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Isolation and Identification of Gram Negative Bacteria from the Outer Surface of the House Fly

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Abstract

Musca domestica specimens were collected from three locations in Salah al-Din Governorate's Al-Dur district, namely sheep breeding places, butcher shops, and vegetable markets. The collection took place between early January 2022 and May of the same year. The aim of the study was to isolate intestinal and Gram-negative bacteria from the outer surface of the flies. Different culture media, microscopic examinations, biochemical tests, and the vatic system were used for diagnosing the bacterial isolates.

A total of 157 bacterial isolates were obtained from the study, representing eight different types of bacteria. The most prevalent type was Escherichia coli, accounting for 44 isolates or 28% of the total. Klebsiella pneumoniae followed with 32 isolates, representing 20.3%. Proteus mirabilis ranked third with 30 isolates, making up 19.1%. Salmonella typhi accounted for 20 isolates, comprising 12.7% of the bacterial isolates. Providencia rettgeri had 15 isolates, representing 9.5%, while Proteus vulgaris had 8 isolates, accounting for 5.1%. Citrobacter freundii numbered 6 isolates, making up 3.8%. Finally, Shigella spp. had 2 isolates, representing 1.2% of the bacterial isolates.

Keywords: *Musca domestica, Escherichia coli, bacterial isolates.*

Introduction

Musca domestica is one of the most important insect which is closely associated with human life and living wherever it is, and it is considered the most widespread type of insect inside homes and the most fertile one[1].Flies, including insects in general, have a significant impact on human health. They are among the most prominent insects that coexist with humans, both indoors and outdoors. Flies are often found in close proximity to humans and can be present in their living spaces. Furthermore, flies have a tendency to be found on various food items consumed by humans, including vegetables, fruits, meat, beverages, and more. Their presence in such contexts can have implications for human health and wellbeing.Therefore, they are considered a disturbing insect for humans and animals, and a mean of transmitting many dangerous diseases that threaten health due to their frequent presence in stables, barns, dirt places and poultry fields, which are considered the appropriate place to lay their eggs which turn into larvae [2].

The association between house flies and microorganisms found in their surroundings is a significant one. Flies have a close connection with the pathogenic microorganisms present in their environment, as they can carry these microbes on their mouthparts, legs, and wings. Additionally, these microorganisms can adhere to the internal organs,

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particularly the digestive system, of flies. Given that flies are highly mobile insects, swiftly moving between human and animal environments, they can effectively transfer pathogens, increasing the risk of transmitting harmful diseases.[3]

House flies mechanically transmit pathogens from contaminated areas to humans through various body parts such as their legs, wings, and fleshy pads located between the claws of the feet. The pathogens can also be transmitted through the fly's proboscis, the mouthparts used for feeding, as well as through its cuticle layer. Additionally, microbes can be transmitted through the fly's regurgitation, feces, or via its mouthparts. It is through these mechanisms that house flies play a role in the dissemination of disease-causing microorganisms.The most important types of bacteria isolated from different parts of the body of house flies and the most common are the intestinal bacteria and it is obvious that the main reason for this is the domestic fly, which is the main food for it is animal waste and feces, and it is a mean of intestinal bacterial types. The most important types of intestinal bacteria that are considered highly virulent are Escherichia coli, which causes cholera, Klebsiella spp and bacteria that cause intestinal and skin anthrax (Bacillus anthrax) and the other types including bovine, enterococcus and these types are considered the most important bacterial one that cause major human diseases [4].

In addition, studies have shown that house flies have the ability to transmit antibiotic-resistant bacteria, especially in hospitals environment [5]. Which proved that flies have a major role in the transfer and spread of strains that have multiple resistance to antibiotics from hospitals to many environments out of the hospital environment, causing the spread of epidemics, especially intestinal ones, such as diarrhea, cholera, salmonella and other diseases [6].

Materials and Methods

1- Insect Inspection

The collected insects were immediately transported to the laboratory and subjected to anesthesia by freezing them at a temperature of zero degrees Celsius for approximately five minutes. Sterilized tubes containing 2 ml of normal saline were prepared, and the flies were shaken using a vibrating device called a Vortex. A sterile loop was then used to transfer a sample from the solution onto various culture media, including Nutrient agar, MacConkey agar, Salmonella agar, Shigella agar, and Mannitol salt agar. These media were prepared according to the instructions provided by the manufacturers. The purpose was to isolate different types of bacteria. The culture dishes were subsequently incubated for 24 hours at a temperature of 37°C, under specific air conditions. The bacterial types were identified using a combination of microscopic, cultural, and biochemical methods. Following identification, an antibiotic test was conducted using a selected number of antibiotics as part of the study.

2- Identification of bacteria

Morphological Characteristics

The phenotypic characteristics and culture characteristics were studied after the completion of the incubation period when bacterial colonies appeared on media (Maconki, mannitol saline medium, and nutrient medium), after that the shape, size, and color of the bacteria were observed, and this is considered a preliminary diagnosis [7].

Microscopically test

From the developed and pure colonies, smears were prepared by placing a drop of the saline solution on a glass slide. A portion of the colonies was transferred using a sterile loop and spread across the slide's surface. The slide was then fixed by passing it through a flame, followed by staining with Gram stain. After staining, the slide was dried using

blotting paper. Subsequently, microscopic examination was conducted using the oil immersion lens of an optical microscope to observe and record the shape, cell type, and response to the Gram stain of the bacterial cells. [8].

3- Biochemical Test

The Methyl Red (MR) test involved inoculating tubes containing a specific medium composed of peptone water, phosphate, and glucose. A small, pure, and fresh sample from a bacterial culture, not older than 24 hours, was added to the tubes. The tubes were then incubated at a temperature of 37°C for a period ranging between 24 to 48 hours. Following incubation, 5 drops of methyl red reagent were added to the medium in the tubes and the tubes were shaken. After a few minutes, a positive test result was indicated if the medium in the tube turned pink. [9].

Indole test-

The indole production test assesses the bacterial ability to metabolize tryptophan, an amino acid, into indole. In this test, the pure bacterial isolates were inoculated into peptone water medium and incubated at a temperature of 37°C for 24 hours. Following the incubation period, 5 drops of Kovacs reagent were added to the medium, and the mixture was gently shaken. A positive test result was indicated by the formation of a red ring in the upper layer of the solution. [10].

-Citrate consumption test

To perform the Simmon's Citrate test, a single, pure colony was inoculated onto the Simmon Citrate medium and incubated at a temperature of 37°C for a period of 24 to 48 hours. A positive result was indicated by a color change in the medium from green to blue. This test is employed to determine the bacteria's capability to utilize sodium citrate as a carbon source.

-Voges-Proskauer test

The Methyl Red Voges-Proskauer (MR-VP) test is utilized to determine the bacteria's ability to produce acetoin, which is a precursor to acetone. The test involves inoculating the MR-VP medium with pure bacterial colonies that are 24 hours old. The inoculated medium is then incubated at 37°C for a period of 24 to 48 hours. Following incubation, six drops of Alfa Naphthol reagent and three drops of potassium hydroxide are added to the culture. The mixture is allowed to react for a duration of 10 to 15 minutes. A positive result is indicated by a change in color to brick red[11].

-Motility test

The inoculation in the tube containing the motility medium for this test was performed using the stabbing method. The tubes were then incubated at a temperature of 37°C for a duration of 48 hours. A positive result was identified by the presence of a cloudy region surrounding the areas where the stabbing was performed, indicating the movement of bacteria[12].

-Growth test on Eosin Methylene Blue medium.

Bacterial colonies were cultured on Eosin Methylene Blue (EMB) medium, and the plates were subsequently incubated at 37°C for 24 hours. The presence of bacterial growth exhibiting a distinct bright green metallic color indicates the identification of E.coli bacteria. [13].

-Catalase Test

The catalase test was employed to determine the bacterial ability to produce the catalase enzyme, which facilitates the breakdown of hydrogen peroxide into oxygen and water. A portion of the bacterial colony was transferred onto a glass slide using a loop.

Subsequently, a drop of hydrogen peroxide (acting as the catalase reagent) was added. The presence of bubbles indicates a positive test result. [14].

Oxides test-

In this test, filter papers of the type (Whattman No.1) were used that were wetted with a solution of the oxidase enzyme reagent for this test, then a pure colony of (24) hours old and growing on the agar medium was transferred by using wooden sticks and then spread on the filter paper. If the color of the colony changes into violet after (10) minutes, this means that this test is positive and this test shows the ability of bacteria to produce the oxidase enzyme.

Urease test-

The urease test was conducted by inoculating pure bacterial isolates diagonally onto a pre-prepared urea medium. The tubes containing the inoculated medium were then incubated at a temperature of 37°C for 24 hours. A positive result was indicated by a color change in the medium from yellow to pink. [15].

- H2S Production test

To perform the Kliger iron agar test, bacterial colonies were inoculated onto tubes containing Kliger medium by streaking them on the surface of the inclined agar. The tubes were then incubated at a temperature of 37°C for 24 hours. A positive result was indicated by the formation of H2O gas, which was observed as a black precipitate at the bottom of the tube [16].

-Carbohydrate Fermentation test

This test was carried out by inoculating the medium of carbohydrate fermentation with a pure and young 24-hour-old colony of bacteria, and then the mediums were incubated at a temperature of (37) °C for a period of (24-48) hours. When the color of the medium changed to yellow as a result of the fermentation of sugars and the production of acid, this means that the test is positive.

The current study included the test of (180) samples taken from the body of the housefly, which were obtained from sheep breeding places, butcher shops, and vegetable markets in the district of Al-Dur,and the number of insects that gave bacterial growth, that is, carries of bacteria, reached 157, or an average of (87.2)%. The growth colonies of bacteria were diagnosed through their growing on Macconkey agar and Mannitol salt agar and conducting laboratory tests after staining it with Gram stain.

The results of Table (1) showed that the highest percentage of flies carrying the bacteria was from sheep breeding places, reaching (93.3)%, followed by butchers' shops, so the percentage was (85)%, and the lowest percentage was in vegetable markets, which amounted to (83.3)%.

Through the results of the table, it was found that the percentage of bacterial ontamination of the current study reached (87.2)%, which is less than what was reached by [17]. on the isolation and diagnosis of bacteria transmitted by house flies, in which the percentage of contamination reached (88.6)%, and also less than the percentage reached by [18] as it reached (100)% of bacterial contamination. This percentage of the current study is different from what is reached by [19]. which is confirmed in the study that the bacterial contamination is (81.3) %

Table (1) Percentages of houseflies contaminated with bacteria from their collecting sites

Insect contaminated with bacteria		Total number of houseflies	Collecting site
%	The number		
93.3	69	60	**Breeding sheep**
85	52	60	**Butcher shops**
83.3	36	60	**Vegetable markets**
87.2	157	180	**The total**

Types of Gram-negative Enterobacteriaceae isolated from house flies

The results showed that (157) insects carrying or contaminated with the bacteria were obtained after examining the samples collected from the aforementioned sites. These isolates are devided into bacterial types such as: Klepsiella Pneumonia, Escherichia coli, , C.freundii, P.vulgaris, Providencia rettgeri, Salmonella. spp, P. mirabilis and Shigella sonni. The results of Table (2) showed that the largest number of isolates was contaminated with E.coli, reaching 44 isolates, at a rate of (28)% among the isolates, at a rate of (27.5)% of the total number of insects, then K.pneumonia came in second place, with a percentage of isolation of (20.3%). Its percentage of the total number of insects amounted to (20)%, then the P.mirabilis, the percentage of isolates was (19.1)% and (18.7)% of the total number of insects, It was followed by Salmonella.spp with a rate of (12.7)% of the bacterial isolates, while the percentage of the total number of insects was (12.5)%, then Providencia rettgeri, which gave a percentage of (9.5)% of the isolates and (9.3)% of the total number of insects, then P.vulgaris bacteria, which reached (5.1)% of the number of isolates and (5)% of the total number of insects, while C.freundii With a rate of (3.8)% of the isolates and (3.7)% of the total number of insects. Finally, the Shigella.spp bacteria came last with a rate of (1.2)% of the number of isolates and (1.1)% of the total number of insects.

The results of the current study showed that E.coli bacteria were superior in number to the rest of the bacterial types during their presence on the external surfaces of the housefly body, and this result agreed with many previous studies that showed the superiority of this bacterial type over the rest. The study [20]. in Basra showed that E.coli bacteria is the largest number comparing with other bacterial types and the mentioned study agreed with [21] one that showed during diagnosis the isolated bacteria from the outer surface of the fly's body that E. coli bacteria are the most present on the outer surface of the insect's body, reaching a percentage of 37.5%.

The results of the current study showed that the K.pneumonia came second after E.coli in the percentage of contamination. This bacteria is a type that is resistant to a number of antibiotics because it has a resistance factor and is surrounded by a capsule. It also has the ability to produce the beta-lactimase enzyme. Therefore, it is one that has the ability to resist antibiotics from the group of penicillin. The results agree with what is conducted by [22]. when isolating this type of bacteria from the Culex mosquito that was collected in AL-Dur district in Salah AL-Din Governorate, where the percentage of isolates was 6.3%.

As for the P.mirabilis bacterial type, it took the third place in terms of contamination rates, which amounted to (19.1)%. As shown in the table, this bacteria is an opportunistic type that has a high ability to resist antibiotics and certain types of disinfectants and is considered a secondary cause of wounds [23]. and it is also one of the main causes of infection, which occurs clearly in the urinary tract, causing inflammation of the urinary tract [24].

The results showed that the Salmonella.spp came after the P.mirabilis, that is, it ranked fourth in terms of contamination rates, as it reached (12.7)%. This type is considered one of the most dangerous bacterial one that is transmitted through food to human and it can

live in dry places for a number of weeks and it can also survive for monthes in aquatic environment [25].

The results showed that the Providencia rettgeri came in the fifth rank in the percentage of contamination on the external surface of body of fly's body, with a percentage of (9.5)%, which was diagnosed by the phytic system, and it is opportunistic types that causes many infections including urinary infection (Uti) especially for patients who use urinary catheters as well as infection resulting from burns and wounds [26], and it is also the main cause of purple urine syndrome [27].

As for the P.vulgaris bacteria, it ranked sixth in the percentage of contamination, as its percentage reached (5.1)%. This bacteria is an opportunistic pathogen, and it abounds in dirt, dust and polluted water. This type of bacteria is common in urinary tract infections and diseases transmitted from hospitals [28].

The results showed that the C. freundii ranked seventh, with a contamination rate of (3.8)%. It was stated by [29]. That this type of bacteria is widespread in wastewater, drinking water and soil. These results differed with what was reached by [30]. where they indicated in their study that this type of bacteria reached a contamination rate of 28.4% when isolated from house flies in slaughterhouses. so, this percentage is small comparing with that shown in the current study.

The results showed that the Shigella.spp bacteria came in the last rank in terms of contamination, with a percentage of (1.2)%. This bacteria is considered one of the dangerous types that affect human health, as this type of germ is transmitted to humans through their food, causing severe diarrhea, which is known as (Shigellosis).

Table (2) the types of negative and contaminated bacteria and their percentage of house fly samples from the sites included in the collection

Percentages of isolates from the total number of insects	**The percentage of isolation**	**number of isolates**	**bacterial type**
27.5	28	44	***E.coli***
20	20.3	32	***K.pneumonia***
18.7	19.1	30	***P.mirabilis***
12.5	12.7	20	***Salmonella.sPP***
9.3	9.5	15	***Providencia rettgeri***
5	5.1	8	***P. Vulgaris***
3.7	3.8	6	***C.freundii***
11.	1.2	2	***Shigella. Spp***
160	99.87	175	**Total**

Gram-negative bacteria was diagnosed based on the approved diagnostic methods in terms of the phenotypic examination of the bacterial isolate, which includes preparing swabs from the colonies and staining them with a Gram-stain to differentiate between the positive and negative type, which is the overall study, as well as observing their shape if they are spherical, sticky, color, texture, smell and arrangement if they are arranged singly, chain, pairs and clusters, as well as microscopic examination and biochemical tests. The diagnostic results of these tests and for some samples were also supported by the Vatic system, and the results were confirmed as shown in table (3).

Table (3) Biochemical tests that were used to diagnose Gram-negative bacteria.

Type of isolates								**Type of test**
Shigella . spp	***C. freundii***	***P.Vulgaris***	***Providencia rettgeri***	***Salmonella.sPP***	***p.mirabilis***	***K. pneumina***	***E. coli***	
Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	(Shape)
-	-	-	-	-	-	-	-	(Gram stain)
+	+	+	+	+	+	+	+	(Catalase)
-	-	-	-	-	-	-	-	(Oxidase)
+	-	+	+	-	-	-	**+**	(Indol test)
+	+	+	+	+	+	**-**	+	(Methyl red)
-	-	-	**-**	**-**	-	+	-	(Voges- proskauer)
-	+	V	+	+	V	+	-	(Citrate utilization)
-	+	+	+	-	+	**-**	+	(Motility)
K/A **-**	A/A +	K/A +	A/A -	K/A +	K/A +	A/A -	A/A -	(H_2S)
+	+	+	+	+	+	+	+	(Fermentation glucose)
-	+	-	-	**-**	-	+	**+**	(Lactose)
+	+	**-**	+	+	-	+	+	(Mannitol)
-	-	+	+	-	+	**-**	-	(Urase Production)

(+) the result is positive, (-) the result is negative, (v) the result is heterologous, (K) basic, (A) acidic.

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