JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ABUNDANCE AND MOLECULAR IDENTIFICATION OF HOUSE FLY *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE) WITH STUDY TO ASSOCIATED PATHOGENIC BACTERIA

Ahmed Z.I. Shehata[1], *Amr A. El-Waseif*[2], *Hassan O. Waheeb*[1], *Deyaa E.M. Emam*[1], *Mohamed A.M. El-Tabakh*[1], *Dina E. El-Ghwas**[3]

Address(es):
[1] Zoology Department, Faculty of Science, Al-Azhar University, Cairo 11651, Egypt.
[2] Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.
[3] Pharmaceutical Industrial Research Institute, Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, 12622 Cairo, Egypt.

*Corresponding author: dinaelghwas7781@yahoo.com

https://doi.org/10.55251/jmbfs.10580

ARTICLE INFO

Received 13. 9. 2023
Revised 2. 2. 2024
Accepted 5. 2. 2024
Published 1. 4. 2024

Regular article

OPEN ACCESS

ABSTRACT

The common house fly, *Musca domestica* L., is a mechanical vector of numerous diseases that may be spread from one living thing to another by sponging their mouthparts on their body and leg hairs after they have vomited. House flies (*Musca domestica*) have been known as a mechanical vector in spreading infectious diseases such as cholera, shigellosis, salmonellosis and skin infections. The present study aims to investigate the abundance of *M. domestica* adults in an urban region of Cairo Governorate, Egypt, throughout one year and bacterial diversity on its body surface. Adults of *M. domestica* were collected monthly by hunting. Collected adults were subjected to molecular identification. Bacteria were isolated from the surface of flies' body pieces and identified by automated identification systems (VITEK). Obtained data revealed that the highest abundance (201.67±15.57) was recorded in May 2022, while the lowest abundance (17.33±0.57) was recorded in April 2023. Also, *M. domestica* abundance positively correlated significantly (P≤0.001) with temperature (r= 0.895) and relative humidity (r= 0.827). *Musca domestica* species were molecularly identified, and the sequences were then banked on NCBI under the accession numbers OQ784762-OQ784764. The highest bacterial pathogen presence were Gram-positive *Staphylococcus* spp. and *Bacillus* spp. (77.0 %). Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonias* had the lowest presence (total of 23.0 %). On the other hand, the results of antibiotic sensitivity revealed that *Bacillus* spp., *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* are more resistant to several antibiotics than *Staphylococcus* spp. and *Escherichia coli*.

Keywords: Abundance, *Bacillus spp.*, *Musca domestica*, *Pseudomonas aeruginosa*, *Staphylococcus* spp.

INTRODUCTION

Most tropical and subtropical developing nations face a major threat from vector-borne illnesses, which account for millions of yearly fatalities in these areas. Attention has recently been drawn to the function of insect vectors' microbiota in the spread of human infections **(Weiss and Aksoy, 2011; Cirimotich *et al.*, 2011; Azambuja *et al.*, 2005).** The microbiota of numerous insects known to transmit human infections, particularly those that feed on blood, has been documented **(Jones *et al.*, 2010; Wei *et al.*, 2014; Osei-Poku *et al.*, 2012; Gupta *et al.*, 2104; Aksoy *et al.*, 2014).** Also, **Dillon and Dillon, (2004)** demonstrated that indirect and direct microbiota-induced phenotypes can affect an insect vector's capacity to spread human illnesses which can impact the host-vector competence. *Musca domestica* L. (Diptera: Muscidae), known as the house fly, is the most widespread species of fly, found in livestock facilities and cattle feedlots all over the globe **(Khamesipour *et al.*, 2018; Neupane *et al.*, 2019).** *Musca domestica* is an earnest a public health pest and pest to livestock; it acts as a mechanical vector of many pathogens to humans and animals through contaminated water and unsanitary food handlers **(Douglass and Jesse, 2002; Mian *et al.*, 2002; Issa, 2019).** Many pathogenic microbes, including viruses, bacteria, parasites, and fungi are known to be carried by *M. domestica* **(Greenberg, 1973).** Adult *M. domestica* remains in areas rich in microbes, such as those polluted with animal excrement, to feed on decaying matter, including manure and animal fluids, as well as spoiled food and drink **(Neupane *et al.*, 2019).** On the other hand, investigating of various aspects of host physiology can be influenced by the microbiota of *M. domestica*, providing resistance to the colonization of pathogenic organisms **(Bahrndorff *et al.*, 2017).** The microbiota of insects can support the host's physiology in a number of ways, including by giving extra nutrients, supplying colonization resistance against harmful organisms, enhancing tolerance to environmental changes, and promoting the growth and maturation of the host immune system **(Bahrndorff *et al.*, 2016; Cerf-Bensussan *et al.*, 2010).** Thus, it has been proposed that changes in variations in the human microbiome can account for variations in the phenotypic, like vector competence. Few research, however, has looked at differences in the microbiota through space and time between individuals in natural settings. Also, studying the microbiota of *M. domestica* helps to understand the relationship between the house fly and pathogenic agents, as well as its ability to transmit disease **(EL-Ghwas *et al.*, 2021).** More than 100 pathogens can be transmitted to humans and animals by *M. domestica* including diarrhoea, cholera, bacillary dysentery, typhoid and ophthalmia, and various bacterial strains such as *Escherichia coli, Salmonella*, and *Shigella* spp. **(Issa, 2019).** Evidence showed that adult house flies might contaminate food and spread illness by ingesting pathogens and then transmitting them through their spongy mouthparts, vomit, body hairs, sticky feet, and digestive tracts **(De Jesús *et al.*, 2004).** Although few studies have addressed the activity pattern of the house fly *M. domestica* and variation in its associated microbes under field conditions, the present study aimed at investigating the abundance of *M. domestica* adults in an urban region of Cairo Governorate, Egypt, throughout one year as well as bacterial diversity on its body surface.

MATERIAL AND METHODS

Survey of *Musca domestica* adults

Collection of adult flies

Musca domestica adults were collected monthly by hunting from El-Muqattam region (30°02'20" N, 31°16'28" E), Cairo Governorate, Egypt **(Figure 1)** through the period from May 2022 to April 2023 using commercial pheromone baited fly trap (produced by AL RAWDA Company). Collected adults were sacrificed using ethanol 70% and immediately transferred to sterile Falcon tubes, kept cool on ice for transportation to the laboratory **(Neupane *et al.*, 2019),** where they were identified morphologically under a binocular microscope according to the description of **Al-Ghamdi *et al.*, (2015).** Only bacterial isolation specimens were put in sterile saline immediately after collection. LCD digital thermometer and hygrometer were used to determine temperature and relative humidity (HTC-1).

Figure 1 The Study area El-Muqattam region in Cairo, Egypt.

Genetic identification of Musca domestica

Extraction of DNA

Tissue samples of *Musca domestica* were put into 1.5 mL Eppendorf tubes. An Invitrogen PureLink® Genomic DNA Kit (Waltham, Massachusetts, USA) was used to extract DNA. To sum up, between 180 and 250 µL of lysis tissue buffer was added to each sample, followed by the addition of proteinase K (10 µL for every 180 µL of tissue lysis buffer) was added and incubated at 56°C for 4 hours. Following the protocol provided by the manufacturer (Invitrogen, Waltham, Massachusetts, USA), the supernatant was put into a new tube. Before vertexing, the lysate was treated with 200 µL of lysis/binding buffer and 200µL of ethanol. The sample was centrifuged for one minute at 10,000 x g. An elution buffer (50 L) was used to elute the DNA after being washed twice with wash buffers and preserved at -20°C.

Polymerase chain reaction (PCR)

The reverse primer LCO1490-R:5′-TAA ACT TCA GGG TGA CCA AAA AAT CA- 3′ and the forward primer LCO1490:5′-GGTCAACAAATCATAAAGATATTGG-3′ were used to amplify the mitochondrial DNA of the COI (cytochrome oxidase subunit I) **(Folmer *et al.*, 1994).** The final reaction volume for the PCR amplification was 2X (50 L), and it contained 14.5 L of nuclease-free water, 0.2 M (2 L) of each primer, 25 L of 2X master mix solution (i-Taq, iNtRON, Seongnam, Korea), 4 L of template DNA, and 0.2 mg/ml of BSA. After that, it went through 40 times each for denaturation for 1 minute at 95ºC, annealing for 1 minute at 46ºC, and extension for one minute at 72ºC. The denaturation phase lasted for ten minutes at 95°C. At 72 degrees, a final 10-minute extension was carried out. A 1% agarose gel stained with ethidium bromide was used in conjunction with a transilluminator to run the PCR amplicon (U.V. transilluminator, Spectroline, Westbury, USA) in order to examine the purity and quantity of the PCR result.
Purification of PCR products was achieved using a macrogen reagent (Seoul, Korea). Alignment of *M. domestica* COI nucleotide sequences followed sequencing of a single strand of DNA.

Bioinformatics

Chromas Pro 1.5 beta was used to compile the obtained sequences (Technelysium Pty., Tewantin, QLD, Australia). For molecular identification, the BLAST (Basic Local Alignment Search Tool) was employed to evaluate the newly gained *M. domestica* COI sequences (accession numbers: OQ784762- OQ784764) to those already present in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To align the sequences, muscle alignment was performed in MEGA 11.0. Sequence divergences are determined using the Tamura-3 parameter **(Tamura, 1992).** NJ trees use the Tamura 3-parameter approach to depict the patterns of species divergence **(Tamura, 1992).** The bootstrapping method with 1000 replicates was carried out in MEGA 11.0 **(Kumar *et al*., 2004).** ITOl software for better visuals **(Letunic and Bork, 2021).** PopArt v.3.0 was utilized to assess the minimal spanning network for haplotype divergence.
The phylogenetic tree was inferred using evolutionary distances, and its branches are drawn to size to reflect these distances. The 3-parameter Tamura approach was used in order to calculate evolutionary distances, which appear as the mean number of base substitutions per location **(Tamura, 1992).** Thirty nucleotide sequences were analyzed in this study. First, second, third, and non-coding positions were all accounted for. The final dataset included 434 positions. MEGA11 was used for evolutionary analysis.

Isolation and identification of associated pathogenic bacteria

Bacteria were isolated from the surface of five flies' body pieces by using sterile saline and centrifugation. The solution spread into nutrient, blood agar, and McKanky (Merck, Germany), incubated for 24 hours at 37˚C. Colonies and differentiation were carried out using Gram stain. The pure bacterial isolates were identified by Automated Identification Systems (VITEK) **(Kassiri *et al.*, 2012; Kababian *et al.*, 2020; El-Ghwas *et al.*, 2021; Nazari *et al.*, 2017).**

Sensitivity Bacteria strains to antibiotics

The disc diffusion technique was used to examine the susceptibility of bacterial strains isolated from fly parts according to the recommendations of the Laboratory Standards Institute (CLSI) and Clinical **(Wayne, 2013; Humphries *et al.*, 2018; El-Waseif *et al.*, 2022).** A suspension was adjusted turbidity at 0.5 McFarland for each bacterial strain. Then, antibiotics disks were added to the surface of the medium and incubated for 24 hours at 37°C. The antibiotics covered the four categories: tobramycin, cefotaxime, novobiocin, ampiclox, imipenem, linezolid, azithromycin, tetracycline, rifamycin, piperacillin/tazobactam, kanamycin, and streptomycin.

Statistical analysis

Data from our results were input and coded using SPSS V.22. Data were tested for satisfying assumptions of parametric tests, and continuous variables were subjected to Shapiro-Wilk and Kolmogorov-Smirnov test for normality. Data were presented as mean and standard deviation. ANOVA analyses were done regarding *M. domestica* abundance, temperature, and relative humidity; post-hoc analysis was evaluated using Tukey pairwise comparison; P-values were considered significant at <0.05 using MiniTab V 14. The correlation coefficient was estimated between species abundance and observed physical parameters using SigmaPlot V12.0. Data were visualized when possible, using R studio V 2022.02.4

RESULTS AND DISCUSSION

Abundance of *Musca domestica* adults

Data in **Table (1)** revealed that the highest abundance of adult Musca domestica (201.67±15.57) was recorded in August 2022, while the lowest abundance (17.33 ± 0.57) was recorded in February 2023. A substantial variation was also detected (P<0.05) in the abundance of *M. domestica* over the studied periods **(Figure 2).**

Table 1 Relative Abundance of adult *Musca domestica* collected from El-Muqattam region, Cairo Governorate, Egypt, from May 2022 to April 2023.

Month	Abundance
May 2022	67.67 ± 5.51^{fg}
Jun 2022	107 ± 8.89^{cd}
Jul. 2022	122.33 ± 6.03^{bc}
Aug. 2022	201.67 ± 15.57^{a}
Sep. 2022	131.33 ± 7.51^{b}
Oct. 2022	97.67 ± 4.04^{de}
Nov. 2022	51.67 ± 3.79^{gh}
Dec. 2022	21.33 ± 2.08^{j}
Jan. 2023	30 ± 2.65^{ij}
Feb. 2023	17.33 ± 0.57^{j}
Mar. 2023	40.67 ± 2.52^{hi}
Apr. 2023	81 ± 4.58^{ef}

This means in a column that does not share a letter is significantly different.

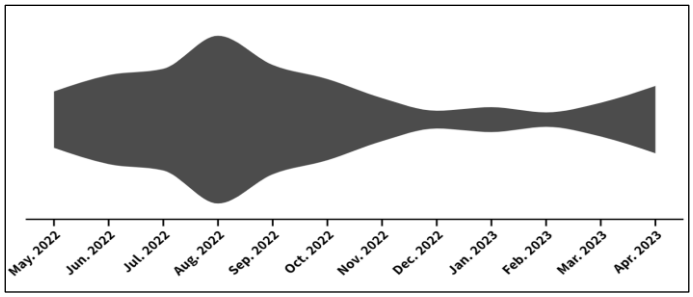

Figure 2 Stream graph represents the total number of adult Musca domestica collected from El-Muqattam region, Cairo Governorate, Egypt, throughout the study period.

In addition, temperature and relative humidity (RH) varied significantly (P<0.05) from one month to another, with the temperature peaking during Aug. 2022 (36.4 ± 0.52a ˚C) and the temperature getting very low during Feb. 2023 (17.26 ± 0.32g ˚C); and the temperature getting very low during Apr. 2023 (31.46 ± 0.32˚C); during the same month relative humidity gets as low as 41.26±0.54% **(Table 2 and Figure 3).**

Table 2 Relative Abundance of adult *Musca domestica* collected from El-Muqattam region, Cairo Governorate, Egypt, from May 2022 to April 2023.

Month	Temperature	Relative Humidity (Rh)
May 2022	30.56 ± 1.65^{c}	63.2 ± 0.37^{b}
Jun 2022	33.03 ± 0.32^{b}	73.93 ± 0.4^{a}
Jul. 2022	35.76 ± 0.45^{a}	73.8 ± 1.17^{a}
Aug. 2022	36.4 ± 0.52^{a}	72.4 ± 0.43^{a}
Sep. 2022	32.83 ± 0.35^{b}	64.06 ± 0.23^{b}
Oct. 2022	27.23 ± 0.65^{d}	$61.6 \pm 0.56b^{c}$
Nov. 2022	22.7 ± 0.75^{e}	58.56 ± 0.34^{cd}
Dec. 2022	19.6 ± 0.55^{f}	53.27 ± 1.05^{e}
Jan. 2023	19.63 ± 0.45^{f}	43.26 ± 0.4^{f}
Feb. 2023	17.26 ± 0.32^{g}	41.26 ± 0.54^{f}
Mar. 2023	22.73 ± 0.37^{e}	52.13 ± 0.75^{e}
Apr. 2023	31.46 ± 0.32^{bc}	56.56 ± 0.48^{d}

This means in a column that does not share a letter is significantly different.

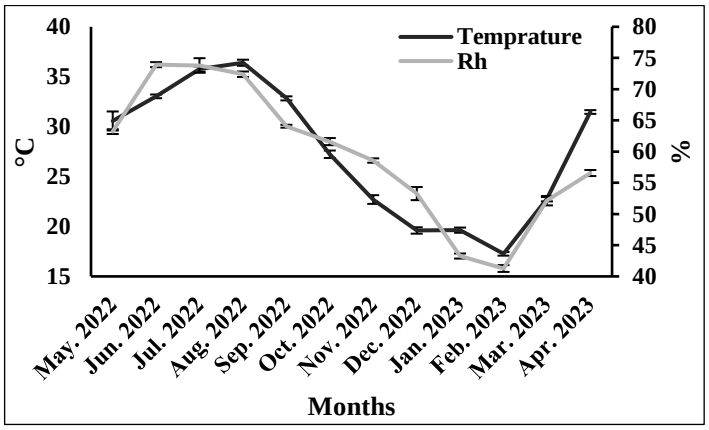

Figure 3 Line chart of recorded temperature and relative humidity in El-Muqattam region, Cairo Governorate, Egypt, throughout the study period.

On the other hand, as shown in **Figure (4),** *M. domestica* abundance positively correlated significantly ($P \leq 0.001$) with temperature ($r= 0.895$) and relative humidity ($r= 0.827$).

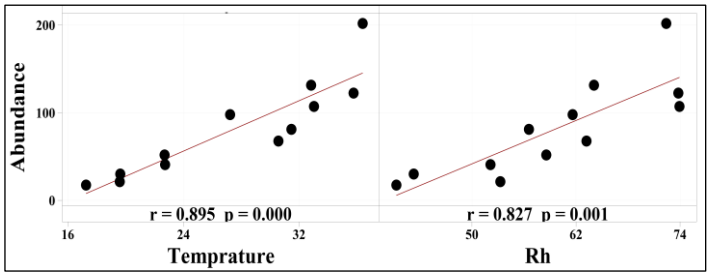

Figure 4 Correlation matrix of *Musca domestica* abundance with temperature relative humidity

Genetic identification of *Musca domestica*

According to **Saitou and Nei, 1987** the neighbor-joining method was employed to deduce the evolutionary history of the species. The best tree choice is shown **(Figure 5a).** The proportion of bootstrapped value (1000 repetitions) that groups together linked taxa is shown as a colour grade for the branches. Using the minimal haplotype spanning network, the studied nucleotide sequences were also mapped in terms of haplotype diversity. A p-value of 0.117941 indicates nucleotide diversity; 46 segregating sites and 38 parsimony-informative sites were found. The value of Tajima's D was -1.97762, and the probability that D is greater than -1.97762 was found to be 0.98698% **(Figure 5b).**

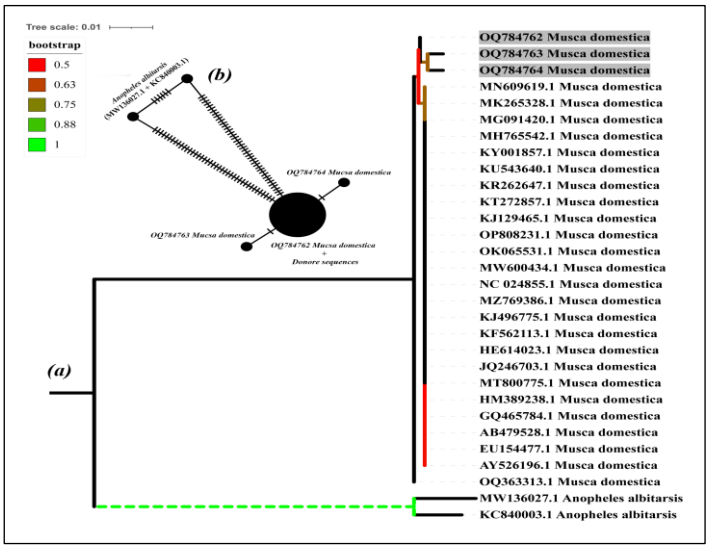

Figure 5 ***(a)*** Neighbor-Joining phylogenetic evolutionary tree ***(b)*** Minimum haplotype spanning network.

Bacterial diversity on *Musca domestica* body surface

The results of bacterial isolation showed that sixty-four (64) bacteria isolates were obtained for this study; forty-nine (49) were Gram-positive with 77% and fifteen (15) were Gram-negative with 23 % from the houseflies' external surfaces. As illustrated in **Figure (6).** On the other hand, the automated identification systems (VITEK) illustrated that there were six (6) different bacterial genera which are *Escherichia coli* (12) with (8.0%), *Pseudomonas aeruginosa* (3) with (2.0%), and *Klebsiella pneumonia* (9) with (5.0%), *Staphylococcus epidermidis* (14) with (9.0 %), *Staphylococcus aureus* (29) with (19.0 %), and *Bacillus spp.* (33) with (21.0 %) as depicted in **Figure (7).**

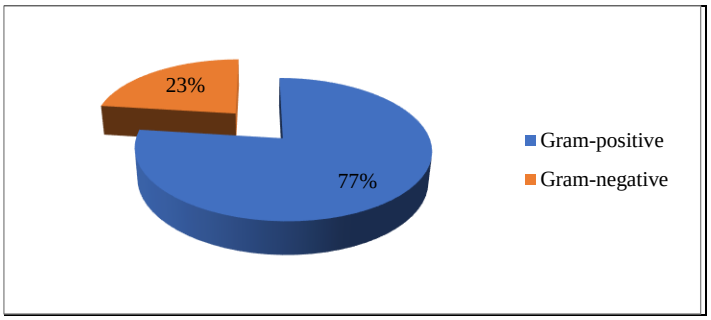

Figure 6 Bacterial isolates diversity and distribution on *Musca domestics*' body surface.

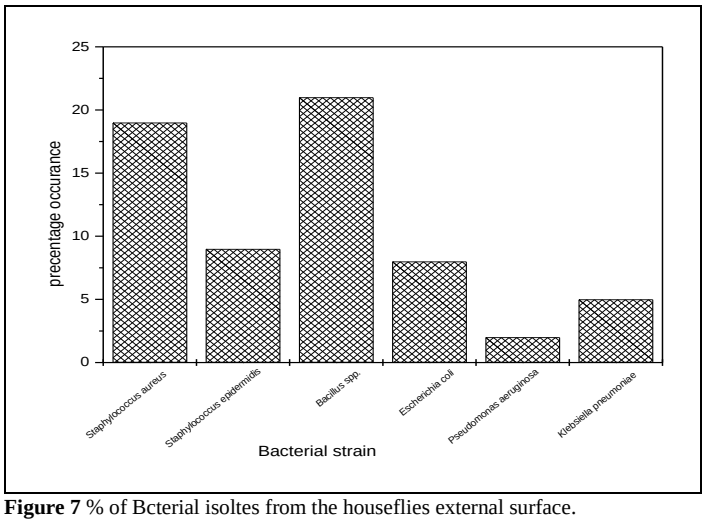

Figure 7 % of Bcterial isoltes from the houseflies external surface.

On the other hand, the results presented in **Table (3)** for antibiotics sensitivity revealed that *bacillus subtilis*., *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* are more resistant to several antibiotics than *Staphylococcus* spp. and *Escherichia coli.* On the other hand, streptomycin, followed by cefuroxime, has recorded potency against almost all tested pathogens.

Table 3 The antibiotics sensitivity of bacterial isolates from *Musca domestics'* body surface.

Bacterial Strains	Antibiotics sensitivity percentage																
	TOB (10)	CTX (30)	NV (30)	AX (25)	KF (30)	IPM (10)	LZD (30)	AZM (15)	CFR (30)	TE (30)	CRO (30)	RF (30)	CL (30)	TZP (110)	CE (30)	K (30)	S (10)
Staphylococcus aureus	34	R	43	R	R	90	100	45	R	21	R	12	R	22	R	52	45
Staphylococcus epidermidis	43	34	R	69	R	R	72	43	R	17	R	9	R	30	R	66	90
Bacillus spp.	R	73	R	R	R	R	R	R	R	R	R	R	R	R	R	R	70
Escherichia coli	14	R	R	R	R	86	R	43	R	R	R	R	R	R	R	63	11
Pseudomonas aeruginosa	R	76	R	R	R	R	R	R	R	R	R	R	R	R	R	R	62
Klebsiella pneumoniae	R	100	R	R	R	R	R	R	R	R	R	R	R	R	R	32	21

DISCUSSION

The findings demonstrated a monthly preponderance of the *Musca domestica*, a common house fly in the Cairo Governorate of Egypt. Differences in the abundance that is significant (P<0.05) of *M. domestica* over the studied months were recorded; these differences can be attributed to climatic changes, especially temperature. These results agree with the previous results recorded by **Abdel Latif *et al.*, (2004),** who found that the highest abundant of *M. domestica* in different localities of Alexandria Governorate, Egypt, was recorded in West Districts and El-Amreya where mean grid counts were 75.0±40.8 and 72.9±46.3, while the lowest abundance was recorded in East Districts and Borg El-Arab where a mean grid counts were 37.7±19.7 and 39.5±28.1, respectively. Also, **Abd El-Halim *et al.*, (2005)** who recorded a high abundance of *M. domestica* in fourteen Egyptian governorates throughout the period from 1999 to 2001. Additionally, according to **Kenawy *et al.*, (2014); Desoky *et al.*, (2020),** *M. domestica* was more plentiful in the summer, autumn, and spring in Cairo and Qalyoubiya Governorates, Egypt, than it was in the winter. Also, they noted seasonal variations in *M. domestica* abundance in Egypt's Sohag Governorate as a result of climate changes.

Moreover, the identification of *M. domestica* has been a challenge for entomologists due to its morphological similarity to other fly species. However, the use of molecular techniques has provided an effective solution to this problem. The cytochrome oxidase subunit I (COI) gene has been widely used for species identification due to its high variability and conserved regions. Several studies have successfully identified *M. domestica* using the COI gene, including a study by **Archana *et al.*, (2016)** that identified *M. domestica* from different regions of India using COI barcoding. Similarly, a study by **Mashaly *et al.*, (2017)** used COI gene sequencing to identify *M. domestica* from different locations in Saudi Arabia. The success of these studies highlights the effectiveness of the COI gene in identifying *M. domestica* and its potential for use in other insect species.

On the other hand, the results of bacterial isolation showed that sixty-four (64) bacteria isolates were obtained for this study; forty-nine (49) were Gram-positive with 77%, and fifteen (15) were Gram-negative with 23 % from the houseflies' external surfaces. Also, the automated identification systems proved the presence of *Escherichia coli* (12) at (8.0%), *Pseudomonas aeruginosa* (3) at (2.0%), *Klebsiella pneumonia* (9) at (5.0%), *Staphylococcus epidermidis* (14) with (9.0 %), *Staphylococcus aureus* (29) with (19.0 %), and *Bacillus* spp. (33) with (21.0 %). These were similar to the findings of **(Babak *et al.*, 2008; Mawak and Olukose, 2006).** According to research by several scientists, the housefly is a potent carrier of infections such *Shigella* spp. and *Campylobacter* spp. and a carrier of bacteria, like *Salmonella* spp. **(Holt *et al.*, 2007),** *Campylobacter jejuni* **(FoÈrster *et al.*, 2009),** *Staphylococcus aureus* **(Fotedar *et al.*, 1992),** *Shigella* spp. **(Cohen *et al.*, 1991),** *Enterococcus faecalis* **(Fotedar *et al.*, 1992),** *Pseudomonas aeruginosa* **(Fotedar *et al.*, 1992)**, and *Escherichia coli* **(Kobayashi *et al.*, 1999).** On the other hand, *Pseudomonas aeruginosa* with an antibiotic resistance strain was discovered in Iran **Bahrndorff *et al.*, (2017).** Furthermore, houseflies in Spanish broiler farms were shown to carry *Escherichia coli* strains that produce β-lactamase. Also, houseflies may be involved in the spread of a disease, the spread of resistance genes, and ecological niches according to **Solar-Gines *et al.*, (2015).** Additionally, **El-Ghwas *et al.*, (2021)** previously examined the microbial variety discovered in *Musca domestica* samples from several places in the Makkah region of Saudia Arabia. Also, there were 18 different bacterial isolates in all; 14 were from *Bacillus* species, two from *Pseudomonas* species, one from *Micrococcus* species, and one from *Staphylococcus* species. Moreover, most isolates belonged to *Bacillus* spp., *Staphylococcus* spp., and *Bacillus* spp. was shown to be the most common species of bacteria found in different environments, accounting for 31.1% and 22.9% of all detected strains, respectively, in prior research **(Nazari *et al.*, 2017).** *Salmonella* and *Shigella* were the most common types of bacteria detected in hospitals, whereas *Enterococcus* spp. were the least common. However, **Kababian *et al.*, (2020)** found that 160 *M. domestica* house flies collected from animal husbandry in the Iranian province of Qom harbored 23 different bacterial species. *P. aeruginosa* (100%) *and E. coli* (73.8% of the total) were the most often isolated bacteria from the skin and the digestive system, respectively.

CONCLUSION

The results demonstrated a monthly preponderance of the *Musca domestica*, a common house fly in the Cairo Governorate of Egypt, where the highest abundance of adult *Musca domestica* (201.67±15.57) was recorded in August 2022, while the lowest abundance (17.33±0.57) was recorded in February 2023. Additionally, the presence of various pathogens, such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus epidermidis, Klebsiella pneumonia, Staphylococcus aureus*, and *Bacillus* spp. suggests a potential risk of the infections being transmitted from houseflies to people, which results in illnesses. Thus, raising awareness of this issue and working toward controlling this pest is vital. They must be managed and various vector control strategies must be used to lower their population's density. Thus, the present study's results considered a step in drawing an updated map for insect vectors in Egypt that helps in national control programs

Acknowledgments: The authors would like to acknowledge the Pharmaceutical Industrial Research Institute, Chemistry of Natural and Microbial Products Department, National Research Centre and the Botany and Microbiology Department, Zoology Department, Faculty of Science, Al-Azhar University for their support.

REFERENCES

Abd El-Halim A. S., Mostafa A. A., & Allam K. A. M. (2005). Dipterous flies' species and their densities in fourteen Egyptian Governorates. *Journal of the Egyptian Society of Parasitology,* 35, 351-362.

Abdel Latif, S., Abou El-Ela, N., Awad, O., & El-Fiki, S. (2004). Monitoring of Housefly [*Musca Domestica*] Population Abundance in Alexandria Governorate and Assessment of its Susceptibility towards some Commonly Used Insecticides. *Journal of High Institute of Public Health,* 34(4), 941–960. https://doi.org/10.21608/jhiph.2004.183674

Aksoy, E., Telleria, E. L., Echodu, R., Wu, Y., Okedi, L. M., Weiss, B. L., Aksoy, S., & Caccone, A. (2014). Analysis of Multiple Tsetse Fly Populations in Uganda Reveals Limited Diversity and Species-Specific Gut Microbiota. *Applied and Environmental Microbiology,* 80(14), 4301–4312. https://doi.org/10.1128/aem.00079-14

Archana M., D'Souza P. E., Ojha R., &Jalali S. K. (2016). DNA barcoding of flies commonly prevalent in poultry farms of Bengaluru District. *Journal of Entomology and Zoology Studies,* 4(4), 228-233.

Azambuja, P., Garcia, E. S., & Ratcliffe, N. A. (2005). Gut microbiota and parasite transmission by insect vectors. *Trends in Parasitology,* 21(12), 568-572. https://doi.org/10.1016/j.pt.2005.09.011

Bahrndorff, S., Alemu, T., Alemneh, T., & Lund Nielsen, J. (2016). The Microbiome of Animals: Implications for Conservation Biology. *International Journal of Genomics*, 1-7. https://doi.org/10.1155/2016/5304028

Bahrndorff, S., de Jonge, N., Skovgård, H., & Nielsen, J. L. (2017). Bacterial Communities Associated with Houseflies (*Musca domestica* L.) Sampled within and between Farms. *PLOS ONE,* 12(1), e0169753. https://doi.org/10.1371/journal.pone.0169753

Bahrndorff, S., Rangstrup-Christensen, L., Nordentoft, S., & Hald, B. (2013). Foodborne Disease Prevention and Broiler Chickens with Reduced *Campylobacter* Infection. *Emerging Infectious Diseases,* 19(3), 425-430. https://doi.org/10.3201/eid1903.111593

Cerf-Bensussan, N., & Gaboriau-Routhiau, V. (2010). The immune system and the gut microbiota: friends or foes?. *Nature Reviews Immunology,* 10(10), 735-744. https://doi.org/10.1038/nri2850

Cirimotich, C. M., Ramirez, J. L., & Dimopoulos, G. (2011). Native Microbiota Shape Insect Vector Competence for Human Pathogens. *Cell Host and Microbe,* 10(4), 307-310. https://doi.org/10.1016/j.chom.2011.09.006

Cohen, D., Green, M., Block, C., Slepon, R., Ambar, R., Wasserman, S. S., & Levine, M. M. (1991). Reduction of transmission of shigellosis by control of houseflies (*Musca domestica*). *The Lancet,* 337(8748), 993-997. https://doi.org/10.1016/0140-6736(91)92657-n

De Jesús, A. J., Olsen, A. R., Bryce, J. R., & Whiting, R. C. (2004). Quantitative contamination and transfer of *Escherichia coli* from foods by houseflies, *Musca domestica* L. (Diptera: Muscidae). *International Journal of Food Microbiology*, 93(2), 259-262. https://doi.org/10.1016/j.ijfoodmicro.2003.12.003

Desoky, ASS, Ahamed, HEM, A., & Eraky SA, (2020). Seasonal Abundance of Fly Species in the Animals Farm of the Faculty of Agriculture, Sohag University, Sohag Governorate, Egypt. *Journal of Aquaculture & Livestock Production,* 1(1), 1-3. https://doi.org/10.47363/jalp/2020(1)106

Dillon R. J., & Dillon V. M. (2004). The gut bacteria of insects: Nonpathogenic interactions. *Annual Review of Entomology,* 49(1), 71-92. https://doi.org/10.1146/annurev.ento.49.061802.123416

Douglass E. S. & Jesse C. (2002). Integrated pest management for fly control in Maine dairy farms. Texas Agricultural Extension Service, 4. 6. pp.
El-Ghwas, D. E., Al-Nasser, A. S., & Al-Sheikhy, A. A. (2021). Musca Domestica: A vector of pathogenic microorganisms and biocontrol approaches. *Global Journal of Science Frontier Research*, 1-14. DWT. https://doi.org/10.34257/gjsfrcvol21is2pg1
El-Waseif, A. A., Eissa, H. A., Abo El Maaty, S. A., & Hassan, M. G. (2022). Anti-Virulence and Cytotoxic Behavior of Copper Nanoparticles against antibiotics-resistant *Staphylococcus aureus*. *Egyptian Journal of Chemistry*, 65(9), 361-367. https://doi.org/10.21608/ejchem.2022.111832.5079
Felsenstein, J. (1985). Confidence Limits on Phylogenies: An approach using the bootstrap. *Evolution*, 39(4), 783. https://doi.org/10.2307/2408678
Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology*, 3(5), 294-299. PMID: 7881515.
Förster, M., Sievert, K., Messler, S., Klimpel, S., & Pfeffer, K. (2009). Comprehensive study on the occurrence and distribution of pathogenic microorganisms carried by synanthropic flies caught at different rural locations in Germany. *Journal of Medical Entomology*, 46(5), 1164-1166. https://doi.org/10.1603/033.046.0526
Fotedar, R., Banerjee, U., Singh, S., Shriniwas, & Verma, A. K. (1992). The housefly (*Musca domestica*) as a carrier of pathogenic microorganisms in a hospital environment. *Journal of Hospital Infection*, 20(3), 209-215. https://doi.org/10.1016/0195-6701(92)90089-5
Greenberg B. (1973). Flies and Diseases vol II, Biology and Disease Transmission. Princeton, NJ: Princeton University Press. https:// https://doi.org/10.2307/j.ctvbcd1ck
Gupta, A. K., Rastogi, G., Nayduch, D., Sawant, S. S., Bhonde, R. R., & Shouche, Y. S. (2014). Molecular phylogenetic profiling of gut-associated bacteria in larvae and adults of flesh flies. *Medical and Veterinary Entomology*, 28(4), 345-354. https://doi.org/10.1111/mve.12054
Holt, P. S., Geden, C. J., Moore, R. W., & Gast, R. K. (2007). Isolation of *Salmonella enterica* Serovar Enteritidis from Houseflies (*Musca domestica*) Found in Rooms Containing *Salmonella* Serovar Enteritidis-Challenged Hens. *Applied and Environmental Microbiology*, 73(19), 6030-6035. https://doi.org/10.1128/aem.00803-07
Humphries, R. M., Ambler, J., Mitchell, S. L., Castanheira, M., Dingle, T., Hindler, J. A., Koeth, L., Sei, K., Hardy, D., Zimmer, B., Butler-Wu, S., Dien Bard, J., Brasso, B., Shawar, R., Dingle, T., Humphries, R., Sei, K., & Koeth, L. (2018). CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests. *Journal of Clinical Microbiology*, 56(4), e01934-17. https://doi.org/10.1128/jcm.01934-17
Issa, R. (2019). *Musca domestica* acts as transport vector hosts. *Bulletin of the National Research Centre*, 43,73. https://doi.org/10.1186/s42269-019-0111-0
Jones, R. T., Knight, R., & Martin, A. P. (2009). Bacterial communities of disease vectors sampled across time, space, and species. *The ISME Journal*, 4(2), 223-231. https://doi.org/10.1038/ismej.2009.111
Kababian, M., Mozaffari, E., Akbarzadeh, K., Shabani Kordshouli, R., Saghafipour, A., & Shams, S. (2020). Identification of Bacteria Contaminating *Musca domestica* (Diptera: Muscidae) Collected from Animal Husbandries. *Shiraz E-Medical Journal*, 21(4), e92018. https://doi.org/10.5812/semj.92018
Kassiri, H., Akbarzadeh, K., & Ghaderi, A. (2012). Isolation of Pathogenic Bacteria on the House Fly, *Musca domestica* L. (Diptera: Muscidae), Body Surface in Ahwaz Hospitals, Southwestern Iran. *Asian Pacific Journal of Tropical Biomedicine*, 2(2), S1116-S1119. https://doi.org/10.1016/s2221-1691(12)60370-0
Kenawy, M., Amer, H., Lotfy, N., Khamis, N., & Abdel-Hamid, Y. (2014). insects associated with hospital environment in egypt with special reference to the medically important species. *Journal of the Egyptian Society of Parasitology*, 44(3), 665-676. https://doi.org/10.21608/jesp.2014.90157
Khamesipour, F., Lankarani, K. B., Honarvar, B., & Kwenti, T. E. (2018). A systematic review of human pathogens carried by the housefly (*Musca domestica* L.). *BMC Public Health*, 18(1), 1049 . https://doi.org/10.1186/s12889-018-5934-3
Kobayashi M., Sasaki T., Saito N., Tamura K., Suzuki K., and Watanabe H. (1999). Houseflies: Not simple mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7. *The American journal of tropical medicine and hygiene*, 61(4), 625-629. https://doi.org/10.1371/journal.pone.0169753
Kumar, S., Dhingra, A., & Daniell, H. (2004). Stable transformation of the cotton plastid genome and maternal inheritance of transgenes. *Plant Molecular Biology*, 56(2), 203-216. https://doi.org/10.1007/s11103-004-2907-y
Letunic, I., & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, 49(W1), W293-W296. https://doi.org/10.1093/nar/gkab301
Mashaly, A., Alajmi, R., Mustafa, A. E.-Z., Rady, A., & Alkhedir, H. (2017). Species Abundance and Identification of Forensically Important Flies of Saudi Arabia by DNA Barcoding. *Journal of Medical Entomology*, 54(4), 837-843. https://doi.org/10.1093/jme/tjx049
Mawak J. D. & Olukose O. J. (2006). Vector potential of houseflies (*Musca domestica*) for pathogenic organisms in Jos, Nigeria. *Journal of Pest, Disease and Vector Management*, 7, 418-423.
Mian L. S., Maag H., & Tacal J. V. (2002). Isolation of Salmonella from muscoid flies at commercial animal establishments in San Bernardino County, California. *Journal of Vector Ecology*, 27(1), 82-85. PMID: 12125877.
Nazari M., Mehrabi T., Hosseini S. M., Alikhani M. Y. (2017). Bacterial Contamination of Adult House Flies (*Musca domestica*) and Sensitivity of these Bacteria to Various Antibiotics, Captured from Hamadan City, Iran. *Journal of Clinical and Diagnostic Research*,11(4), DC04-DC07. http://doi:10.7860/JCDR/2017/23939.9720
Neupane, Nayduch, & Zurek. (2019). House Flies (*Musca domestica*) pose a risk of carriage and transmission of bacterial pathogens associated with bovine respiratory disease (BRD). *Insects*, 10(10), 358. https://doi.org/10.3390/insects10100358
Osei-Poku, J., Mbogo, C. M., Palmer, W. J., & Jiggins, F. M. (2012). Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from Kenya. *Molecular Ecology*, 21(20), 5138-5150. https://doi.org/10.1111/j.1365-294x.2012.05759.x
Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4), 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
Solà-Ginés M., González-López J. J., Cameron-Veas K., Piedra-Carrasco N., Cerdà-Cuéllar M., & Migura-Garcia L. (2015). Houseflies (*Musca domestica*) as Vectors for Extended-Spectrum β-Lactamase-Producing *Escherichia coli* on Spanish Broiler Farms. *Applied and Environmental Microbiology*, 81(11), 3604-3611. https://doi.org/10.1128/aem.04252-14
Tamura K, Stecher G, & Kumar S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7), 3022-3027. http://doi:10.1093/molbev/msab120
Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Molecular Biology and Evolution*, 9(4), 678-687. http://doi:10.1093/oxfordjournals.molbev.a040752.
Vazirianzadeh B., Solary S. S., Rahdar M., Hajhossien R., & Mehdinejad M. (2008). Identification of bacteria which possible transmitted by *Musca domestica* (Diphtera: Muscidae) in the region of Ahvaz, SW Iran. *Jundishapur Journal of Microbiology*, 1(1), 28-31 https://brieflands.com/articles/jjm-72510.html
Wayne P. (2013). Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. M100-S23. http://doi.org/10.7860%2FJCDR%2F2017%2F23939.9720
Wei, T., Ishida, R., Miyanaga, K., & Tanji, Y. (2014). Seasonal variations in bacterial communities and antibiotic-resistant strains associated with green bottle flies (Diptera: Calliphoridae). *Applied Microbiology and Biotechnology*, 98(9), 4197-4208. https://doi.org/10.1007/s00253-013-5498-1
Weiss B, & Aksoy S. (2011). Microbiome influences on insect host vector competence. *Trends in parasitology*, 27(11), 514-522. https://doi.org/10.1016%2Fj.pt.2011.05.001