Role of House Fly, Musca domestica (Diptera: Muscidae) as a Mechanical Vector of pathogenic Bacteria in Thi Qar Province

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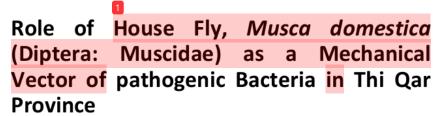
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ABTRACT

The common house fly, known as Musca domestica, has been acknowledged to carry and spread numerous contagious illnesses. The purpose of this investigation is to uncover the extent of the house fly's involvement as a carrier of harmful bacteria that can lead to diseases in both humans and animals. To accomplish this goal, a collection process was conducted using manual traps from March 2023 until July 2023. I collected house flies from various locations in Thi Qar province. A total of 370 samples were gathered, out of which 270 flies were utilized to isolate bacteria from both the outer and inner surfaces. To identify the house flies, I sent 100 of them to the Natural History Museum at the University of Basra. The current study recorded among 270 flies 104 (38.52%) have not bacteria, while (61.48%) have bacteria. The results were recorded the highest isolated bacteria from outer surface 94 (34.81%) and 72 (26.67%) from inner surface, in contrast the lowest negative bacteria were from outer surface 41 (15.19%), and 63 (23.33%) inner surfaces. The study recorded the highest number of isolated bacteria were from Arido 23 (8.52%), followed in both Al-Sharqiyah and Altathhia 22 (8.15%), followed by Shuhadda, Ur and Summer 21 (7.78%), while the lowest isolated bacteria were in Shmoukh 4 (1.48%), followed in both Aledara almahaleia 14 (5.19%). The current study recorded the most isolated bacteria was P. vulgaris 21 (20.19%), followed by P. mirabilis 18 (17.31%), followed by S. aureus 11 (10.58%), in contrast the lowest isolated bacteria were P. stuartii 1 (0.96%), followed by both S. paucimobilis and S. maltophilia 2 (1.92%).

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INTRODUCTION

The common house fly, scientifically known as M. domestica Linnaeus, can be found buzzing around in human houses, places where food is made, hospitals, restaurants, food markets, poultry and livestock farms, and various other domestic spaces or structures Klakankhai (2022). These little buggers are quite a nuisance to people, as well as to poultry, livestock, and other farm animals. Due to their habits and way of life, flies are known to be carriers of disease-causing microorganisms both mechanically and biologically. What makes the housefly particularly worrisome is its fondness for our food Wilson (2021). Flies have a natural inclination towards decomposing plants and animal organisms, which leads them to interact with various ecosystems, litter, and animal feces Davies et al., (2016). The areas that witness a substantial presence of manure or compost, particularly those lacking in human sanitation and kennels, serve as the primary breeding grounds for house flies and the concurrent propagation of bacteria El-Ghwas et al., (2021).

House flies are notorious carriers of various dangerous diseases, including anthrax, ophthalmia, tuberculosis, cholera, typhoid fever, and infantile diarrhea. They have the ability to transmit these illnesses between humans, animals, and vice versa Akter et al., (2017). Researchers have discovered over 130 pathogens on the surface of house flies, primarily bacteria. This highlights the critical role played by these insects in spreading harmful diseases Manickkam and Moses (2023).

When it comes to the transfer of disease-causing organisms by insects, such as house flies, every nook and cranny on their exterior can potentially serve as a mode of transportation for these tiny germs Ranasinghe (2023). Thus, the extent to which a specific body part of a house fly contributes to the spread of these microbes depends on its ability to carry out various tasks: capturing the germs, retaining them during travel, ensuring their infectivity remains intact, and ultimately depositing them onto either a new host or a surface that may come into contact with the host Yap et al., (2008). Grown-up flies function as carriers for certain diseases, carrying them from one place to another by interacting with the host. The transmission of diseases happens when the pathogens are dislodged from the fly's outer covering, through regurgitation or by contaminating surfaces with their feces. It has been reported that houseflies play a substantial role in spreading viruses Fayyaz et al., (2021).

The conduct of house flies heightens their ability to transmit bacterial pathogens. They dwell in close proximity to individuals (synanthropic) or within their abodes (endophilic cosmopolitan), and they chiefly sustain themselves by consuming animal and human excrement (coprophagic) as well as decaying substances, like trash Iqbal et al, (2014). Consequently 3 ll stages of their life cycle may be exposed to various pathogens in unsanitary environments, which can then be mechanically transferred to humans. In the span of their lives, adult flies can traverse up to 20 miles, signifying their ubiquitous presence in the

surroundings and their adeptness at dispersing pathogens from unhygienic zones into people's residences and places of employment and recreation Yin et al., (2022)

METHOD

Collection of flies

I went around various places in Thi Qar province, like Aledara almahaleia, Al Sharqiyah, Arido, Batha, Shmoukh, Shuhadda, Sumer, Tathhiah and Ur to gather house flies. I managed to collect a total of 370 samples by using a manual insect trap. As soon as I caught the flies, I sorted them into equal groups and placed them in glass tubes. Without wasting any time, I swiftly transported the tubes to the laboratory. To render the flies inactive, I put them in a freezer set at a chilling temperature of 0°C for a duration of 3 minutes Hasaballah (2021). House flies were identified by sending 100 flies to the Natural History Museum at the University of Basra. The flies have been identified as belonging to the class: Insect, order: Diptera, suborder: Cyclorrhapha, family: Muscidae, Genus: Musca, Species: *M. domestica*.

Culture and isolation of bacteria from house flies

The bacteria were cultured from 270 adult house flies that were collected in two ways:

The first: By adding 5 ml of distilled water to a test tube containing the adult house fly's external surface, we then cultured the bacteria. The next step involved shaking the mixture and taking a swab with a loop to streak it on MacConkey and blood agar. After incubating for 24 hours at 37°C, we obtained our results Hassan et al., (2022).

The second: The process involved collecting the bacteria from inside the adult flies, which was done by putting it in a test tube and sterilizing the outer surface with ethanol. After letting it dry, the fly was crushed and its internal entrails were extracted. Then, 5 ml of d.w. were added and shaken before taking a swab with a loop and streaking it on MacConkey medium and blood agar. Finally, it was incubated for 24 hours at 37°C Nwangwu et al., (2013)

Laboratory Diagnosis

Characteristic of Cultural Isolations

We examined the traits of the colonies that were separated and studied after we cultivated and purified the bacterial isolates on culture media. Our investigation involved using various types of agar, such as MacConkey Agar, Blood

Agar, and Mannitol Salt Agar. We looked at characteristics such as shape, size, texture, color, edges, and heights to understand more about these isolated bacterial colonies Yaseen et al., (2019).

Microscopic Characteristics

We took a closer look at the microorganisms by using a microscope once we had stained them with Gram stain. To do this, we spread a few bacteria from a colony onto a clean slide with a drop of normal saline. Then, we applied heat to fix them in place and proceeded to stain them with crystal violet, Iodine, alcohol, and safranine. Finally, we examined them under oil immersion.

Identification of Bacterial Isolates using Confirmatory Tests API-20 E System

According to the Manufacturer's Instruction Kit (BioMrieux), the API 20E system was utilized for Enterobacteriaceae. The 20 tests included in the kit were Beta-galactosidase test (ONPG), decarboxylase test (ODC), H2S production test (H2S), Lysine decarboxylase test (LDC), Urease test (URE), Indole production test (IND), Arginine dehydrolase test (ADH), Citrate utilization test (CIT), Tryptophan deaminase test (TDA), This test was conducted in accordance with the manufacturer's guidelines. (BioMerieux, France) as following:

A single isolated colony (from a pure culture) was suspended in sterile D.W.

- 1. Please fill these compartments completely with the bacterial suspension.
- 2. Please ensure that the ADH, LDC, ODC, H2S, and URE compartments are filled with sterile oil.
- 3. The container containing the strip was sealed shut and placed in an incubator set at a temperature of 37°C. It remained there for a period of 18-24 hours.
- 4. After the incubation period, the necessary substances were introduced into their respective tubes: a drop of Kova's reagent was added into the IND tube, and a drop of TDA reagent was added into the TDA tube. The result was then observed without delay. Additionally, a drop of VP1 reagent followed by a drop of VP2 were directly introduced into the VP tube. After waiting for 10 minutes, the result was read. Subsequently, all the obtained results were carefully documented and compared with the company index based on numerical profiles. Every strip was divided into seven segments, and within each segment, there were three tests labeled as 1, 2, and 4. While the positive tests were assigned distinct numbers, the negative test was assigned the number zero. Ultimately, in the end result, there are seven numbers that can be compared with the index to determine the genus and species of the bacterial polates.

Identification of Bacterial Isolates using Vitek 2 System

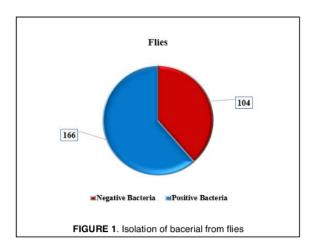
The Vitek 2 system was employed to verify the recognition of bacterial isolates belonging to both gram negative and

gram positive categories. To accomplish this task, a distinctive diagnostic kit tailored for this particular system was utilized. This kit includes a diagnostic card specifically designed for gram-negative and gram-positive bacteria, comprising a total of 64 slots. Each slot contains a dry color indicator that interacts with the provided sample. The system meticulously registers these alterations, which are caused by the growth of bacteria within the holes. By analyzing the resulting changes in color, the Vitek 2 system then proceeds to identify the bacterial sample in accordance with the instructions laid out by BioMerieux.

RESULT AND DISCUSSION

Isolation Bacteria from Flies

The current study involved 270 flies were classified in to two part 135 of flies used for isolation bacteria from outer surface and the other 135 flies used for isolation bacteria from inner surface, the current study recorded among 270 flies 104 (38.52%) have not bacteria, while 166 (61.48%) have bacteria, the results also noted a significant difference between bacterial isolation at p. value < 0.05 as in Figure 1.



Prevalence of Bacteria According to Their Site on

The results of the current study recorded the highest isolated bacteria from outer surface 94 (34.81%) and 72 (26.67%) from inner surface, in contrast the lowest negative bacteria were from outer surface 41 (15.19%), and 63 (23.33%) inner surfaces, the study also a significant difference between bacterial isolation according to site of sample at p. value < 0.05, as in Table 1.

TABLE 1. Prevalence of bacteria according to site sample flies

Site	Outer surface		Inner	surface	Total		
Sample	No.	%	No.	%	No.	%	
Negative bacteria	41	15.19	63	23.33	104	38.52	
Positive bacteria	94	34.81	72	26.67	166	61.48	
Total	135	50.0	135	50.0	270	100	

CalX²= 7.52 TabX²= 3.84 DF=1 p. value 0.006

Prevalence of Bacteria According to the Site of the Flies Sample

 TABLE 2. Prevalence of bacteria depends on location of fly sample

Sample Location	Negative bacteria		Positive bacteria		Total	
Location	No.	%	No.	%	No.	%
Ur	9	30.0	21	7.78	30	11.1
Sumer	9	30.0	21	7.78	30	11.1
Batha	12	40.0	18	6.67	30	11.1
Shmoukh	26	86.67	4	1.48	30	11.1
Al-Sharqiyah	8	26.67	22	8.15	30	11.1
Tathhia	8	26.67	22	8.15	30	11.1
Arido	7	23.33	23	8.52	30	11.1
Aledara almahaleia	16	53.33	14	5.19	30	11.1
Shuhadda	9	30.0	21	7.78	30	11.1
Total	104	38.52	166	61.48	30	100
CalX ² = 4	1.4 Tal	$X^2 = 15.51$	DF=8	B p. va	lue < 0.	001

The current results recorded the highest isolated bacteria were from Arido 23 (8.52%), followed in both Al-Sharqiyah and Altathhia 22 (8.15%), followed in Shuhadda, Ur and Summer 21 (7.78%), while the lowest isolated bacteria were in Shmoukh 4 (1.48%), followed in both Aledara almahaleia 14 (5.19%), the study also a significant difference between bacterial isolation according to location of sample at p. value < 0.05, as in Table 2.

Prevalence of Bacteria According to Location of Flies Sample

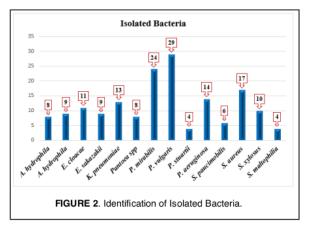
The current results recorded the most isolated bacteria were from outer surface of flies 94 (56.63%), while the lowest isolated bacteria from inner surface 72 (43.37%), also, recorded the most flies have bacteria were that collected from Shmoukh, Aledara almahaleia, Sumer, Ur, and Shuhadda, the study also a significant difference between 12 terial isolation according to location of flies' sample at p. value < 0.05, as in Table 3.

TABLE 3. Prevalence of bacteria according to location of flies' sample

	Inner	Inner surface		uter rface	Total		
-	No. %		No. %		No. %		
Ur	9	42.86	12	57.14	21	12.65	
Sumer	10	47.62	11	52.38	21	12.65	
Batha	7	38.89	11	61.11	18	10.85	
Arido	2	50.00	2	50.00	4	2.41	
Al- Sharqiyah	15	68.18	7	31.82	22	13.25	
Aledara almahaleia	5	22.73	17	77.27	22	13.25	
Shmoukh	8	34.78	15	65.22	23	13.86	
Tathhiah	8	57.14	6	42.86	14	8.43	
Shuhadda	8	38.10	13	61.90	21	12.65	
Total	72	43.37	94	56.63	166	100	
CaIX2= 55.	55.7 TabX ² = 15.51			B p.va	p. value < 0.001		

Identification of Isolated Bacteria

The current study recorded the most isolated bacteria was P. Vulgaris 21 (20.19%), followed by P. mirabilis 18 (17.31%), followed by S. aureus 11 (10.58%), in contrast the lowest isolated bacteria were P. stuartii 1 (0.96%), followed by both S. paucimobilis and S. maltophilia 2 (1.92%), as in Figure 2.



Prevalence of Isolated Bacteria According to Site of Flie Sample

The results of present study showed that the isolated bacteria were P. vulgaris and P. mirabilis from outer surface 17 (10.24%) and 14 (8.43%), respectively. Also, the most isolated bacteria from inner surface were P. vulgaris and P. mirabilis 12 (7.23%) and 10 (6.02%), while the lowest isolated bacteria were S. maltophilia 2 (1.20%), from both outer and inner surface, this study also a non-significant difference between bacte 2 l isolation according to location of flies' sample site at p. value < 0.05, as in Table 4.

TABLE 4. Prevalence of isolated bacteria according to site of flies'

sample							
Bacterial Species	Inner surface			Outer surface		Total	
opecies	No.	%	No.	%	No.	%	
A. hydrophila	3	1.81	5	3.01	8	4.82	
A.hydrophila	3	1.81	6	3.61	9	5.42	
E. cloacae	5	3.01	6	3.61	11	6.63	
E. sakazakii	4	2.41	5	3.01	9	5.42	
K. pneumoniae	6	3.61	7	4.22	13	7.83	
Pantoea spp	3	1.81	5	3.01	8	4.82	
P. mirabilis	10	6.02	14	8.43	24	14.46	
P. vulgaris	12	7.23	17	10.24	29	17.47	
P. stuartii	3	1.81	1	0.60	4	2.42	
P. aeruginosa	6	3.61	8	4.82	14	8.43	
S. paucimobilis	3	1.81	3	1.81	6	3.61	
S. aureus	8	4.82	9	5.42	17	10.24	
S. xylosus	4	2.41	6	3.61	10	6.02	
S. maltophilia	2	1.20	2	1.20	4	2.41	
Total	72	43.37	94	56.63	166	100	
CalX2= 2.68 Ta	abX2= 2	2.23 DF	=13	p. value	0.999		

M. domestica, commonly known as the common house fly, has a strong association with humans and can be found in various regions across the globe Dawaye (2024). The growth of its larvae occurs in decaying organic matter like fecal sources, while adults have a diverse diet that includes human and domestic animal food, waste, and excrement Salem and Attia (2021). Moreover, these adult flies serve as carriers for disease-causing agents that pose threats to both humans and domestic animals Hassan et al, (2021).

House flies possess the ability to mechanically transmit harmful pathogens that have an adverse effect on the well-being of both humans and animals. The daily flight behavior of house flies, which includes dispersal flights, undoubtedly plays a significant role in their capacity to not only cause annoyance but also propagate disease-causing agents Brewer et al., (2021).

House flies are most active during the day, but they prefer to find a place to rest overnight as the sun begins to set. During these resting hours, they take a break from their buzzing and flying, possibly because they sense the temperature changing with the arrival of night. Interestingly, their flight patterns differ depending on the season. In cooler months, their activity follows a single peak, while in hotter months, it shows a two-peaked.

CONCLUSION

- The prevalence and occurrence of bacteria in all studied environments is evidence of house fly prevalence.
- Healthcare and hygiene play an important role in reducing environmental pollution and limiting the spread of disease-carrying insects.
- House flies can spread disease germs through various parts of the body, both internally and externally.

AUTHOR CONTRIBUTIONS

All authors played a role in the preparation of this article.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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