

A Study on the bacterial flora and its significance in the housefly, *Musca domestical L. (Diptera : Muscidae)*

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Abstract

The microbial flora associated with the housefly, *Musca domestica* were isolated and their influence on some biological parameters of the fly was studied. The microbial flora isolation was carried out by using different selective and non-selective media. The examination of the housefly gut revealed a heavy and diverse microbial flora, these were : Enteric bacteria (*Escherichia coli*, *Salmonella sp.*, *Shigella sp.*, *Pseudomonas sp.*, *Klebsiella sp.*, *Proteus sp.*, *Serratia sp.*); Cocci form bacteria (Haemolytic Staphylococci and Streptococci, non-haemolytic Staphylococci and Streptococci) and *Bacillus sp.* Actinomycetes, yeasts and filamentous fungi were isolated only from external surface of the fly. Aposymbiotic (the gut-bacterial free) flies were obtained by feeding female flies on norfloxacin antibiotic. Hatchability, larval and pupal mortality, pupation and adult emergence were affected by elimination of bacteria from the fly gut. It was concluded that gut bacteria of the housefly are not pathogenic but their association might be mutual or even symbiotic.

Introduction

Houseflies have adapted to domestic living, feeding and breeding on human food, organic wastes and faeces. The movement of houseflies between faeces and food makes them ideal transmitters of human disease. A vast literature has developed on this subject and was summarized by West (1951) for work published before 1950. Greenberg (1971, 1973) has given a full list of the organisms which have been recovered from houseflies and a detailed consideration of their relationship to human and animal diseases.

Houseflies have been found to harbor about 100 different pathogens and charged with transmitting 65 of these (Greenberg, 1965). The pathogens recovered from flies range from viruses to helminthes, and include the viruses of

poliomyelitis and infectious hepatitis, the bacteria associated with cholera (*Vibrio*), enteric infections caused by species of *Salmonella* and *Shigella*, pathogenic *Escherichia coli*, haemolytic streptococci, *Staphylococcus aureus*, agents of trachoma, bacterial conjunctivitis, anthrax, diphtheria, tuberculosis, leprosy and yaws. In addition flies can carry the cysts of protozoa, including those of *Entamoeba histolytica* which causes amoebic dysentery, and the eggs of the threadworm *Trichuris trichiura*, the hookworm *Ancylostoma duodenale* and of other nematodes and cestodes.

The present work aimed firstly, to isolate and identify the different microbial flora associated with the female housefly, *Musca domestica*, Secondly; to study the effect of gut microbial flora on preoviposition

period, hatchability, larval and pupal mortality percent, pupation and adult emergence.

Materials And Methods

Insect used and maintenance of culture:

The insect used in the present study were the housefly, *Musca domestica* L. A stock colony of this fly species was raised for several generations in the laboratory of Medical Entomology, Faculty of Science, Al-Azhar University, Cairo at $25 \pm 2^\circ\text{C}$ and 50-60% R.H. the rearing technique was carried out according to the method of Busvine (1962).

Elimination of gut bacteria of *Musca domestica* females:

Newly emerged fifteen flies were collected from established colony and placed in a glass cylinder measured 15x10 cm, its top covered with a muslin tissue with a small hole. These flies were left to fed on 10% sugar solution added to it 14 µg/ml Norofloxacin antibiotic for three days. After this period each fly were transferred to another cylinder. Two small Petri dishes (2.5cm. diam.) were placed inside each cylinder. The first dish contains a cotton pad saturated with 10% sugar solution, and the second dish contains a cotton pad saturated with 10% milk solution.

Examination of gut microbial flora of *Musca domestica*:-

Ten specimens of housefly, *Musca domestica* were carefully kept alive in sterile stoppered glass tubes. Then they were separately dipped in 95 % ethanol for two seconds, and then placed for equal period in 10 % sodium thiosulfate (Poinar, *et al*, 1978 and Hassan *et al.*, 2001). After the external surface has been sterilized, the specimens were

rinsed for three consecutive times in sterile distilled water and were dissected under aseptic condition using sterile minute needles. The gut of each specimen was taken separately and diluted in 5 ml. of sterile saline solution. Fifty µl of 10^{-3} dilution of each sample was streaked on plates of different selective and non-selective microbiological agar media. Vital counts of the developed microorganisms of each specimen were recorded after an incubation time of 24 – 48 h. for bacteria at 30°C . Identification of microbial flora was carried out according to Keys constructed by (Kreig, 1989 and Holt *et al.*, 1994).

Antimicrobial susceptibility:-

The antimicrobial susceptibility of selected 10 purified bacterial culture of gut flora of different investigated flies was examined by disk diffusion method (Bauer *et al*, 1966). Bacterial cultures maintained on nutrient agar slants or blood agar slant in case of fastidious organisms were grown in nutrient broth or nutrient broth supplemented with 5 % sheep blood for 18 h. at 37°C . A volume of 0.1 of the diluted culture equivalent to 10^6 CFU/ml. was mixed with sterile nutrient agar (or blood agar) plates. The disks of the following antimicrobial agents (BBL) were used.: Ampicillin (AM, 10mg), Kanamycin (K,30mg), Carbencillin (CB,100 mg), Rifampicin (RA, 30 mg), Sulfa (SXT, 25mg), Nitrofurantoin (FD, 300mg), Tetracycline (TE,30mg), Natidixic acid (NA, 30mg) and Norofloxacin (NOR, 30mg).

Effect of gut bacteria on some biological aspects of *Musca domestica*:

After 48 h. the males flies were excluded and the female flies were left to study the effect of bacterial

elimination from the gut on number of eggs laid, preoviposition period hatchability, larval and pupal mortality, pupation and adult emergence. A control group was used as the treated one and fed on the same media, without adding antibiotic and the previous developmental aspects were recorded. Statistical analysis of the data was carried out according to the method of Lentner *et al.* (1982).

Results

Microbial flora associated with the housefly *Musca domestica*:

The microbial examination of the housefly, *Musca domestica* revealed a heavy and very diverse microbial flora which can be summarized in table (1) and (2). The isolated bacteria from the housefly either externally or internally were found to belong to *Escherchia coli*, *Salmonella sp.*, *Shigella sp.*, *Pseudomonas sp.*, *Klebsiella sp.*, *Proteus, serratia sp.* and *Bacillus sp.*

Tables (1) and (2) showed the absence of *Haemophilus* bacteria either externally or internally (in the gut), while non-haemolytic *Staphylococci* and *Streptococci* represented 100% in both isolated sites. Also it was noticed from table (1) that actinomycetes, yeasts and filamentous fungi were isolated only from the external surface of the housefly, while they were completely disappeared from the housefly gut.

Data in tables (1) and (2) showed that enteric bacteria represented 20 and 79% of all isolated bacteria from the external surface and the gut; respectively, while cocci form bacteria recorded the lowest percent in the two examined sites. It was also noticed that *Escherchia coli* represented 73% of all enteric bacteria isolated, while

Pseudomonas: Klebsiella, Proteus and *Serratia* represented 11% of the enteric bacteria in the gut of the housefly.

As regards, *Salmonella* and *Shigella* were represented only by 1% and 16% of enteric bacteria isolated from external surface and gut; respectively.

Elimination of gut bacterial flora of female *Musca domestica*:

The data presented in table (3) showed that the norfloxacin antibiotic was the most effective agent against: *Escherchia coli*, *Salmonella sp.*, *Pseudomonas sp.*, *Staphylococcus sp.* and *Streptococcus sp.* thus it is used in the present work to eliminate these bacterial species from the gut of *Musca domestica* female. Other antimicrobial agents tested were less effective than norfloxacin on the previous bacterial species.

Effect of the isolated midgut bacteria on different biological aspects of *Musca domestica*:

The data in table (4) showed that the mean number of eggs laid was decreased in the midgut-bacterial free females compared to 136 ± 7.82 for the control ones to 111.4 ± 8.92 . The bacterial elimination from the gut was found to greatly affect on the hatchability percent, where the hatchability percent was highly significantly reduced to 59.6% compared to 96.5%.

The data recorded in table (5) revealed that the larval mortality was increased to 8.4% in midgut-bacterial free flies, while it was 3.5% in the control group. On the other hand the pupal mortality was 8.0% in the midgut-bacterial free flies and 1.8% in the control group.

Table (1) Percentage of Microbial flora associated with external surface and gut of the house fly, *Musca domestica*.

Microbial flora	External surface %	Gut %
<i>Haemophilus sp.</i>	0	0
Enteric bacteria	20	79
1- <i>Eschericia coli</i>	70	73
2- <i>Salmonella sp.</i> and <i>Shigella sp.</i>	1	16
3- <i>Pseudomonas sp.</i> , <i>Klebsiella sp.</i> , <i>Proteus sp.</i> and <i>Serratia sp.</i>	29	11
Cocci form bacteria	20	21
1- Haemolytic staphylococci and Streptococci	0	0
2- Non-haemolytic staphylococci and streptococci	100	100
<i>Bacillus sp.</i>	60	10
Actinomycetes	1.5×10^6	0
Yeasts	0.04×10^6	0
Filamentous Fungi	0.1×10^6	0

*Percentage of enteric bacteria, Cocci form and Bacilli form was calculated relatively to the total count on nutrient agar

*Percentage of *Haemophilus sp.* and Haemolytic cocci form bacteria was calculated to the total count on blood agar

*Amounts of Actinomycetes, Yeasts and Fungi was calculated as viable count

Table (2) The viable count of gut bacteria associated with the house fly, *Musca domestica* on different selective media.

Media used	External surface	Gut bacteria
NA	5.0x10 ⁶	1.2 x 10 ⁷
SGA	0.8x10 ⁶	0.25 x 10 ⁷
BGA	1.0x10 ⁶	0.95 x 10 ⁷
SSA	0.01x10 ⁶	0.15 x 10 ⁷
EMB	0.7x10 ⁶	0.7 x 10 ⁷
BA	1.2x10 ⁶	-
AZBA	-	-
HA	-	-
Litt	-	-

Where: NA = Nutrient agar, SGA = Ston gelatin agar, BGA = Brilliant green agar, SSA = *Salmonella and Shigella* agar, EMB = Eosin methylene blue agar, BA = Blood agar, AZBA = Azid blood agar, HA = *Haemophilus* agar and Litt = Luttman agar,

Table (3) Susceptibility test of the selected 12 bacterial isolates for 10 antimicrobial agents using the disk diffusion method.

Bacterial strain	Antimicrobial agents									
	NOR	AM	K	CB	RA	C	SXT	FD	TE	NA
<i>Eschericia coli</i>	3+	2+	3+	2+	4+	2+	3+	-	1+	2+
<i>Salmonella sp.</i>	3+	3+	2+	1+	2+	2+	2+	1+	4+	2+
<i>Pseudomonus sp.</i>	3+	1+	2+	2+	1+	-	-	2+	3+	-
<i>Staphylococcus sp.</i>	2+	3+	3+	3+	3+	2+	3+	4+	3+	3+
<i>Streptococcus sp.</i>	3+	3+	3+	2+	3+	3+	2+	1+	1+	3+

Where : (NOR) Norofloxacin, (Am) Ampicillin, (K) Kanamycin, (CB) Carbenicillin, (RA) Rifampicine, (C) Chloromphenicol, (SXT) Sulfa, (FD) Nitrofurantion, (TE) Tetracycline, (NA) Nalidixic acid.

Norofloxacin was used to eliminate the gut bacterial flora where it was proved to be the most effective on different bacterial strains.

Table (4) Effect of the midgut bacterial flora on the preoviposition period, fecundity and hatchability of the house fly, *Musca domestica*

Treatment	No. of flies used	Preoviposition period (days)		No. of eggs laid		Non hatched eggs			Hatched eggs		
		range	mean±SE	range	mean±SE	range	mean±SE	%	range	mean±SE	%
Normal females	8	4 - 5	4.5±0.18	104 - 160	136±7.82	2 - 9	4.75±0.85	3.5	98 - 158	131.35±8.16	96.5
Midgut bacterial free females	7	4 - 5	4.4±0.2	70 - 135	111.4±8.92	19 - 61	44.9±8.03**	40.4	10 - 88	66.42±10.42**	59.6

Table (5) Effect of the midgut bacterial flora on the larval and pupal mortalities, pupation and adult emergence percent of *Musca domestica*

Treatment	Larval mortality			Pupation			Pupal mortality			Adult emergence		
	range	mean±SE	%	range	mean±SE	%	range	mean±SE	%	range	mean±SE	%
Normal females	2 - 8	4.5±3.59	3.5	26 - 150	126.8±48.1	96.5	1 - 3	2.25±0.88	1.8	93 - 147	124.5±46.9	98.2
Midgut bacterial free females	2 - 10	5.57±2.57	8.4	8 - 79	60.85±26.5**	91.6	1 - 23	5.14±3.6	8.4	7 - 78	55.71±23.6*	91.6

Discussion

The present data revealed that the female housefly, *Musca domestica* harbored both gram-negative and gram-positive bacteria, also other microorganisms in her midgut. A variety of bacterial species and genera were isolated. These bacteria were enteric bacteria which include: *Escherichia coli*, *Salmonella sp.*, *shigella sp.*, *Pseudomonas sp.*, *Klebsiella sp.*,

Proteus sp. also cocci form bacteria which include haemolytic and non haemolytic *Staphylococci* and *Streptococci*. *Bacillus sp.* was also isolated. Beside to these bacteria actinomycetes, yeasts and filamentous fungi were also isolated.

The present data confirm previous reports on the occurrence of bacteria in the midgut of the housefly (Greenberg,

1965, 1973 Ghanem *et al.*, 1986; Radi *et al.*, 1988; Benoit *et al.*, 1990; Schmidtman 1992). The functional role of midgut bacteria in the adult female housefly is unknown. As regards the available literature there are no any reports concerning the effect of midgut bacterial flora on the different biological aspects of the housefly, *Musca domestica*. Up till now the role of these bacteria either symbiotic or non-symbiotic is unknown. The present data throw some light on the role of the midgut bacteria in the housefly. The present results showed that the occurrence of bacteria in the midgut of the fly play a very important role in the fecundity, where the number of eggs laid is affected by the elimination of bacteria. Also the hatchability percent was greatly affected by the absence of bacteria. Thus the midgut bacteria may be beneficial to the flies where they may provide the flies with some essential nutritional elements.

The reproduction inhibition induced by the elimination of digestive tract bacteria of female *Musca domestica* is supported by earlier investigations of Wigglesworth (1952) for the sucking lice, *Pediculus* and *Reduviidae*, *Triatona* and *Rodinius* ; Nogge (1975, 1976) for tsetse flies, Fouda (1984) for *Hippobosca equina*, Douglas (1990) and Sasaki *et al.* (1991) for aphid, Hassan *et al.* (1996) for *Cx. pipiens*, Hassan *et al.* (1998 a & b) for the sand fly *Phlebotomus papatasi*, Fouda *et al.* (2001) and Hassan *et al.* (2001) for *Cx. pipiens*.

The decrease in the percentage of hatchability for eggs laid by midgut-free bacterial female *Musca domestica* may be comparable with the results obtained by Awahmukalah and Brooks (1983 & 1985) and Hassan *et al* (2001) for anaerobic strain of *Cx. pipiens*, where they reported that eggs of

bacterial free females produced anaerobically did not hatch. Moreover, Hassan *et al.* (1998b) observed a decrease in the hatchability percent, which reached to 24.0% for eggs laid by aposymbiotic female sand fly, *Phlebotomus papatasi*.

Concerning the larval survivorship and pupation, the present data showed that the elimination of midgut bacteria of the female housefly, *Musca domestica*, increased mortality percent of the produced larvae and pupae. Seitz *et al.* (1987) reported that the presence of *Serratia marcescens* bacteria in *An. stephensi* infected with *Pseudomonas berghei* and *S. marcescens* caused a significant increase in mortality. They found that *S. marcescens* strain produced the exoenzymes chitinase and lecithinase which might have been responsible for the observed mortality. Thus it can be concluded that the bacteria present in the housefly midgut are not pathogenic to the flies but their association might be mutual or even symbiotic. These results are more or less in agreement with that of Awahmukalah and Brooks (1983), Pumpuni *et al.* (1996), Hassan *et al.* (2001) and Fouda, (2001).

In addition, the present data revealed that the bacterial elimination from the housefly gut reduced the percentage of adult emergence. These data are in agreement with that of Hassan *et al.* (2001) where they observed similar results for the aposymbiotic *Cx pipiens*.

Acknowledgement: the authors wish to express their sincere gratitude and appreciation to Dr. Hesham Mahdy assistant professor of Microbiology, Faculty of science, Al-Azhar University, for isolation and identification of bacteria used in the present work.

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دراسة علي الفلورا البكتيرية و معنويتها في الذبابة المنزلية مسكا دومستيكا (ذات الجناحين : مسيدي)

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تم عزل الفلورا البكتيرية المصاحبة للذبابة المنزلية ، مسكا دومستيكا وتم دراسة تأثيرها علي بعض الظواهر البيولوجية للذبابة. تم عزل الفلورا البكتيرية باستخدام أوساط اختيارية وغير اختيارية. بفحص معى الذبابة المنزلية تم عزل وتعريف أنواع متنوعة وكثيفة من الفلورا الميكروبية مثل *اشرشيا كولاي* ، *سالمونيلا* ، *شيجلا* ، *بسيكوموناس* ، *كليبسيلا* ، *بروتس* ، *سيراتيا*. كما تم عزل أنواع محللة وغير محللة للدم مثل *ستافيلوكوكاي* و *ستربتوكوكاي*. وتم عزل *باسيلس*. هذا بالإضافة إلى *اكتينومايتس* ، المميزة والفطريات المنيطية وتم عزلها من السطح الخارجي فقط للذبابة. تم التخلص من البكتريا الموجودة في معى الذبابة باستخدام المضاد الحيوي نورفلوكساسين و ذلك لمعرفة دور هذه البكتريا في دورة حياة الذبابة. وجد ان التخلص من البكتريا الموجودة في المعى قد اثر سلبيا علي النسبة المئوية للفقس ، النسبة المئوية لوفيات اليرقات والعدارى ، كما قلل نسبة التعذر. ويمكن استنتاج ان البكتريا المتواجدة في معى الذبابة المنزلية ليس لها تأثير مرض علي الذبابة ويمكن مصاحبته للذبابة ربما يكون تبادل منفعة او معايشة.