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ISOLATION OF SOME PATHOGENIC CAUSATIVE AGENT FROM SOME SPECIES OF DIPTERA

Jalal Y. Mustafa*, Muna K. Marzoq **, Nadia K. Thamer*
Iman A. Saadoon, Iman K. Sultan

*College of Veterinary Medicine , University of Basrah,Basrah,Iraq

** Department of Biology ,College of Education, University of Basrah,Basrah,Iraq

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ABSTRACT

Collected truefly samples *Musca domestica* (housefly) and *Culex pipiens* (mosquitoes) from diptera to investigation the bacterial and fungal causative agents transmitted outside or inside the body of it. Recorded fifteen bacterial species from the outer surface and midgut of the *Musca domestica* (housefly) and thirteen from the outer surface and midgut of *Culex pipiens* (mosquitoes). Ten species of fungi isolated from the outer surface and midgut of the housefly, seven of it isolated from the midgut, while isolated seven species from the outer surface of mosquitoes, five of it isolated from the midgut. The animal house and farm of veterinary medicine recorded the largest number of bacterial isolate. *Micrococcus luteus* are the maximum percentage in housefly infected (39.2 %) while in the mosquitoes are *Staphylococcus aureus* (35.2 %), *Penicillum spp.* are the maximum percentage in truefly infected (8.8 %), while in the mosquitoes are *Candida albicans* (8.8 %).

INTRODUCTION

Diptera is an order of winged insects commonly known as true flies, which are one of the most successful groups of organisms on earth in diversity and distribution [1]. The most important damage related with dipteral species the annoyance and the indirect damage produced by the potential transmission of pathogens viruses, bacteria, fungi, protozoa and nematodes associated with this fly [2, 3]. House flies is a good example of this order, the behavioral characteristics of it ensure its contact with food and wastes of man and his animals [4, 5]. This communicative behavior that allows it to easily move from infected materials to human populated areas and mechanical transmission of bacterial, fungal, viral pathogens [6]. It has been demonstrated that some microorganisms may live inside and \ or on the house fly body surface from 5-6 hours up to 35 days [7].

Mosquitoes are vectors of a large number of animal and human pathogens, as for all insects, the successful spreading of mosquitoes worldwide might be partly linked to their symbiosis with microorganisms, notably with bacteria [8]. The larval form of mosquito feed on organic matter and microorganisms like bacteria, The pupa does not have a mouth and hence do not feed, so several studies suggest that transstadial transfer of bacteria from larvae to adult, otherwise there was no studies have been performed on the origin of the mosquito mid gut bacteria in nature and hence, it is not clear where the adult mosquitoes obtain their bacteria [9].

There are many bacterial disease that are transmitted by some of adult flies that may be able to spread pathogen via their sponging mouth part, vomits, intestinal tract, sticky pads of their feet or via their body or leg hairs or by some form of fecal contamination of food and water either directly or indirectly [10].

Among the pathogens commonly transmitted by truefly are many species yeast and filamentous fungi that cause illness [11]. The majority of these fungi caused opportunistic infections that may cause life threatening infections and especially occurring in immunocompromised patients admitted in hospital [12, 13]. Dirt, soil, body discharges and excreta from animals in holding pens are the main Sources of fungal contamination of house flies [14]. *Aspergillus spp*. and *Candida spp*. are commonly isolated from the soil, plant debris and the indoor environment, including hospitals [13]. The association of fungi and insects has been verified by several authors [11, 14, 15, 16].

The aim of this study is isolating and identifying the probable pathogen that transmission by houseflies and mosquitoes.

MATERIALS AND METHODS

Collected 250 adult of *Musca domestica* and 250 *Culex pipiens* from Al-Zubair, Abu Alkhaseeb, the animal house and farm of veterinary medicine (Vet. Med.), city center (Ashar) and Al- Fayhaa, which identify according to [1, 19]. Fifty sample of houseflies and fifty sample of mosquitoes for each situation above. The truefly were captured with a sterile nylon net and transferred to the laboratory and placed in the sterile dishes in freeze temperature for 15 min to anaesthetize them.

Identification was made by examining the fly (inside test tube) under a dissecting microscope and following standard taxonomic keys [17].

For external of the fly, immersed the two sample (two of houseflies or two mosquitoes) in normal saline and shaken for 5-10 Minute. The washed fly bodies where filtered out and the fluid was tested for bacteria and fungi [18].

For internal (inside the fly body), sterilized the houseflies and mosquitoes by immersed in 3 % cloroxin (sterilized solution) for one minute, then broyer the fly by sterilized rood, then washing by autoclaved distal water, this protocol did twice times, then immersed the sample like external was write above [20].

Bacterial and fungi isolates:

Bacterial isolates growing on nutrient agar, blood agar, manitol salt agar, eosin mathel blue agar and MacConkey agar after incubation for 24-48 hours at 37° C which were identified according to [21].The following biochemical tests were used for identification: coagulase test, catalase test, DNase test, catalase test, triple-sugar iron test, oxidase test, citrate utilization test, indole test, methyle red test, urase test, H_2S production test and motility test. For further identification: three types of API techniques (Bio Meriux, Frunce) were used for rapid identification of varies bacterial isolated based on enclosed instruction of supplied company [22, 23, 24]:

- API Staph ID 32 test: Identification system for staphycocci.
- Api 20 strep: Identification system for streptococci.
- Api 20 E: Identification system for Enterobacterioccace and other Gram negative rods.

Fungi growing on sabouraud dextrose agar containing chloramphenicol to inhibit bacterial growth under hood and sterile conditions. The plates were incubated at 25°C and daily observations were made for 15 days. The resulting growth (if occurs) was identified by standard mycological methods [25]. The grown fungi were identified by standard mycological techniques based upon gross cultural and microscopic morphology. The fungi that could not be identified by this manner were subcultured on potato dextrose agar, water agar and / or slide cultures for further study were identified according to [26] after examination of colonies characteristics and microscopic slides techniques. Staining the bacteria by gram stain and lactophinol for the fungi [27].

RESULTS

Figure (1) the *Musca domestica* (A) and *Culex pipiens* (B) which were isolated in this study.

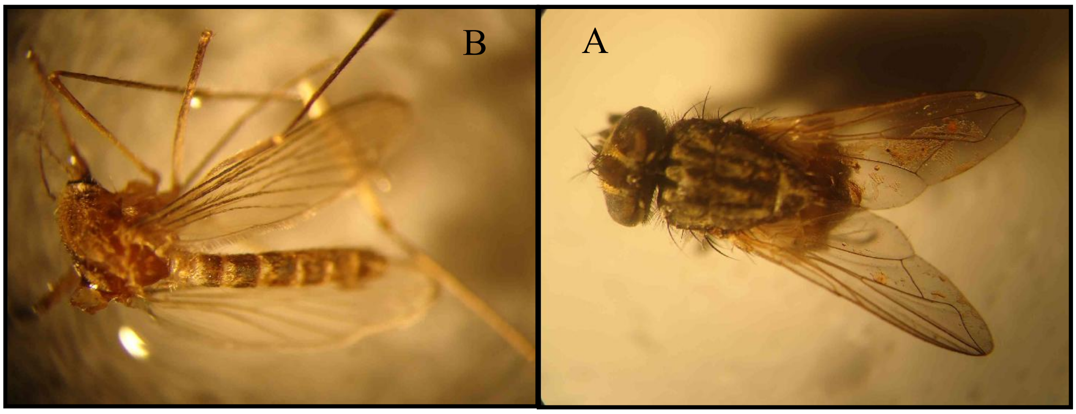

Fig. (1) *Musca domestica* (A) and *Culex pipiens* (B)

The result showed in table (1) and diagram (1) bacterial and fungi isolated from the houseflies 323 (64.6 %) from the outer side and 172 (34.4 %) from the internal part, while the bacterial and fungi isolated from the mosquitoes 307 (61.4 %) from the outer side and 148 (29.6 %) from the internal part. All 250 houseflies and 250 mosquitoes collected were infected by different type of bacteria in outer surface (100 %) and 153 only (61.6 %) infected the internal part of body of the houseflies while 130 only (52 %) internal part of the mosquitoes. In the same table the result shoed 73 (29.2 %) infected the external part of body of the houseflies and 19 (7.6 %) from the internal part, while 57 (22.8 %) infected the external part of body of the mosquitoes and 18 (7.2 %) from the internal part.

Table (1) the number and percentage of bacteria and fungi isolated from houseflies and mosquitoes

	Houseflies				Mosquitoes				Total			
	Out body		Inside body		Out body		Inside body		Out body		Inside body	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Bacteria	250	100	153	61.6	250	100	130	52	500	100	283	56.6
Fungi	73	29.2	19	7.6	57	22.8	18	7.2	130	26	37	14.8
Total	323	64.6	172	34.4	307	61.4	148	29.6	630	63	420	42

- No: number of true fly infected by bacteria or fungi.
- %: percentage of infection.

Diagram (1) the number and percentage of bacteria and fungi isolated from houseflies and mosquitoes

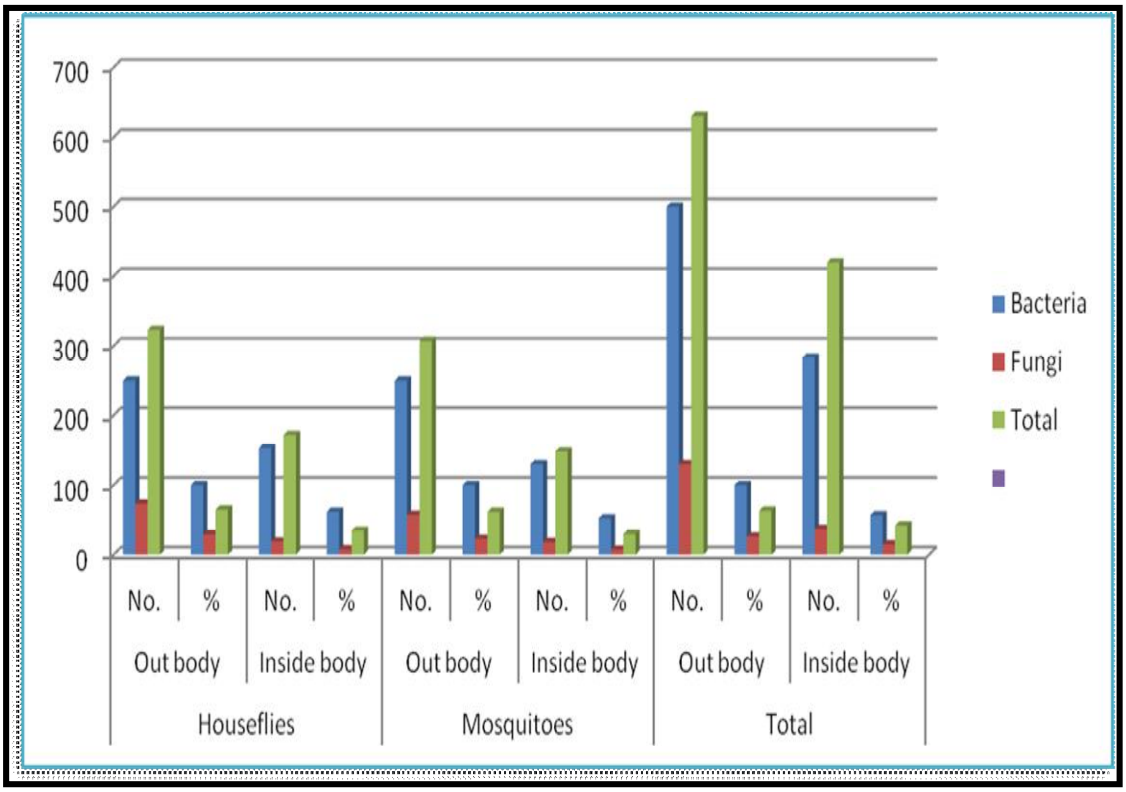

Table (2) shows the bacterial isolated of identification results in this table, fifteen bacterial species from the outer surface and midgut of the housefly and thirteen from the outer surface and midgut of mosquitoes table (3). Ten species of fungi isolated in this study from the outer surface of the housefly table (4), seven of it isolated from the midgut, while isolated seven species from the outer surface of mosquitoes, five of it isolated from the midgut table (5). The animal house and farm of veterinary medicine recorded the largest number of bacterial isolate. *Micrococcus luteus* are the maximum percentage in housefly infected (39.2 %) while in the mosquitoes are *Staphylococcus aureus* (35.2 %), *Penicillum spp.* are the maximum percentage in housefly infected (8.8 %), while in the mosquitoes are *Candida albicans* (8.8 %).

Table (2) the number and percentage of bacteria isolated from houseflies in different region in Basra

Area	Site of sample	Zubair		Abu Alkhaseeb		Vet. Med.		City center		Fayhaa		Total	
Bacteria		No	%	No	%	No	%	No	%	No	%	No	%
Micrococcus luteus	External	20	40	18	36	28	56	10	20	22	44	98	39.2
	Internal	16	32	10	20	20	40	6	12	16	32	68	27.2
Staphylococcus epidermidis	External	12	24	10	20	22	44	12	24	8	16	64	25.6
	Internal	8	16	6	12	16	32	6	12	6	12	42	16.8
Staphylococcus aureus	External	12	24	10	20	30	60	8	16	10	20	70	28
	Internal	10	20	10	20	28	56	6	12	8	16	62	24.8
Staphylococcus xylosus	External	0	0	0	0	8	16	2	4	0	0	10	4
	Internal	0	0	0	0	6	12	2	4	0	0	8	3.2
Staphylococcus capitis	External	2	4	0	0	6	12	2	4	2	4	12	4.8
	Internal	2	4	0	0	6	12	2	4	2	4	12	4.8
Staphylococcus saprophyticus	External	6	12	8	16	14	28	4	8	6	12	38	15.2
	Internal	4	8	8	16	10	20	4	8	2	4	28	11.2
Shigella dysenteriae	External	4	8	6	12	20	40	6	12	8	16	44	17.6
	Internal	4	8	2	4	14	28	4	8	4	8	28	11.2
Klebseilla spp	External	2	4	4	8	18	36	4	8	4	8	32	12.8
	Internal	2	4	4	8	12	24	4	8	2	4	24	9.6
Escherichia coli	External	18	36	14	28	22	44	10	20	8	16	72	28.8
	Internal	16	32	14	28	14	28	4	8	2	4	50	20
Enterococcus fuecium	External	0	0	2	4	4	8	0	0	0	0	6	2.4
	Internal	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus subttfis	External	4	8	10	20	18	36	2	4	2	4	36	14.4
	Internal	4	8	4	8	10	20	2	4	0	0	20	8
Proteus mirabilis	External	2	4	0	0	4	8	2	4	0	0	8	3.2
	Internal	2	4	0	0	2	4	2	4	0	0	6	2.4
Diphtheroid bacilli	External	2	4	0	0	4	8	0	0	0	0	6	2.4
	Internal	2	4	0	0	0	0	0	0	0	0	2	0.8
pseudomonas arigenosa	External	0	0	2	4	8	16	2	4	2	4	14	5.6
	Internal	0	0	2	4	8	16	2	4	0	0	12	4.8
Streptococcus mitis	External	4	8	2	4	8	16	0	0	2	4	16	6.4
	Internal	0	0	0	0	2	4	0	0	0	0	2	0.8

Table (3) the number and percentage of bacteria isolated from mosquitoes in different region in Basra

Area	Site of sample	Zubair		Abu Alkhaseeb		Vet. Med.		City center		Fayhaa		Total	
Bacteria		No	%	No	%	No	%	No	%	No	%	No	%
Micrococcus luteus	External	10	20	12	24	22	44	10	20	18	36	72	28.8
	Internal	8	16	8	16	18	36	6	12	14	28	54	21.6
Staphylococcus epidermidis	External	6	12	8	16	20	40	14	28	6	12	54	21.6
	Internal	4	8	6	12	18	36	10	20	4	8	42	16.8
Staphylococcus aureus	External	18	36	18	36	26	52	8	16	18	36	88	35.2
	Internal	14	28	14	28	16	36	4	8	12	24	60	24
Staphylococcus xylosus	External	2	4	0	0	10	20	4	8	0	0	16	6.4
	Internal	0	0	0	0	4	8	2	4	0	0	6	2.4
Staphylococcus capitis	External	0	0	0	0	2	4	0	0	2	4	4	1.6
	Internal	0	0	0	0	2	4	0	0	0	0	2	0.8
Staphylococcus saprophyticus	External	4	8	2	4	10	20	2	4	0	0	18	7.2
	Internal	2	2	0	0	4	8	0	0	0	0	6	2.4
Shigella dysenteriae	External	10	20	12	24	32	64	8	16	4	8	72	28.8
	Internal	6	12	8	16	24	48	4	8	2	4	44	17.6
Klebseilla spp	External	0	0	2	4	10	20	2	4	0	0	14	5.6
	Internal	0	0	0	0	4	8	0	0	0	0	4	1.6
Escherichia coli	External	14	28	16	32	24	48	16	32	4	8	74	29.6
	Internal	10	20	8	16	20	40	16	32	2	4	56	22.4
Enterococcus fuecium	External	0	0	0	0	4	8	0	0	2	4	6	2.4
	Internal	0	0	0	0	2	4	0	0	0	0	2	0.8
Proteus mirabilis	External	0	0	0	0	8	16	12	24	0	0	20	8
	Internal	0	0	0	0	4	8	8	16	0	0	12	4.8
Diphtheroid bacilli	External	0	0	0	0	0	0	0	0	2	4	2	0.8
	Internal	0	0	0	0	0	0	0	0	0	0	0	0
pseudomonas arigenosa	External	4	8	2	4	4	8	0	0	0	0	10	4
	Internal	2	4	2	4	2	4	0	0	0	0	6	2.4

Table (4) the number and percentage of fungi isolated from houseflies in different region in Basra

Area	Site of sample	Zubair		Abu Alkhaseeb		Vet. Med.		City center		Fayhaa		Total	
Bacteria		No	%	No	%	No	%	No	%	No	%	No	%
Cladosporium	External	4	8	0	0	8	16	0	0	2	4	14	5.6
	Internal	0	0	0	0	2	4	0	0	0	0	2	0.8
Penicillum spp.	External	6	12	2	4	8	16	2	4	4	8	22	8.8
	Internal	2	4	0	0	2	4	0	0	0	0	4	1.6
Fusarium spp.	External	0	0	2	4	4	8	0	0	2	4	8	3.2
	Internal	0	0	0	0	2	4	0	0	2	4	4	1.6
Aspergillus flavus	External	2	4	4	8	6	12	2	4	0	0	14	5.6
	Internal	0	0	0	0	2	4	0	0	0	0	2	0.8
Aspergillus niger	External	0	0	0	0	0	0	0	0	2	4	2	0.8
	Internal	0	0	0	0	0	0	0	0	0	0	0	0
Candida albicans	External	4	8	4	8	4	8	2	4	0	0	14	5.6
	Internal	2	4	0	0	2	4	2	4	0	0	6	2.4
Mucor	External	4	8	0	0	6	12	0	0	2	4	12	4.8
	Internal	2	4	0	0	2	4	0	0	0	0	4	1.6
Ulocladium preuss	External	0	0	0	0	0	0	2	4	0	0	2	0.8
	Internal	0	0	0	0	0	0	2	4	0	0	2	0.8
Alternria spp.	External	2	4	0	0	0	0	0	0	0	0	2	0.8
	Internal	0	0	0	0	0	0	0	0	0	0	0	0
Acremonium	External	0	0	0	0	0	0	0	0	2	4	2	0.8
	Internal	0	0	0	0	0	0	0	0	0	0	0	0

Table (5) showed the number and percentage of fungi isolated from mosquitoes in different region in Basra

Area	Site of sample	Zubair		Abu Alkhaseeb		Vet. Med.		City center		Fayhaa		Total	
Bacteria		No	%	No	%	No	%	No	%	No	%	No	%
Cladosporium	External	2	4	2	4	6	12	0	0	0	0	10	4
	Internal	0	0	0	0	2	4	0	0	0	0	2	0.8
Penicillum spp.	External	2	4	4	6	4	8	0	0	2	4	12	4.8
	Internal	2	4	0	0	2	4	0	0	0	0	4	1.6
Fusarium spp.	External	0	0	0	0	2	4	0	0	0	0	2	0.8
	Internal	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus flavus	External	4	8	2	4	4	8	0	0	2	4	12	4.8
	Internal	2	4	0	0	2	4	0	0	0	0	4	1.6
Candida albicans	External	6	12	4	8	8	16	2	4	2	4	22	8.8
	Internal	2	4	0	0	4	8	0	0	0	0	6	2.4
Mucor	External	6	12	2	4	4	8	2	4	0	0	14	5.6
	Internal	0	0	0	0	2	4	0	0	0	0	2	0.8
Alternria spp.	External	0	0	0	0	2	4	0	0	0	0	2	0.8
	Internal	0	0	0	0	0	0	0	0	0	0	0	0

DISCUSSION

In the recent years much attention has been given to the housefly as a potential mechanical vector of disease transmitting agent [28]. Diptera, truefly are one of the largest and most divers order of insect and with the medical and veterinary significant, so some of the truefly species affect human and livestock health indirectly through disease transmission and transmission of disease agent occur when diptera physically carriers pathogen from one place or host to another host often via body parts that collect contaminates as the insect feed on dead animals or excrement, and the main point about the mechanical transmission in that the pathogen undergoes no development and no multiplication [29]. Mechanical transmission pathogens may be carried or transmitted by flies via contaminated appendages, usually month parts, on the hair of the feet and body or regurgitated in the saliva during feeding [1].

Our study agreed with Land and Crosskey [30] in diagnosed *Shigella spp.* which causing dysentery and diarrhea, and *Escherichia coli* causing urgently and intestinal infection are wide spread enteric disease.

In bacterial isolated, this study diagnosed many bacteria *Micrococcus luteus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *staphylococcus xylosu, staphylococcus capitis, Staphylococcus Saprophyticus*, *Shigella dysenteriae*, *Klebseilla spp*., *Escherichia coli*, all these bacteria are similarity in recorded to Mufeed and Mohammed study but different their in some other bacteria because different time and situation [31]. *Staphylococcus spp*., *Bacillus spp.* and *Escherichia coli* are very harmful bacteria, its one causes of diarrhea, which we were obtained from external and internal parts of housefly body and this result will be similar to the result of Nazni *et al.,* (2005) and Bouamama *et al.,* (2010) [28, 32].

The genera *Staphylococcus* and *Streptococcus* both are recreational and tourist sites, could pose a danger in the spread of diarrhea diseases. Control measures must be undertaken urgently in order to suppress the fly population. According to Chavasse [33] thousands of people had died due to diarrheal in diarrheal epidemic areas.

The results showed similarity in recorded fungi isolates by Davari [8], these fungi are *Aspergillus flavus, Aspergillus niger, Penicillum spp., Fusarium spp.* and *Alternria spp*, as well as *Mucor, Cladosporium, Candida albicans, Ulocladium* *preuss*, and *Acremonium*, this give very bad and serious image about the truefly in Basra because they have many types of fungi and bacteria.

There is abundant opportunity for house fly, *Musca domestica*, to become contaminated and in turn to contaminated the patient environment [5]. Most of the organisms recovered from the housefly are serious pathogens, known to produce diseases such as meningitis, food poisoning, diarrhea, abscesses, bloodstream infections, and hemorrhagic colitis [6].

Finally adult truefly are vectors pathogen of disease such as dysentery have frequently been associated with disease transmission in human and animals as well as myiasis and these flies thought to be responsible for the spread of such disease as those already mentioned above also diarrhea, anthrax, eye inflammation and possibly tuberculosis, that throated to public health [2], these flies bread in bacteria Laden-environments such as feces, carrion and human food waste so the bacteria enter the digestive system and pads through it, unharmed and fall on food with feces of the fly and other in close proximity to human and animals [29].

For all that harmful of bacteria and fungi which transmitted to human and animal, must be put some rules to dissolved this problem like killed fly or using some protocols to limited it reproduction to control the hug number especially spring and summer seasons in the markets and houses.

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عزل بعض المسببات المرضية من بعض أنواع حشرات رتبة ثنائية الأجنحة

جلال ياسين مصطفى* ،منى خضير مرزوق** ،ناديه ثامر كاظم* ،ايمان عبد الباسط سعدون**

ايمان خلف سلطان**

*كلية الطب البيطري، جامعة البصره ، البصره ،العراق

قسم علوم الحياة ،كلية التربيه للعلوم الصرفه، جامعة البصره ، البصره ،العراق

الخلاصة

جمعت عينات الذباب المنزلي *Musca domestica* والبعوض المنزلي *Culex pipiens* من رتبة ثنائية الأجنحة للتحري عن المسببات المرضية المحمولة في سطحها الخارجي والداخلي. إذ عزلت خمسة عشر نوعا من البكتريا من السطح الخارجي والأحشاء الداخلية للذباب، كما تم تسجيل ثلاثة عشر نوعا من السطح الخارجي والأحشاء الداخلية للبعوض، كما عزلت عشرة أنواع من الفطريات من للذباب كانت سبعة منها من الأحشاء الداخلية، فيما وجدت سبعة أنواع أخرى للفطريات في البعوض، خمسه منها في الأحشاء الداخلية. أظهر كل من البيت والحقل الحيواني في كلية الطب البيطري أعلى نسب إصابة للبكتريا والفطريات، وكانت لبكتريا *Micrococcus luteus* أعلى نسبة إصابة في الذباب المنزلي و بنسبة (39.2 %)، فيما كانت بكتريا *Staphylococcus aureus* هي الأعلى إصابة في البعوض بنسبة (35.2 %)، فيما كانت فطريات *Penicillum spp.* هي أعلى نسبة إصابة في الذباب (8,8 %) وفطريات *Candida albicans* هي الأعلى في البعوض بنسبة (8.8 %).

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