

ANTAGONISTIC ACTIVITY BETWEEN BACTERIAL SPECIES CARRIED BY ADULT HOUSE FLY MUSCA DOMESTICA

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الخلاصة

تناولت الدراسة عزل وتشخيص الأنواع البكتيرية الموجبة والسالبة من 300 عينة من الذباب المنزلي جمعت من بيئات مختلفة في مدينة كركوك تضمنت أسواق بيع الخضراوات والفواكه والمجازر ومكبات النفايات ومن داخل بيئة المستشفيات . عزلت من الجناح الأيمن الأنواع البكتيرية التالية : Pseudomonas ، Micrococcus luteus ، Staph. xylosum ، Staph. aureus ، B. subtilis ، Acinetobacter. spp ، P. vulgaris ، K. oxytoca ، E. coli ، fluorescence . Acinetobacter. spp و P. fluorescence ، Aerococcus viridans ، Strep. iniae ، Bacillus. spp أما من سطح جسم الذبابة فقد عزلت كل من : P. aeruginosa ، Staph. aureus ، Staph. saprophyticus ، B. cereus ، Enterobacter. spp و Hafnia alvei ، Serratia fonticola ، K. oxytoca ، E. coli . تم التحري عن الفعالية التضادية المايكروبية الفعالية التضادية المايكروبية للعزلات البكتيرية المعزولة من الجناح الأيمن على العزلات البكتيرية المعزولة من الجناح الأيسر والعكس ، أظهرت النتائج أن بعض العزلات أبدت قدرتها في تثبيط نمو بعض الأنواع الأخرى وتميزت العزلة البكتيرية B. subtilis والمعزولة من الجناح الأيمن والعزلة البكتيرية Pseudomonas. fluorescence المعزولة من الجناح الأيسر فعالية تضادية عالية ضد الأنواع الأخرى من البكتيريا .

ABSTRACT

This study dealt with the isolation and identification of Gram-positive and Gram-negative bacterial species for 300 samples house fly *Musca domestica* L. associated with external surface and its body parts, collected from different areas of kirkuk city including (Kirkuk general hospital, AL-Tapa waste near the house, Khan- Al-Tamr area, Domez area, which included vegetable and meat markets). The following bacterial species were isolated from the right wing: *B. subtilis*, *Staph. aureus*, *Staph. xylosum*, *Micrococcus luteus*, *Pseudomonas fluorescence*, *E. coli*, *K. oxytoca*, *P. vulgaris*, *Acinetobacter. spp*. from the left wing the following species were isolated: *Bacillus. spp*, *Strep. iniae*, *Aerococcus viridans*, *P. fluorescence*, *Acinetobacter. spp* and the body surface these species were isolated: *B. cereus*, *Staph. saprophyticus*, *Staph. aureus*, *P. aeruginosa*, *E. coli*, *K. oxytoca*, *Serratia fonticola*, *Hafnia alvei*, *Enterobacter. spp*. The microbial antagonistic activity was also studied of the bacterial isolates isolated from the right wing was studied on bacterial isolates from the left wing and vice versa. the results showed that some isolates showed their ability to inhibit the growth of some other species, the isolates of *B. subtilis* isolated from the right wing and *Pseudomonas fluorescence*.

Introduction

The house fly, *Musca domestica*, is one of the most medically important insects spread all over the world. It found in both rural and urban areas with tropical and temperate climates. It has been present since the inception of mankind. It belongs to a group of flies referred to as Filthflies. *M. domestica* has morphological and behavioral traits that make it not only annoyance-inducing, but also a mechanical vector for over 100 infections [1-7]. Studies have shown that the isolation of pathogens in high percentages from the surfaces of house flies is an indication that the house flies have morphological and behavioral characteristics that made them act as a mechanical vector which is the most common mechanism. These mechanisms are linked to the fly's morphology and feeding habits, as well as their frequent association with unclean substances like feces and garbage [15]. External surfaces of the fly, particularly the tarsi and pulvilli (the 'sticky pads' on fly 'feet' where infections are known to adhere), as well as the mouthparts, can get contaminated when they come into contact with pathogens [2,16]. The fly's external surfaces are coated in spines, hairs (setae), and microtrichia, which allow material to attach and be carried. Several studies using agricultural and genetic diagnostic methods have been able to detect more than hundred pathogens including bacteria, fungi, parasites, protozoa, and viruses that cause serious disease threaten human life, they were isolated from the body parts of adult house fly [7,11,14,23]. The results of those studies that focus on isolating bacteria from the body parts of house flies showed that among the bacterial species, intestinal bacteria were the most common. The bacterial species were highly virulent including Enteropathogenic *E. coli* strains that cause various diarrheal cases and vibrio cholera which causes cholera, *B. anthracis* which causes cutaneous and intestinal anthrax and *Klebsiella. spp* and many other bacterial species including *Pseudomonas. spp*, *Staphylococcus. spp*, *Streptococcus. spp*, and *Enterococcus. spp*. to name a few which are important causes human disease including nosocomial infections [27,30]. The researchers noted that most of the

bacterial isolates from house flies collected from inside and around the environment of hospitals and animal farms were multi resistant to antibiotics ,which indicates that house flies play amajor role in the spread of antibiotic- resistant strains from the hospital inviroments to different enviroments outside hospitals and thus caused epidemic spread of many disease especially intestinal disease .There fore , house flies have been considered by the US food and drug administration (FDA) as a major vector for the transmission of diseases such as cholera ,shigella disease and salmonellosis[17,30].

Materials and Methods

Collecting flies

300 house flies, *M. domestica*, were collected from Kirkuk city in six locations(Kirkuk general Hospital, garbage near the house,vegetable market, meat market, rural area (Al amal alshaby area,and khan -al tamr area) . Collected flies were transported to the laboratory in the college of science\ Biology department\ postgraduate labs with sterile cups and then saved in a wooden sterile cages till we dissecting them they were morphologically identified. These flies were used as a stock for the experimental work.

Bacterial isolation using differential media

This study conducted during the period from the beginning of October 2020 to April 2021. The flies were collected in a sterile containers and then transferred to the laboratory to preform the necessary operations ,as the parts of the flies were separated from right ,left wing and the surface of fly body (Head, Abdomen, legs) and placed each part in tubes containing 1ml of physiological solution (Normal saline) and left them for a while and then was taken out parts of the fly and a series decimal dilutions of the solutions were conducted using different and discriminatory culture media and appropriate growth methods to isolate them and identify the numbers and types of microbes in each part of fly body . Table (1) shows the bacterial species that isolated from house flies' wings and body parts

Bacterial species were isolated and diagnosed using different culture microscopic and biochemical tests , and then diagnostic results were supported using API 20E diagnostic system for diagnosing negative bacterial species ,and API Staph for diagnosing *Staphylococcus.spp* ,as well as Vitek 2 system .

Table (1) Most important microscopic species isolated from the House fly wings

Right wing	Left wing	Body surface
Enterobacter.spp	Pseudomonas.Florescence	Bacillus . cereus
Pseudomonas. Florescence	Bacillus.spp	Pseudomonas. aeruginosa
E. coli	Aerococcus.viridans	Staph. Saprophyticus
Bacillus .subtilis	Streptococcus. iniae	Hafnia .alvei
Staph. xylosus	Acintobacter. Spp	Serratia. Fonticola
Staph. aureus		Klebsiella. Oxytoca
Proteus .spp		Enterobacter. spp
Micrococcus. Luteus		E.coli
Klebsiella. Oxytoca		Staph. aureus

Results and Discussion

Musca domestica, is one of the most common fly species found worldwide. It has been considered as vector of more than 100 pathogens [1,6] . From the total of 300 house fly samples taken from six sites

,168 different growth positive bacterial isolates were obtained . The house flies samples taken from the six locations and with 50 flies for each site were adopted as a source for isolating 17 types of germs .The collected flies from the hospital showed bacterial growth and the number reached 32 positive samples with growth rate of 64 % , as for the samples of Khan- AL - Tamr , 36 samples showed positive bacterial growth with rate 72% , Domiz area (vegetable and meat market) positive samples were 34 and 38 positively with rate , 68 % and 76 % respectively . and the site of waste near the house showed a growth of 20 samples with rate of 40 % , while AL – Amal Alshaby has the less growth only 8 samples with 16 % . Characterization of bacterial strains, Isolates were definitely characterized as 16 genus and species; *Escherichia.coli*, *Bacillus.subtilis* and *Pseudomonas.fluorescence*, *Aerococcus.viridans*, *Streptococcus.iniae*, *Staphylococcus.aureus*, *Staphylococcus.xylosum*, *Enterobacter.spp*, *Serratia.fonticola*, *Proteus.spp*, *Klebsiella oxytoca* , *Acinetobacter.spp*, *Micrococcus luteus*. The data in table (1) reveal that the microorganisms discovered on the wings and body surface of domestic flies, particularly bacteria, are dense and diverse, and that the microbial variety and numerical density in both wings of flies reflect the environment in which they live [6,22,24].

Fig (1) showed percentage of the bacterial growth on the fly body and wings. The bacterial isolates on the flies' wings and body were of particular medical significance because of their capacity to transmit a variety of diseases affecting humans, animals, plants, and food corruption [16].

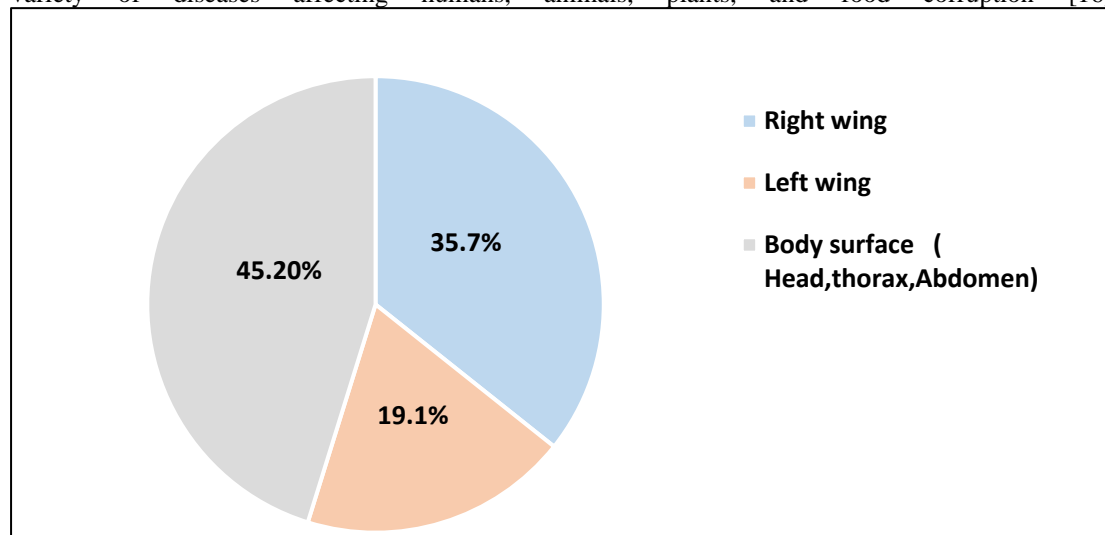


Fig (1) Percentage of the contamination on House fly (Wings and body surface)

Antagonistic activity of the bacterial isolates :

- **Antagonistic activity of isolates from the right wing against isolates from the left wing :**

The results shown in table (2) that *B.subtilis* showed high antagonistic efficiency in inhibiting isolates of *Pseudomonas.spp* isolated from the left wing, good efficiency in inhibiting *Aerococcus viridans* isolates and medium efficiency inhibition *Streptococcus iniae* isolates , while it didn't affect the species *B.cereus*, *Acinetobacter.spp* and isolates of *Staph.aureus* and *K.oxytoca* , showed antagonistic effects against bacterial isolates isolated from the left wing . Members of the genus *Bacillus* including *B.subtilis* have a great role in the production of antibiotics ,enzymes , and other secondary metabolites, and these antimicrobials showed a wide range of antimicrobial activity against bacteria , fungi , and pathogenic parasites [31].

It also has many applications in the field of genetic engineering ,medicine , vaccines production ,and industry ,and was used in biological control against many plant and human pathogens [32].

Table (2) Antimicrobial activity between the isolates from the right and left wing of *M. domestica* fly.

In view of the importance of this bacterium in terms of its production of antibiotics and other secondary metabolites, its effect on bacteria isolated from the same right wing has been studied, and medium inhibitory efficiency against *E.coli* isolates, while it didn't affect *P.vulgaris* and *M.luteus* isolates and good inhibition against *P. fluorescens* and *K. oxytoca* isolates, table (3) present the results between *B.subtilis* isolated from right wing and some isolates from the same wing.

Table (3) antagonistic activity of *B.subtilis* against bacteria isolates from right wing

Right, wing isolates	<i>Pseud.fluorescence</i>	<i>E.coli</i>	<i>K.oxytoca</i>	<i>P.vulgaris</i>	<i>M.luteus</i>
Bacillus .subtilis	++	+	++	-	-

Also antagonistic effect was studied on some isolates isolated from the body surface of the fly (head, abdomen, and legs) and it showed medium antagonistic activity against each of isolates *Pseudomonas.spp* and *Staph.saprophyticus*. while it didn't show any antagonistic activity against other tested isolates as shown in table (4).

Table (4) antagonistic activity of *B.subtilis* against bacteria isolates from body surface

Bacterial isolates	<i>Pseudomonas .spp</i>	<i>E.coli</i>	<i>K.oxytoca</i>	<i>Serratia.fonticola</i>	<i>Staph.saprophyticus</i>
Bacillus .subtilis	+	-	-	-	+

- **Antimicrobial activity of bacterial species isolated from left wing against species isolated from the right wing**

The results shown in table (5) that *Aerococcus viridans* and *P.fluorescence* showed good inhibitory activity against most isolates isolated from the right wing while isolates of unknown bacilli isolates from this wing did not show any antagonistic effect against isolates isolated from the wing as neither *Strept.iniae* nor *Acintobacter.spp* showed antagonistic activity against the tested isolates.

Right wing Isolates \ Left wing Isolates	<i>Micrococcus Luteus</i>	<i>Proteus .spp</i>	<i>Staph. aureus</i>	<i>Staph. xylosus</i>	<i>Bacillus .subtilis</i>	<i>Kleibsiella. Oxytoca</i>
Pseudomonas. Spp	-	-	++	-	+++	++
Bacillus.cereus	-	-	+	-	-	-
Aerococcus.viridans	-	-	++	-	++	+
Streptococcus. iniae	-	-	-	-	+	-
Acintobacterspp.	-	-	+	-	-	-

Pseudomonas fluorescens is considered one of the effective agents in biocontrol to inhibit plant pathogenic fungi, as it is characterized by its production of many secondary metabolites such as antibiotics and siderochromide hydrogen enzymes, which have an antagonistic role against bacterial, fungal, and parasitic pathogens that cause plant disease in horticultural

crops ,cereals and oil seeds , Its antagonistic role for pathogenic bacteria is stronger compared to fungi [33].

Table (5) Antagonistic activity of left wing isolates against right wing isolates

Left.wing isolates Right wing isolates	Bacillus.spp	Strep.iniae	Aerococcus viridans	Pseudo.flurescence	Acintobacter.spp
Bacillus .subtilis	-	-	++	+++	-
Staph. aureus	-	-	+	+++	-
Staph. xylosus	-	-	+	-	-
M.luteus	-	-	+	-	-
P.vulgaris	-	-	-	+	-
K.oxytoca	-	-	+	++	-

Many researchers have studied the efficiency of the antagonistic activity of isolates isolated from right wing on isolates isolated from left wing and vice versa ,such as [27] , and study [6] ,study [14] in order to reach scientific facts and identify disease and medicine in the wings of fly accordance with hadith of the messenger Muhammed ,may Allah prayers and peace be upon him (If a fly falls into the vessel of one of you dip it and throw it , for one of its wings is a disease and the other is a healing) Including as medicines for the treatment of disease transmitted by the cause of house fly . The results of these researchers showed antagonistic activity of number of isolates isolated in there study from wings of house fly , and they found that some isolates had a high antagonistic effect in eliminating other types of bacteria,In a study by the researcher [27] five types of bacteria showed B.subtilis ,B.circulans ,Lactobacillus animalis ,Pseudo.aeruginosa ,Staph aureus, but the isolate B.circulans that was isolated from right wing was the strongest among other species and researcher investigated the active metabolite in these bacteria and reached the results , the analyzes indicated that the substances is an aromatic compound of acyclic nature ,and when he study the effect of the minimum inhibitory concentration is 5 µg\ ml sufficient to kill many types of bacteria . In the study of [6] they found that the bacteria Streptomyces.spp isolated from the right wing showed a high efficiency inhibiting against other types of bacteria isolated from the left wing and the right wing it self . the researchers indicated that these species of this genus are characterized by their ability to antibiotics production . From the left wing the researchers found that B.mycooids showed a high ability in producing antibiotics and inhibition the growth of bacterial species isolated from the right and the left wing it self.

In the researchers study [14] the results of her study showed that bacteria Pseudo.aeruginosa isolated from right wing of the fly had a good antagonistic activity against bacterial isolates that isolated from left wing and right wing it self such as Klebsiella ,and Staph. aureus as well as against P.vulgaris and E.coli isolated from right wing only. Despite the discrepancy in the type of isolates isolated from two wings of the fly that gave antagonistic activity against other types of isolated bacteria .

The results of the current study came in agreement with them in presence of microbial isolates isolated from the wings of a house fly that carry disease and medicine .

For that prophet Muhammed (peace be upon him) in one of his hadiths say :

(If a fly falls into the vessel of one of you ,let him dip it and then throw it away ,for one of its wings there is a disease and in the other there is a cure) forbade dipping the fly quickly because it attaches to the surface of the liquid due to the presence of surface tension and a word and then to drop it after dipping gives an opportunity for the beneficial types bacteria and fungi to excrete the counter productive secondary metabolic products (medicine) to eliminate harmful bacteria (disease)

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