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Research Article

Role of the Housefly as a Biological Vector for Bacteria and Fungi at Some Slaughterhouses

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Abstract

Background and Objective: The housefly, *Musca domestica* L. is the most widespread species of fly in the world. It is in close association with many human pathogens that which can cause serious and life-threatening diseases are known to be carried by house flies, including bacteria, viruses, fungi and parasites, Therefore, this study came intending to determine the role of house flies isolated from some slaughterhouses in the city of Al-Diwaniyah/Iraq in the spread of bacterial and fungal pathogens to humans.

Materials and Methods: The 120 insects of house flies were collected randomly from some slaughterhouses for the period September to November, 2021 by special networks prepared for this purpose and transferred to the laboratory and then the bacteria and fungi were isolated and identified from the external surface of house flies by using appropriate culture media. **Results:** In this study, 148 bacterial isolates belonging to 15 species of bacteria were isolated and diagnosed from the external body of house flies that isolated, 91 samples had given positive growth of bacteria and *Escherichia coli* was the most occurrence and frequent from the other bacterial isolates, the occurrence rate of it was 16/91 (17.582%) with a frequency rate of 27/148 (18.243%). The 154 fungal isolates belonging to 16 species of fungi were isolated and diagnosed from the external body of house flies, 87 samples had given a positive growth of fungi and *Aspergillus niger* was the most occurrence and frequent from the other fungal isolates, as its occurrence rate reached 14/87 (16.091%) and the frequency rate of 21/154 (13.636%). **Conclusion:** House flies transmit many pathological microorganisms such as bacteria, fungi, viruses, etc., which causes various diseases for humans and animals and it may help the spread of antibiotic-resistant species and there is an urgent need to conduct many studies to know the new types of microorganisms that house flies can transmit.

Key words: Housefly, *Musca domestica*, bacteria, fungi, slaughterhouses

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Musca domestica is one of the insects that belong to the Muscidae family, which belongs to the Diptera order, which is one of the largest orders of insects and which includes many species. Several laboratory studies have been conducted on species of this order for their medical, veterinary and economic importance, for their ease of breeding in the laboratory and their short life cycle¹. House flies cause many harms, including direct harm to humans, such as annoyance and indirect through contact with food and human and animal waste and thus it is considered a biological vector for many microorganisms such as bacteria, fungi, viruses, protozoa and nematodes^{2,3}. The main phenotypic characteristics that helped house flies to be a good vector for pathogens are that the proboscis of the mouthparts contains many fine capillaries, which enabled it to easily pick up pollutants from the middle, in addition to containing six feet containing tiny capillaries that act as adhesive parts for the pathogens, which gives it high efficacy in transmitting pathogens⁴. Some studies indicate that about 500,000 different microorganisms exist on the parts and legs of the body of house flies⁵. Among the important bacterial and fungal diseases that house flies helped spread are Cholera, Typhoid, Food poisoning, Bloody diarrhoea, Tuberculosis, Anthrax, Aspergillosis, Penicilliosis, Alternariosis, Onychomycosis and other various diseases^{6,7}. The different microorganisms can live inside the body of the insect or on its external surface for a period ranging from 5 hrs to approximately 35 days⁸. These insects spread in different environments such as hospitals, houses, slaughterhouses and other places and cause mechanical transmission of pathogens, which is the most famous mechanism and this occurs when pathogens move from one vertebrate host to another without the organism developing inside the vector⁹. House flies usually feed and reproduce in faeces, animal waste and other decomposing organic materials and thus it is in close association with many microorganisms including human pathogens, which may adhere to the body surfaces of the fly during its movement and may lead to the transmission of various pathogens to humans and leading to infection¹⁰. They are also potential vectors for the spread of antimicrobial resistance (AMR)¹¹. Therefore, this study came intending to determine the role of house flies isolated from some slaughterhouses in the city of Al-Diwaniyah in the spread of bacterial and fungal pathogens to humans as the first steps to control house flies and reduce their density in different human environments.

MATERIALS AND METHODS

Collecting house flies: The 120 insects of house flies were collected randomly from some slaughterhouses in Al-Diwaniyah city/Iraq for the period September to November, 2021 by special networks prepared for this purpose and then they were placed in special sterile glass bottles and transferred to the laboratory.

Isolation of bacteria and fungi: The sterile glass bottles containing the flies were placed in the freezer at a temperature of 0°C for 15 min to anaesthetize the flies, then they were washed twice by placing them for 1 min in 1 mL sterile distilled water and then placed in 2 mL of saline solution at a concentration of 0.85% for soaking and then transfer 0.1 mL of the yield solution to plastic Petri dishes containing the appropriate culture media, which are Blood agar, MacConkey agar for bacteria, Potato dextrose agar and Sabouraud dextrose agar with the addition of the antibiotic Chloramphenicol at a concentration of 250 mg L⁻¹ to prevent bacterial growth for fungi and the dishes were incubated at 37°C For 24 hrs for bacteria and 25°C for 3-7 days for fungi¹².

Diagnosis of bacteria: The bacteria were identified after the end of the incubation period according to the phenotypic characteristics of the colonies growing on the medium of blood agar and MacConkey, such as colony shape, colour and nature and a glass slide of growth was prepared and examined microscopically to identify the shape of the bacteria and the type of staining with Gram stain, some biochemical tests such as Indole test, Sugar fermentation test, Methyl Red/Voges-Proskauer test, Motility test, Urease test, Catalase test, Coagulase test, Blood Hemolysis test, Gelatin hydrolysis test, Oxidase test and growth on Mannitol salt agar^{13,14}.

Diagnosis of fungi: Molds were identified based on the phenotypic characteristics of colonies growing on Potato dextrose agar media, such as the shape, colour, texture and diameter of the colony in addition to the microscopic characteristics such as fungal filament shape, size, colour and conidia^{15,16}. As for the yeasts, some biochemical tests were used to confirm their diagnosis, such as growth at 37°C, Germ tube formation, hydrolysis of urea test and a fermentation and sugars assimilation test¹⁷. Then the percentage of occurrence and frequency of bacteria and fungi was calculated separately by the following equations:

$$\text{Occurrence (\%)} = \frac{\text{Number of samples that appeared the same genus or species}}{\text{Total number of samples}} \times 100$$

$$\text{Frequency (\%)} = \frac{\text{Number of isolates of the same genus or species}}{\text{Total number of isolates}} \times 100$$

RESULTS

Diagnosis of bacteria and fungi: The results showed isolation and diagnosis of 148 bacterial isolates belonging to 15 types of bacteria from the external body of house flies isolated from some slaughterhouses in Al-Diwaniyah city and 91 samples had given positive growth of bacteria and *E. coli* was the most occurrence and frequent from the other bacterial isolates, as the occurrence rate reached 16/91 (17.582%) and the frequency rate 27/148 (18.243%), followed by *S. aureus* with

an occurrence rate of 11/91 (12.087%) with a frequency of 19/148 (12.837%), while *B. subtilis* was the least occurrence and frequent from the other bacterial isolates, with an occurrence rate of 2/91 (2.197%) with a frequency rate of 3/148 (2.027%) in Table 1, also, the results showed isolation and diagnosis of 154 fungal isolates belonging to 16 species of fungi and 87 samples had given positive growth of fungi and *A. niger* was the most occurrence and frequent from the other fungal isolates, as its occurrence rate reached 14/87 (16.091%) and the frequency rate of 21/154 (13.636%), followed by *P. aurantiogriseum* with an occurrence rate of 11/87 (12.643%) with a frequency rate of 18/154 (11.688%), while *A. parasiticus*, *C. lunata* and *S. brevicaulis* were the least occurrences fungi with an occurrence rate of 2/87 (2.298%) and *S. brevicaulis* was the less frequent, with a frequency rate of 3/154 (1.948%) in Table 2.

Table 1: Percentages of occurrence and frequency of bacteria isolated from houseflies

Bacteria	Number of samples that appeared the bacteria	Occurrence (%)	Number of isolates	Frequency (%)
<i>Bacillus alcalophilus</i>	5	5.494	5	3.378
<i>B. subtilis</i>	2	2.197	3	2.027
<i>B. cereus</i>	9	9.890	11	7.432
<i>Enterococcus faecalis</i>	6	6.593	13	8.783
<i>Escherichia coli</i>	16	17.582	27	18.243
<i>Klebsiella aerogenes</i>	7	7.692	13	8.783
<i>K. pneumonia</i>	4	4.395	6	4.054
<i>Micrococcus halobius</i>	3	3.296	6	4.054
<i>Proteus mirabilis</i>	4	4.395	8	5.405
<i>P. vulgaris</i>	3	3.296	5	3.378
<i>Pseudomonas aeruginosa</i>	8	8.791	9	6.081
<i>Salmonella typhi</i>	4	4.395	8	5.405
<i>Staphylococcus aureus</i>	11	12.087	19	12.837
<i>S. epidermidis</i>	4	4.395	6	4.054
<i>Streptococcus pyogenes</i>	5	5.494	9	6.081
Total	91		148	

Table 2: Percentages of occurrence and frequency of fungi isolated from houseflies

Fungi	Number of samples that appeared the fungus	Occurrence (%)	Number of isolates	Frequency (%)
<i>Alternaria alternata</i>	7	8.045	12	7.792
<i>Aspergillus flavus</i>	9	10.344	15	9.740
<i>A. fumigatus</i>	4	4.597	9	5.844
<i>A. niger</i>	14	16.091	21	13.636
<i>A. ochraceus</i>	3	3.448	6	3.896
<i>A. parasiticus</i>	2	2.298	4	2.597
<i>Candida albicans</i>	6	6.896	11	7.142
<i>C. tropicalis</i>	3	3.448	7	4.545
<i>Curvularia lunata</i>	2	2.298	5	3.246
<i>Fusarium oxysporum</i>	5	5.747	12	7.792
<i>F. proliferatum</i>	4	4.597	7	4.545
<i>Mucor circinelloides</i>	6	6.896	9	5.844
<i>Penicillium aurantiogriseum</i>	11	12.643	18	11.688
<i>P. verrucosum</i>	5	5.747	7	4.545
<i>Rhizopus stolonifer</i>	4	4.597	8	5.194
<i>Scopulariopsis brevicaulis</i>	2	2.298	3	1.948
Total	87		154	

DISCUSSION

The harm of house flies is not only limited to being an inconvenience insect, but that it poses health risks by transferring it to microorganisms to humans and animals and these results are in agreement with some previous studies which indicated the possibility of isolating different bacterial species from house flies, as Vazirianzadeh *et al.*¹⁸, who isolated some bacterial species from the outer surface of house flies and found that *E. coli* was more frequent bacteria with a rate of 36.5%, followed by *S. aureus* with 26.9%, also in another study in Morocco Bouamamaa *et al.*¹⁹ was able from isolating some bacterial species from the outer surface of house flies, *E. coli* were found to be the most frequent, with a rate of 43.3%, as mentioned by Kassiri *et al.*²⁰ that *Pseudomonas* spp. and *E. coli* were the most isolated species from the outer surface of house flies isolated from some hospitals in Ahwaz city in Iran. Baker *et al.*²¹ were also able of isolating some bacterial species from the outer surface of house flies isolated from three different locations in Tikrit city in Iraq, which are the vegetable market, the butchers market and sheep pens and *E. coli* was the most frequent, at a rate of 23.5%. The soil contents are responsible for the spread of several fungal spores and the natural infection of the larvae of the Muscidae family and the transmission of infection with these fungal pathogens from larvae to pupae and adults is through contact with these fungal pathogens²², these results are in agreement with Zarrin *et al.*²³ who was able to isolate some fungi from the outer surface of house flies, including *Aspergillus* spp., at a rate of 30%, *Penicillium* spp., at a rate of 25%, as agreed with Srivoramas *et al.*²⁴, who was able to isolate some fungi from adult house flies, including *A. niger* and *Penicillium* spp. and also agrees with Phoku *et al.*²⁵, which isolated from the outer surface of house flies a wide range of fungi such as *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp., were more frequent than the other fungal species and agree also with Nwankwo *et al.*²⁶, who mentioned that *Aspergillus* spp., were more frequent than the other fungal species, with a frequency of 28.3%.

CONCLUSION

House flies transmit many pathological microorganisms such as bacteria, fungi, viruses, etc., which cause various diseases for humans and animals and it may help the spread of antibiotic-resistant species and there is an urgent need to conduct many studies to know the new types of microorganisms that house flies can transmit, as well as isolation house, flies from different environments such as schools, hospitals and homes.

SIGNIFICANCE STATEMENT

This study confirms that the harm of house flies is much more than a source of a nuisance and that it poses great health risks for humans especially patients with immunodeficiency as mechanical vectors of many bacteria and fungi, so care must be taken to control house flies and reduce their density in the different human environments.

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