>
PP-2	<i>E. coli</i>
PP-3a	<i>Klebsiella sp.</i>
PP-3b	<i>E. coli</i>
PP-4	<i>E. coli</i>
PP-5	<i>Klebsiella sp.</i>
PP-6	<i>E. coli</i>
PP-7	<i>Klebsiella sp.</i>
PP-8	<i>Klebsiella sp.</i>
PP-9	<i>Klebsiella sp.</i>

Table 2 shows the distribution of enteric bacteria isolated from the external surfaces of houseflies collected across three locales. Each entry corresponds to an individual housefly, indicating the specific bacterial species identified. Indeed, houseflies are known vectors of a wide range of human pathogens (Geden, 2021). The data also highlight the microbial diversity

associated with houseflies in different environments and reveal variations in the prevalence of certain enteric bacteria—such as *E. coli*, *Klebsiella sp.*, *Enterobacter sp.*, and *Citrobacter sp.*—between the sampled areas. This finding aligns with the research of Khamesipour et al. (2018), where the results showed that the type of pathogens carried by houseflies vary based

on the location from which they are collected. Due to their behavior and preference for pathogen-rich substrates, all developmental stages of houseflies expose them to diverse bacterial pathogens from various unsanitary settings (Yin et al., 2022).

Identification processes were primarily based on two criteria: colony morphology observed on selective culture media and the

results of gram staining. Bacterial species were confirmed through a series of biochemical tests, which included Triple Iron Sugar (TSI), Gas, Hydrogen production, Motility, Methyl Red, and Voges-Proskauer (MRVP) tests. The results have either supported or refined initial identifications based on morphology and Gram reaction.

Table 3. Frequency of the identified enteric bacteria found in houseflies.

Bacterial Species	Agdao	Bankerohan	Piapi	Total Frequency
<i>E. coli</i>	7	5	5	17 (48.57%)
<i>Klebsiella sp.</i>	4	3	5	12 (34.28%)
<i>Enterobacter sp.</i>	2	3	0	5 (14.29%)
<i>Citrobacter sp.</i>	0	1	0	1 (2.86%)
Total	13	12	10	35 (100%)

Table 3 presents the frequency distribution of enteric bacteria isolated from the collected houseflies of each locale, expressed in percentage of the total number of samples examined using the formula: $F/N \times 100$. Among the identified enteric bacteria, *Escherichia coli* has the highest frequency, at 48.57%, followed by *Klebsiella sp.* (34.28%), *Enterobacter sp.* (14.29%), and *Citrobacter sp.* (2.86%). These findings are consistent with similar studies by Al-A' Dhami et al. (2023), Ibrahim et al. (2020), and Zuhora et al. (2023), which reported *Escherichia coli* as the most frequently isolated bacterial species. Well-documented causes of watery diarrheal disease include various *E. coli* pathotypes, such as enterotoxigenic (ETEC) and enterohemorrhagic (EHEC) strains. The symptoms of such infections are typically acquired through ingestion of unsanitary food and water resources (Mueller & Tainter, 2023).

The consistent detection of *Klebsiella sp.* across all three locales it is evident that houseflies are not merely passive carriers but active mechanical vectors capable of disseminating clinically significant pathogens within the

environment. *Klebsiella pneumoniae*, a prominent subspecies of the *Klebsiella* genus, is recognized as a notable opportunistic pathogen responsible for various infections, including urinary tract infections and septicemia (Isam-Eldem et al., 2022). Similarly, *Klebsiella species* have also been linked to various uncommon infections, including endocarditis, peritonitis, septic arthritis, and acute cholecystitis (Zainal, 2020).

Although *Enterobacter sp.* and *Citrobacter sp.* were identified at lower frequencies, their presence remains significant, given their role in disease transmission. The detection of these bacteria is consistent with the recent study of Adesewa et al. (2024), who also identified these bacteria in houseflies collected from areas with proximity to sources of enteric bacteria. Interestingly, a study conducted in Khartoum State, Sudan, investigating bacteria in houseflies from hospitals and slaughterhouses did not detect the presence of *Enterobacter* and *Citrobacter* among the isolates (Isam-Eldem et al., 2022). Suggesting that while houseflies are carriers of a wide range of pathogenic bacteria, they may vary depending on environmental factors and location.

Table 4. Data analysis between bacterial frequency and sampling location

	Chi-square tests		
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	5.586	6	.471

Chi-square tests			
	Value	df	Asymptotic Significance (2-sided)
Likelihood Ratio	7.007	6	.320
N of Valid Cases	35		
Symmetric Measures			
		Value	Approximate Significance
Nominal by Nominal	Phi	.399	.471
	Cramer's V	.282	.471
N of Valid Cases		35	
Chi-square statistics: 5.56		p-value: 0.471	

The frequency of the identified bacteria from houseflies collected in public eateries in Davao City was statistically assessed using the Chi-square test for independence to determine any significant relationship between the frequency of the identified bacteria and sampling location. The test yielded a p-value of 0.471, meaning there is no significant difference between bacterial distribution and sampling location at the 0.05 conventional threshold of significance. This data analysis suggests that although pathogenic bacteria, such as *E. coli* and *Klebsiella sp.*, were detected across all locations, the variation in their frequencies is not large enough to be considered statistically significant. This result still underscores the potential threat of widespread bacterial contamination and highlights the importance of preventive interventions in public eateries such as proper waste disposal, food covers and maintaining clean spaces to reduce the potential risks of foodborne illnesses linked to houseflies.

Implication of the Study

This study implies that, even without the statistical significant difference among locales, the consistent detection of *Escherichia coli*, *Klebsiella sp.*, *Enterobacter sp.*, and *Citrobacter sp.*— regardless of location, confirms that houseflies are reliable vectors of these enteric pathogens in public eateries. The continuous risks of food contamination are common across these different locales, suggesting that environmental factors, such as poor sanitation and improper waste management contributed to these findings by creating favorable breeding ground for houseflies (Kitole et al., 2024). In light of these results, greater emphasis on proper waste disposal management and fly

control practices are recommended to local health agencies. Regular sanitation inspections of public eateries should be institutionalized to guide targeted and evidence based interventions. Additionally, issuance of and renewal of sanitation permits as a requirement for continued food service operations could strengthen the compliance to food safety regulations. These regulatory interventions help in reducing the potential transmission of foodborne pathogens and significantly contribute to the global efforts of Sustainable Development Goal 3: Good Health and Well Being.

Conclusion

A total of nine houseflies (*Musca domestica*) were examined from each of the selected public eatery locales in Davao City. Through standard microbial techniques, including Gram staining and a series of biochemical tests, *Escherichia coli*, *Klebsiella sp.*, *Enterobacter sp.*, and *Citrobacter sp.* were the enteric bacterial species identified on the exterior surfaces of the houseflies. Of the four identified species, *E. coli* emerged as the most frequently isolated, followed by *Klebsiella sp.*, *Enterobacter sp.*, and *Citrobacter sp.* To determine whether the bacterial frequency varied significantly among the sampling locations, statistical analysis was conducted. The results showed that there was no significant difference, which implies that bacterial presence in houseflies is not significantly influenced by the specific area in which they were collected. Even so, in the context of public health, non-significant findings can still contribute to identifying epidemiologically important trends. This study suggests that local government units, in collaboration with public health authorities, should enforce stricter

sanitation standards in public eatery establishments.

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