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Isolation and Identification of some Species of Bacterial Pathogens from *Musca Domestic* and Test their Susceptibility Againts Antibiotics

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Abstract

The current study was conducted during the period from the beginning of October 2016 to April 2017 to isolate and diagnose the bacteria on the outer surface of the domestic fly (*Musca domestica*) and to determine the sensitivity of bacterial isolates against antibiotics using different agricultural mediums. Insects were collected from three sites in the Tikrit city which are: vegetable market, butchers market and sheep sheds. 50 samples were collected from each site. Bacterial isolates obtained from the external surface of insects were subjected to morphological and biochemical tests and API 20 E system was used for diagnosis.

153 isolations were obtained belonging to 9 bacterial species . *Escherichia coli* reached 36 isolations that prevailed the rest of the bacterial species with an isolation rate of 23.5%, followed by *Salmonella typhi* 32 isolates by 20.9% , and *Proteus mirabilis* 31 isolates by 20.3% . *Staphylococcus aureus* has 25 isolations by 16.3%. *Klebsiella pneumonia* has 11 isolations by 7.2% , and *Shigella spp* has 7 isolates by 4.6%, followed by *Citrobacter freundii* which has 6 isolates by 3.9% , Then *Hafina spp*. Has 3 isolations by 1.9% , *Serratia marcescens* has only two isolates by 1.3% .

The results showed that the highest contamination rate was in the vegetable market which is 92%, then the sheep sheds is 88%, and finally Butchers market is 86%.

The Gram-negative bacterial isolates were tested for sensitivity to 8 types of antibiotics, *E.coli* showed the highest resistance to both Erythromycin and Ceftriaxon by 77.8% , while *S.typhi* showed resistance to Amikacin and Ceftriaxon by 53.1%, *P.mirabilis* was resistant to Tetracyclin by 64.5%, *Shigella spp*. was resistant to Ceftriaxon by 57.14%, *K.Pneumonia* bacteria were resistant to Erythromycin by 72.7% , *C.freundii* showed resistance to the antibiotic Trimethoprim + Sulfamethoxazol and 100%, *Hafnia* was 33.33% resistant to Chloramphenicol, Finally, *Ser.marcescens* were 100% resistant to Erythromycin.

The sensitivity of *Staph.aureus* bacteria was tested against 5 types of antibiotics with the highest resistance to Vancomycin by 80% .

Introduction

Insects are one of the most widespread and successful creatures on earth. They exist in different environments and spread throughout the world and feed on many types of plants and animal remains. They represent the environmental causes of diseases throughout the centuries [1]. There are more than 1 million species of insects in the nature and about 5000 of them are harmful to humans, animals and plants [2]. Therefore, some of these insects are considered important medical pest, which transmit serious diseases to humans and animals. Especially as a result of neglect in all areas of public health in developing countries, as such countries are suitable place for the proliferation of these pests and their

spread, and thus the infection of human with dangerous diseases [3].

Musca domestica L is one of the important household and health pests It got its name because of its human accompaniment almost in all clean and dirty environments . Domestic flies are considered to be one of the most dangerous insects living in the human environment. This is aided by the rapid reproduction and production of eggs in massive numbers and the difficulty of controlling them. The pathogens are mechanically transported from the places of contamination to humans through the wings and the end of the legs, and with the help of mouth parts [4]. The relationship between microbes and insects is a phoresity-only relationship [5]. The pathogens are transmitted through cuticle, proboscis, vomiting or through insect feces[6]. The flies carry more than 100 pathogens which infect humans and animals, and there is a clear link between the number of human and animal disease cases and the increase in the number of flies, including typhoid, cholera, Bacillary dysentery, anthrax opthalmia and Infantile diarrhea [7,8] examined the types of bacteria on the outer surface of the fly body, which transported Salmonella spp. [9] noted that domestic flies have the ability to transport bacteria resistant to high-intensity antimicrobials.

Objectives of the study:

1-Isolate bacteria from adult fly bodies collected from collection sites (vegetable market, butchers market and sheep sheds)

2- Purification and diagnosis of isolates by their usual bacterial methods (Diagnostic culture media, biochemical tests and staining)

3- To test the sensitivity of the bacterial isolates for some antibiotics.

Materials and Methods of Work

The insects used in the study, the domestic flies, were collected from three sites located in the Tikrit city (Buchers market, vegetable market and sheep sheds in the Faculty of Agriculture / University of Tikrit) as 50 insects per site. The flies were collected by the net and the use of traps made of nylon bags by placing sugar inside the bag to be an attractive component to the fly .In the Research Center and Museum of Natural History / University of Baghdad, the insects were identified, and a live and fresh insects were used for the experiments, in the same day to avoid the possibility of the death of some of the bacteria on the insects because to their being away from the original environment for a long time Insects were put in sterile plastic bottles and one insect only was put in each vial to avoid the transfer of bacteria from one insect to another.

The samples that were lost during transfer from the collection sites to the laboratory were removed. The collected insects were transported directly to the laboratory and numb by freezing at 0°C for 5 minutes, then 2 ml of the normal saline was added to sterile tubes and Vortex[10]. Then a Loop full of the wash solution was taken with a Loop sterilizer and Cultivate by streaking method using culture media on the MacConkey agar, Blood agar , Mannitol salt agar, Salmonella-shigella agar, previously prepared (as per manufacturer's instructions) for the purpose of isolating the bacteria. The petri dishes of media were incubated at 37 °C for 24 hours in aerobic conditions. After incubation, bacterial isolates were identified based on morphological, microscopic and biochemical characteristics. The sensitivity of bacterial isolates was tested against the commonly used antibiotics shown in Table 1 and 2 which are used as ready discs. The diffusion method was used on the Mueller Hinton agar medium by the [11] By the World Health Organization [12].

Table 1: Types of antibiotic used in the study (32)(Gram negative)

Number	Antibiotics	Symbol	Concentration disc/ug	Zone of Inhibition(mm)		
				Resistant	Intermediate	Sensitive
1	Amikacin	AK	30	≤ 14	15–16	≥ 17
2	Chloramphenicol	C	30	≤ 12	13-17	≥ 18
3	Erythromycin	E	15	≤ 13	14-22	≥23
4	Trimethoprim 1.25 Sulfamethoxazol 23.75	TS	25	≤ 10	11-15	≥ 16
5	Amoxycillin	AMX	30	≤ 13	14–17	≥ 18
6	Tetracyclin	T	30	≤ 11	12–14	≥ 15
7	Ciprofloxacin	CIP	5	≤ 20	21-30	≥ 31
8	Ceftriaxon	CRO	30	≤ 19	20–22	≥ 23

Table 2: Types of antibiotic used in the study (32)(Gram positive)

Number	Antibiotics	Symbol	Concentration disc/ug	Zone of Inhibition(mm)		
				Resistant	Intermediate	Sensitive
1	Amikacin	AK	30	≤14	15–16	≥ 17
2	Ciprofloxacin	CIP	5	≤ 15	16-20	≥ 21
3	Vancomycin	VA	5	≤ 13	14-22	≥23
4	Gentamycin	GEN	10	≤12	13–14	≥ 15
5	Oxacillin	OX	5	≥ 4	-	≤ 2

Results and Discussion

The bacteria were isolated from the outer surface of the insect body wall of the sites(Vegetable market, Sheep sheds and Butchers market) the number of pollinated insects that gave the growth of bacteria is 133 insects at an average of 88.6%. The results

showed that the highest percentage of pollution in the vegetable market was 92%, followed by sheep sheds by 88% and finally Butchers market, which is 86% as shown in Table (3).

Table (3): Percentage of polluted flies with bacteria from three sites

Collection site	Total number of insect	Insects contaminated with bacteria	
		Number	%
Vegetable market	50	46	92
Sheep sheds	50	44	88
Market Butchers	50	43	86
Total	150	133	88.6

The number of isolates obtained was 153 isolates, and the highest percentage was isolated in the vegetable market by 35.3%, followed by the butchers market by 34%, followed by sheep sheds by 30.7% as shown in Table (4).

Table (4): Number and percentage of isolates Bacteria in each site

	Bacterial isolates of domestic flies	
Collection site	Number of isolates per site	%
Vegetables market	54	35.3
Market Butchers	52	34
Sheep sheds	47	30.7
Total	153	100

The difference in percentages of pollution and isolation is due to many reasons including the differences of sewage systems in vegetable and butchers market sites, in addition to the increase of waste in each of the above sites, which all lead to the spread of flies carrying these bacteria as well as the spread of bacteria itself, the negative results of the samples examined may be attributed to the presence Bacterial pathogens which have not been diagnosed, and to non - bacterial such as fungi, viruses or parasites. The results showed a high percentage of bacterial contamination carried by domestic flies on its outer surface, This indicates the obvious effect of this insect on the general health of humans as a result of the important role it plays in carrying and transmitting bacterial pathogens that cause various diseases for humans especially intestinal diseases. This study shows that domestic flies are considered as a typical mechanic carrier for human and animal pathogens. The results of this study indicate that the domestic fly is more harmful than being an annoying insect as it represents a definite health risk through its role in the transfer of bacteria among humans and animals.

The percentage of total pollution domestic flies in the current study 88.6% , Which is higher than the percentage recorded by[10] where the pollution rate was 81.3% , This percentage was also different from the study of [13] where the infection rate was 36.5% , The differences in the recorded ratios is due to several factors, including sampling locations, differences in the number of samples examined, environmental conditions, health awareness and personal cleanliness, which play an important role in reducing pollution and reducing the spread of vector insects [14].

Table 5: Types of polluted bacteria of domestic fly samples and percentages of sites collected

Types of bacteria	Total number of isolates	Percentage of bacterial isolation	Insulation percentage of total insect count (150)
E.coli	36	23.5	24
S. typhi	32	20.9	21.3
P.mirabilis	31	20.3	20.7
Staph.aureus	25	16.3	16.7
K.pneumonia	11	7.2	7.3
Shigella spp.	7	4.6	4.7
C.freundii	6	3.9	4
Hafina spp.	3	1.9	2
Ser. Marcescens	2	1.3	1.3
Total	153	99.9	

After examining the samples collected from the sites (Vegetable market, butchers and sheep sheds), results showed 153 isolates distributed on the following species *E.coli, Staph.aureus, S.typhi, P.mirabilis, Shigella spp., K.penumonia, Serritia marcescens, Citrobacter freundii* and *Hafina spp.* More than *E. coli* bacteria were contaminated with 36 isolates and 23.5% of bacterial isolates, followed by *S.typhi* bacteria with 20.9% of bacterial isolates, followed by *P.mirabilis* with 20.3% of the bacterial isolates, And *Staph.aureus* bacteria by 16.3% among bacterial isolates, followed by *K.penumina* with 7.2% of bacterial isolates, And bacteria *Shigella spp*. by 4.6% of the bacterial isolation, followed by *Citrobacter freundii* with 3.9% of bacterial isolation, Then *Hafina spp.* bacteria by 1.9% among bacterial isolates, The final rank bacteria *Serratia marcescens* and 1.3% of the bacterial isolation.

From the observation of the results, *E. coli* was the first to cause pollution on the outer surface of the domestic fly, This study was consistent with the studies that indicated the isolation of the bacteria from the outer surface of the insect, It was agreed with the study of the researcher [15] in Morocco recorded a number of bacterial species, and *E.coli* bacteria were the most present and by 43.3%.

The results of the current study showed that *S. typhi* bacteria ranked second in the percentage of pollution, and is one of the worlds most dangerous food-borne bacteria that threaten human[16].As agreed with the results of researcher[17], which was able to isolate the bacteria *S.typhi* from the outer surface very close to the current study, reaching 25.2%.

P.mirabilis is the third most common pollutant in the current study, and it is the main cause of the complicated urinary tract infections these infections are mainly in the urinary tract[18]. The researcher [10] in the province of Salah aldin infection rate of these bacteria less than the current study, which amounted to 8.4%.

The results of the current study occupied *Staph.aureus* bacteria ranked fourth in the proportion of pollution, and can be considered bacteria of opportunistic opportunists, as the injury occurs when the appropriate conditions due to their presence naturally on the skin and nose and throat[19]. A number of researchers have isolated *Staph.aureus* bacteria from the outer surface of a domestic fly, such as [20] in Sulaymaniyah.

K. pneumonia ranked fifth in pollution in the current study, A number of researchers have isolated the *Klebsiella* genus from the outer surface of the fly body, including [21], which isolated five species of bacteria, including *Klebsiella*, from the outer surface of the domestic fly.

Shigella spp. bacteria were ranked sixth in pollution in the current study. The bacteria are intravenous gastrointestinal pathogens that cause severe diarrhea, known as Shigellosis[22], A number of researchers have isolated these bacteria from the outer surface of the fly, In the [23]study, they isolated different types of bacteria from the outer surface of the fly, including *Shigella spp*

C.freundii was ranked seventh in causing contamination on the outer surface of the domestic fly body, A number of researchers have isolated these bacteria, including the study [24] in Iran, where it was isolated from flies, the total of hospitals and slaughterhouses at different rates, the proportion of hospitals in 5.7%, which is an approach to the results of the current study, the rate of isolation of slaughterhouses was 28.4%, which is higher than the results of the current study.

And came in eighth place of isolation of *Hafina spp.* bacteria in causing pollution on the outside of the body of the fly. And ranked ninth and final with *Ser. marcescens* in contamination on the outer surface of the fly body.

The results of the insect examination collected from the different sites indicated that the Gram-negative bacterial species were more common than the Gram-positive species In causing pollution on the surface as showed (5), this result corresponds to the results of many researchers, [24] and [17], The prevalence of bacterial species of Gram-negative antibiotic resistance is due to their having different virulence factors[25]. Because of the nature of domestic flies that are very close to the human environment, which is one of the most important epidemiological factors which is responsible for the carrying and transmission of these bacteria. Also, the composition of the fly body is adaptable well to capture the pathogenesis, as the six legs equipped with hairy structures and pads that secrets sticky material ,and the proboscis is equipped with many tiny filaments that gather things on its external surface [26, 27], and the placement of excrement and vomit parts during the feeding process represent an opportunity to spread diseases [27]. Moreover the adhesion of small molecules sticks easily to the surface of the home fly becomes its electrostatic charge as the body of the fly has a stable charge, and any molecule carrying a different or neutral charge will stick its body [14]. The results of the study showed that all bacterial isolates obtained were of particular medical importance in their ability to cause many diseases that affect human and animal , food , corruption and others, as species are isolated pathogens are important, this result corresponds to a number of studies which have confirmed that [10,28,29,6,30,31]. The sensitivity of isolated bacterial isolates of domestic flies was tested towards a number of antibiotics as shown in Table 5 and antibiotic resistance was determined by measuring the diameter of the inhibition area (mm) according to[32].

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Table 5: Sensitivity of isolated bacteria from domestic flies to antibiotics

Bacterial isolates	R&S	Amikacin	Chloramphenicol	Trimethoprim+ Sulfamethoxazol	Erythromycin	Ciprofloxacin	Ceftriaxon	Tetracyclin	Tobramycin	Vancomycin	Gentamycin	Oxacillin
E.coli 36	R (%)	21 (58.3)	23 (63.9)	20 (55.6)	28 (77.8)	25 (69.4)	28 (77.8)	25 (69.4)	20 (55.6)	/	/	/
	S (%)	15 (41.7)	13 (36.1)	16 (44.4)	8 (22.2)	11 (30.6)	8 (22.2)	11 (30.6)	16 (44.4)	/	/	/
S.typhi 32	R (%)	17 (53.1)	15 (46.9)	12 (37.5)	15 (46.9)	12 (37.5)	17 (53.1)	12 (37.5)	14 (43.8)	/	/	/
	S (%)	15 (46.9)	17 (53.1)	20 (62.5)	17 (53.1)	20 (62.5)	15 (46.9)	20 (62.5)	18 (56.2)	/	/	/
P.mirabilis 31	R (%)	12 (38.6)	17 (54.8)	12 (38.7)	15 (48.4)	12 (38.7)	17 (54.8)	20 (64.5)	13 (41.9)	/	/	/
	S (%)	19 (61.3)	14 (45.2)	19 (61.3)	16 (51.6)	19 61.3)(	14 (45.2)	11 (35.5)	18 (58.1)	/	/	/
Shigella spp 7	R (%)	1 (14.3)	0	0	3 (42.8)	0	4 (57.14)	0	0			
	S (%)	6 (85.7)	7 (100)	7 (100)	4 (57.14)	7 (100)	3 (42.86)	7 (100)	7 (100)			
Ser. Marcescens 2	R (%)	0	0	0	2 (100)	0	0	0	0			
	S (%)	2 (100)	2 (100)	2 (100)	0	2 (100)	2 (100)	2 (100)	2 (100)			
K. Pneumonia 11	R (%)	7 (63.6)	5 (45.5)	7 (63.6)	8 (72.7)	4 (36.4)	6 (54.5)	6 (54.5)	7 (63.6)			
	S (%)	4 (36.4)	6 (54.5)	4 (36.4)	3 (27.3)	7 (63.6)	5 (45.5)	5 (45.5)	4 (36.4)			
C.freundii 6	R (%)	5 (83.3)	3 (50)	6 (100)	4 (66.7)	3 (50)	2 (33.3)	5 (83.3)	5 (83.3)			
	S (%)	1 (16.7)	3 (50)	0	2 (33.3)	3 (50)	4 (66.7)	1 (16.7)	1 (16.7)			
Hafina spp. 3	R (%)	0	1 33.33	0	0	0	0	0	0			
	S (%)	3 (100)	2 66.67	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)			
Staph.aureus 25	R (%)	10 (40)	/	/	/	7 (28)	/	/	/	20 (80)	15 (60)	14 (56)
	S (%)	15 (60)	/	/	/	18 (72)	/	/	/	5 (20)	10 (40)	10 (40)

E.coli showed the highest resistance to both Erythromycin and Ceftriaxon by 77.8% that the cause of *E.coli* resistance to antibiotics may be due to changes in the permeability barrier, making it difficult to reach the antibody to the site of work, a special characteristic of Gram negative bacteri [33], while *S.typhi* showed resistance to Amikacin and Ceftriaxon by 53.1%, *P.mirabilis* was resistant to Tetracyclin by 64.5% the reason for the resistance of many antibiotics to this type of bacteria is the ability to form a biochemical membrane that plays an important role in the resistance of antibiotics[34]., *Shigella spp*. was resistant to Ceftriaxon by 57.14%, *K.Pneumonia* bacteria were resistant to Erythromycin by 72.7% , *C.freundii* showed resistance to the antibiotic Trimethoprim + Sulfamethoxazol and 100% that these bacteria are characterized by their susceptibility to the phenomenon of antigen variability and have the ability to change the surface structures, in order to avoid the mechanisms of the defensive host and of these structures cilia[35]., *Hafnia spp.* was33.33% resistant to Chloramphenicol, Finally, *Ser.marcescens* were 100% resistant to Erythromycin the reason for the resistance is that the outer membrane of their baskets contains a large amount of stable fat and fat, as well as containing their strains on resistance plasmids.

The sensitivity of *Staph.aureus* bacteria was tested against 5 types of antibiotics with the highest resistance to Vancomycin by 80% the reason for the resistance of Gram positive bacteria to antibiotic is due to its ability to produce ß. lactamase which breaks the ß.Lactam ring of the antibody and makes it ineffective and is under the control of Transdusable plasmid.

The results showed that bacteria isolated from the outer surface of domestic flies showed resistance to some antibiotics used in the current study, some isolates also varied in their susceptibility to antibiotics, bacteria have several antibiotic resistance mechanisms, such as alteration of the antibody's target location, altering bacterial cell permeability to prevent entry of the antibody or by acquiring the antibody for effux system [36]. The results of this study are close to a number of studies, including the [24] study in Iran they denoted that bacteria isolated from domestic flies in hospitals and slaughterhouses were resistant to most antibiotics and thus increased human exposure to these resistant bacteria, And agreed with the results of[10], which found that the bacteria found on the outside of the flies in hospitals showed resistance to most antibiotics .This corresponds to what is found by [37] and [38]. This can be attributed to the increased random use of these antibiotics, as well as the lack of awareness of these antibodies. The difference in antibiotic resistance between the different studies in the world is due to the difference in geographical distribution and the time of isolation of bacteria [39]. In addition to a number of other studies that confirmed the possibility of transmission of antibiotic-resistant bacteria by flies [40,1].

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عزل وتشخيص بعض انواع المسببات المرضية البكتيرية من الذباب المنزلي Musca domestica واختبار حساسيتها تجاه المضادات الحيوية

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الملخص

أجريت الدراسة الحالية خلال المدة من بداية شهر تشرين ألاول 2016 ولغاية شهر نيسان 2017 ،بهدف عزل البكتريا المحمولة على السطح الخارجي للذبابة المنزلية *Musca domestica* وتشخيصها، ودراسة مدى حساسية العزلات البكتيرية للمضادات الحيوية باستخدام أوساط زرعية مختلفة، جمعت الحشرات من ثلاثة مواقع ضمن مدينة تكريت وهي: سوق الخضار، سوق القصابين، حظائر الاغنام.

إذ جمعت 50 حشرة من الكاملات من كل موقع، خضعت العزلات البكتيرية التي تم الحصول عليها من السطح الخارجي للحشرات للفحوصات المظهرية والاختبارات الكيموحيوية واستخدام نظام API 20 E system لغرض تشخيصها.

تم الحصول على 153 عزلة تابعة لـ 9 أنواع بكتيرية وهي *Escherichia coli* بلغت 36 عزلة والتي سادت على بقية الأنواع البكتيرية وبنسبة عزل 23.5%، تلتها *Salmonella typhi* عددها 32 عزلة بنسبة 20.9%، وبكتريا *Proteus mirabilis* عددها 31 عزلة بنسبة 20.3% ، ثم *Staphylococcus aureus* عددها 25 عزلة وبنسبة 16.3%، وبكتريا *Klebsiella pneumonia* عددها 11 عزلة بنسبة 7.2%، ثم بكتريا *Shigella spp.* عددها 7 عزلة بنسبة 4.6% ، تلتها بكتريا *Citrobacter freundii* عددها 6 عزلة وبنسبة 3.9%، ثم بكتريا *Hafina spp.* بعدد 3 عزلات بنسبة 1.9%، وجاءت في المرتبة الاخيرة بكتريا *Serratia marcescens* وعددها 2 عزلة وبنسبة 1.3%.

أظهرت النتائج أنَّ أعلى نسبة تلوث كانت في سوق الخضار بنسبة 92 %، ثم حظائر الأغنام بنسبة 88 %، وأخيراً سوق القصابين بنسبة 86 %.

أجريت للعزلات البكتيرية السالبة لصبغة كرام اختبار الحساسية تجاه 8 أنواع من المضادات الحيوية، اذ أعطت بكتريا *E.coli* أعلى مقاومة تجاه كل من المضاد الحيوي Erythromycin و Ceftriaxon وبنسبة 77.8%، بينما أظهرت بكتريا *S.typhi* مقاومة تجاه كل من المضاد الحيوي Amikacin و Ceftriaxon وبنسبة 53.1%، أما بكتريا *P.mirabilis* فكانت مقاومة للمضاد الحيوي Tetracyclin بنسبة 64.5%، في حين كانت بكتريا *Shigella spp.* مقاومة للمضاد Ceftriaxon بنسبة 57.14%، أما بكتريا *K.Pneumonia* فكانت مقاومة للمضاد Erythromycin بنسبة 72.7%، وأبدت بكتريا *C.freundii* مقاومه تجاه المضاد Trimethoprim+ Sulfamethoxazol وبنسبة 100%، أما بكتريا *Hafnia spp.* فقد كانت مقاومة للمضاد Chloramphenicol بنسبة 33.33%، وأخيراً بكتريا *Ser.marcescens* إذ كانت مقاومة للمضاد Erythromycin بنسبة 100%.

واختبرت أيضاً حساسية بكتريا *Staph.aureus* تجاه 5 أنواع من المضادات الحيوية إذ أبدت أعلى مقاومة تجاه المضاد الحيوي Vancomycin بنسبة 80%.