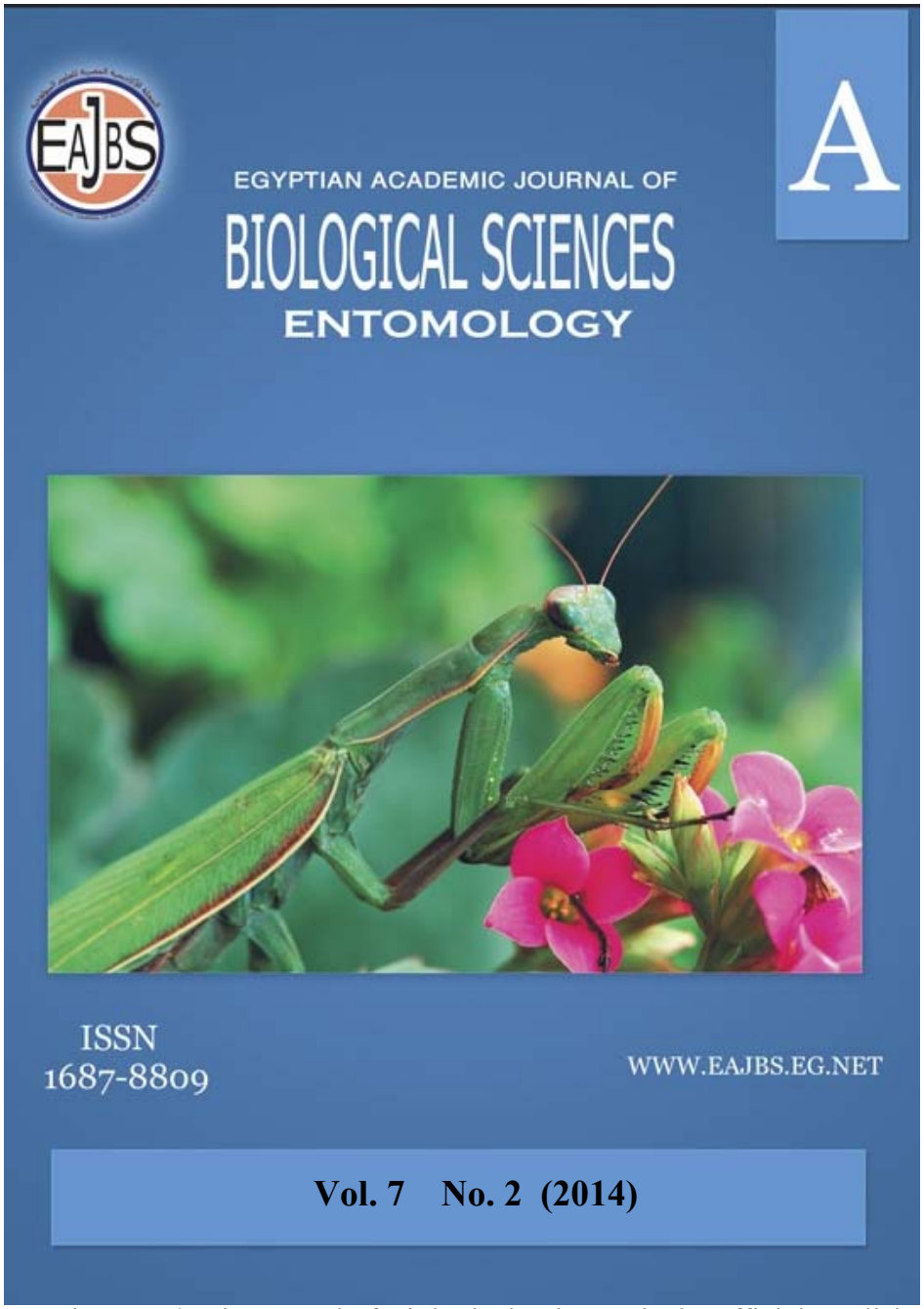

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Detection of Enteric Pathogenic Bacteria Transmitted by Housefly (*Musca domestica*) in Riyadh

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ABSTRACT

The house-fly, *Musca domestica L*., not only is a nuisance pest, but also acts as an important mechanical vector for lots of pathogenic microorganism agents, including: bacteria protozoa, worms, fungi and viruses amongst humans and animals. The aim of this study was to use Microbial methods for identification of bacteria that are pick up by house-fly over the human. In this study totally 200 houseflies were collected to isolate their bacteria from the centre, east, and west of Riyadh. The common isolated bacteria were *Escherichia coli* with 85 cases (42.5%). Were Enteric pathogenic bacteria *Helicobacter pylori* was 63cases (31.5%), and other isolated bacteria was *Staphylococci aureus*21 cases (10.5%), *Pseudomonas spp.* was 20 cases (10 %) and *Proteus spp*. Was 11 cases (5.5%). The results of the current study confirm that flies are much more than a nuisance and that they pose potentially serious health risks. Consequently, the population of houseflies has to be controlled.

Keywords: House fly *Musca domestica*, *Enteric Pathogenic Bacteria, Microbial identification.*

INTRODUCTION

The behavioral characteristics of the house fly *Musca domestica,* ensure its contact with food and wastes of man and his animals (Ahmad A, *et al*. (2007). In this manner the house flies are able to transport pathogenic organisms from infected materials to human. *Escherichia coli*, *Staphylococci aureus*, *Pseudomonas* and *Proteus spp.* are play role in diarrhea diseases in children (Ahmad A, *et al*. (2011). *Helicobacter pylori* are a major pathogenic factor in gastro duodenal disease, including chronic type B gastritis, duodenal ulcers, and gastric adenoid-carcinoma (Ahmad A, *et al*. (2011), Alam MJ & Zurek L (2004). *H. pylori* infection is one of the commonest chronic bacterial infections of humans and affects most populations throughout the world. However, the route by which individuals become infected remains speculative. The available studies suggest the fecal-oral or oral-oral route or a common environmental source as a possible mode of transmission (Ahmad A, *et al*. (2011), Cave. (1997). Houseflies (*Musca domestica*) frequently come into contact with human food and excrement and have been reported to be involved in the dissemination of numerous diseases such as cholera, shigellosis, and salmonellosis

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(Bessen, D.E and S. Lizano (2010), Brady CL *et al*. (2009), Broderick NA *et al*. (2004)). Since houseflies habitually produce and feed on excrement, it is possible that they act as vectors in the transmission of *H. pylori* if they carry the bacterium and contaminate human food (Grubel, P and D.R Cave (1997).

MATERIALS AND METHODS

Two hundred housefly samples (from the centre, east, and west of Riyadh houses) were disinfected in 5% phenol solution for 10 min to reduce bacterial contamination, washed in saline, dried, placed in well-ventilated aseptic buckets, and incubated at 25°C. Adult flies emerged within 3 to 4 days, were fed on a 1:1 mixture of autoclaved granulated sugar and powdered milk, and were kept on a 16-h photoperiod. A bowl filled with autoclaved wood chips and water served as a source of water. The houseflies were removed and divided into groups of approximately 100flies each. They were transferred to aseptic buckets each containing either one blood agar plate (5% [vol/vol]; Remel, Lenexa, Kans.), with freshly grown *H. pylori* or one sterile agar plate (control) and Maconky agar for isolation of enteric bacteria.

The *H. pylori* as well as control plates in the fly cages were removed after 6 h and were replaced with sterile Petri dishes containing one sterile drop of brucella broth (Difco, Detroit, Mich.), which served as a nutrition and water source for the flies as well as a medium for bacteria released from the flies during their feeding. At intervals of 6 h, the flies were an etherized by keeping them at 4°C for 5 min. Ten houseflies from each cage were removed and five houseflies each were transferred to sterile test tubes and into neutral buffered 10% formalin. To determine the viability of *H. pylori* under ambient conditions (room air and 20°C), the lids of some plates were also left open for 48 h. Samples were taken every 6 h and were inoculated on to the surface of brucella agar plates with lyses sheep blood and Skirrow's supplement, and the plates were incubated under microaerophilic conditions at 37°Cfor up to 5 days (Butler JF *et al*. (2010), Cave (1997)).

Isolation of bacteria from external surfaces of houseflies:

One milliliter of sterile saline (0.9%) was added to each test tube containing five flies, and the uses were thoroughly shaken for 2 min. A loop full of the washing was inoculated on to the surface of agar plates (Mac Conkey's medium, blood agar, and brucella agar with lyses sheep blood and Skirrow's supplement). The plates containing Mac Conkey's medium and blood agar were incubated aerobically and anaerobically 37°C for 24 h. The bacteria were identified by colonial morphology, Gram staining, and biochemical phenotype with an automated Micro scan Walka way system (Dade, Sacramento, Calif.). The selective brucella plates were incubated microaerophilically for 48 to 72 h and were regularly screened for small *H. pylori*-like colonies. Typical colonies were sub-cultured and were subsequently identified as *H. pylori* by their macroscopic morphology, phase-contrast microscopy, Gram staining, and urease, oxidase, and catalase activities as described previously (BouamamaaL, *et al*. (2010)).

Isolation of bacteria from alimentary tract of houseflies:

After external washing, the flies were washed in reagent alcohol for 5 min to decontaminate the external surfaces and were dried. The flies were then washed with sterile saline to remove traces of alcohol, and the alimentary tract was dissected out aseptically under a dissecting microscope. The excised gut was then homogenized in 1.0 ml of sterile saline. A loop full of the resulting homogenate was then processed as described above.

Isolation of bacteria from excreta:

The surface of the removed Petri dishes, which showed numerous fly droplets (vomits and feces) (Fig. 1), was streaked with a sterile cotton applicator and was transferred to agar plates, and the plates were processed as described above.

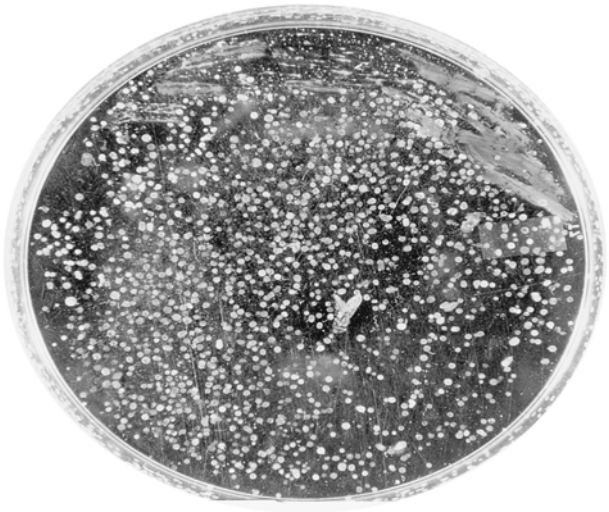

Fig. 1: Housefly sitting on a Petri dish that was left for 12 h in a fly cage containing approximately 100 flies. The surface of the plate is covered with abundant released white droplets of fly excrement.

RESULTS

Bacteria isolated from the house flies were from the feces, vomits, the external body surface and the internal gut content. Nutrient agar medium as well as Blood Agar medium gave the same types of bacterial growth hence the bacteria cultures were plated and maintained on Nutrient Agar media. The bacteria isolated from house flies as presented in Table 1 and Table 2, respectively.

Table 1: Characterisation of internal and external micro-organisms isolated from the house flies on nutrient agar.

Sample No	Gram stain	Shape	Motality	Catalase	Oxidase	Campulase	Urease	MethylRed	Voges Proskaeur	Starch hydrolysis	Oxygen relationship	Indole	Glucose	Lactose	Raffinose	Sucrose	Maltose	Xylose	Probable Identification
Internal (Gut)																			
Nutrient agar	+	S	-	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Streptococcus aureus*
	+	S	-	+	-	-	-	-	-	+	a	-	A	A	A	A	A	A	*Streptococcus pyogenes*
	+	S	+	-	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Bacilius cereus*
Internal (Gut)																			
Maconky	-	R	+	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Pseudomonas aeruginoso*
Nutrient agar	+	S	-	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Streptococcus pyogenes*
	+	R	+	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Bacillius cereus*
	+	R	+	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Bacillius cereus*
External (Body Surface)																			
Nutrient agar	+	S	-	-	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Streptococcus faecalis*
	+	R	+	+	-	-	-	-	-	+	a	-	A	A	A	A	A	A	*Bacillus cereus*
External (Body Surface)																			
Nutrient agar	+	S	-	-	-	-	-	-	-	+	a	A	A	A	A	A	A		*Streptococcus faecalis*
	+	R	+	-	-	-	-	-	-	+	a	A	A	A	A	A	A		*Bacillus cereus*

Key+ = Positive **-** = Negative **S** = Sphere
R = Rod **A** = Acid **G** = Gas
a = aerobic

Table 2: Biochemical and morphological characteristics of bacteria isolated from the maggots on blood agar.

Source of bacteria	Gram stain	Shape	Modility	Catalase	Coagulase	Oxidase	Methy red	Voges Proskaeur	Starch hydrolysis	Indole	Glucose	Sucrose	Lactose	Probable Identification
External (Body Surface)														
From tryptone broth to blood Agar	+	C	-	+	-	-	-	-	-	-	A	A	-	*Streptococcus* sp.
From Selenite broth to blood Agar	+	C	-	+	-	-	-	-	-	-	A	A	-	*Streptococcus* sp.
Internal (Gut)														
From tryptone broth to blood Agar	+	C	+	+	-	-	-	-	-	-	A	A	A	*Micrococcus* sp.
From Selenite broth to blood Agar	+	C	-	+	-	+	-	-	-	-	A	A	-	*Streptococcus* sp.

The bacteria identified to the genus level were *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp. and *Streptococcus* sp. There were a number of gram +ve *coccobacilli* isolates. Most of the bacteria isolated were gram positive with the exception of few gram negative. In some sites in Riyadh yeast cells were also isolated. From the total isolates, 41% were *Bacillus* sp. and 37.3% were *coccobacillus* isolates. The *Streptococcus* sp. was isolated from the external body surface of the house flies from the wastes collection area of some hospitals in Riyadh. The gram negative bacteria were generally from house flies in some food restaurant in Riyadh. The bacteria isolated from the external body surface of the house flies were *Acinetobacter*sp, *Bacillus* sp., *Enterobacter* sp., *Proteus*sp., *M. domestica* collected from various sites in some schools in Riyadh and isolated from the gut contents were *Bacillus* sp, *Enterobacter* sp., *Klebsiella* sp. and *Proteus* sp.

Isolation of *H. Pylori*.

Viable *H. pylori* could be isolated from the body washings for 12 h, from alimentary tracts for 30 h, and from the excreta droplets for up to 30 h (Table 3). After that point, despite the initial disinfection of the pupae and aseptic handling, other gram-negative bacteria from both gut and excreta overgrew the plates and eventually swamped the selective culture plates, making the isolation of *H. pylori* thereafter impossible.

Table 3: Demonstration of viability of *H. pylori* in houseflies[a]

Flies and sampling time (h)	Detection of *H. pylori*[b]			
	Body	Gut	Excreta	Agar plate
Test flies				
0	-	-	-	+
6	+	+	+	+
12	+	+	+	–
18	–	+	+	–
24	–	+	+	–
30	–	+	+	–
36 and more	–	–	–	–
Control flies				
0 – 30	——	—	—	
>30	——	—	—	

a One hundred flies were housed in cages. The flies were exposed to agar plates containing *H. pylori* or sterile agar plates (control) placed in the cage for 6 h and were then replaced with sterile Petri dishes. Six flies and the Petri dishes were removed from each cage at 6-h intervals and were examined for the presence of *H. pylori*. To determine the viability of *H. pylori* at ambient conditions *H. pylori* agar plates were simultaneously exposed to room air, and samples were taken at 6-h intervals for re-culture. *b* 2, negative; 1, positive; —, overgrowth of gram-negative bacteria, rendering isolation of *H. pylori* impossible.

The following bacteria were grown from test and control flies during the experimental period of 5 days: *Serratia marcescens, Providencia rettgeri*, and *Staphylococcus aureus* from the bodies, *Morganella morganii*, and *Pseudomonas aeruginosa* from the gut; and *S. marcescens*, *P. rettgeri, Providenci astuartii, S. aureus, M. morganii,* and *P. aeruginosa* from excreta. The bacterial colonization of both test and control flies showed increasing bacterial numbers with time. Samples taken from H. pylori plates exposed to room air could only be recaptured when they were transferred within 6 h to a microaerophilic environment. We were not able to re culture H. pylori from plates exposed to room air for 12 h or longer (Table 3).

DISCUSSION

These studies show for the first time that houseflies ingest *H. pylori* and can release the organisms as viable bacteria in excreta. In addition, houseflies can carry viable *H. pylori* on their external surfaces. In contrast to the fly gut, we could not passage *H. pylori* from plates left at room air for 12 h or longer, suggesting either that *H. pylori* is replicating within the fly's gut or that the gut is capable of functioning as a medium that preserves viability. The mechanism of transmission of *H. pylori* has been a subject of much debate. Epidemiological studies have suggested that the fecal-oral (Cave.1997) and oral-oral (Ahmad A *et al.* (2011)) routes likely represent the major pathways. The role of the housefly in the transmission of pathogens and gastrointestinal diseases such as shigellosis, salmonellosis, cholera, and yaws has already been firmly established (Broderick NA, *et al*. (2004). The vector role of houseflies is not a surprise if one considers their life cycle. The adult housefly deposits its eggs mainly on excrement, and its larvae feed on this material and the microorganisms in that material (Cave (1997)). The adult housefly can fly as far as 20miles from its source (Wang, Y.C *et al.* (2011)) and can freely enter houses and areas where people congregate, as well as markets, stores, and other places where human food is available. It just as freely frequents human and animal excrement alike. Since the fly can only swallow liquid food, it usually regurgitates ingested material in order to liquefy solid materials to facilitate digestion. In addition, droplets of feces may be deposited during the feeding process. This remarkable behavior of flies in which excreta is deposited (Fig. 1) may particularly contribute to their ability to spread bacterial infections. Structurally, the fly is well adapted for picking up pathogens. Its proboscis is provided with a profusion of fine hairs that readily collect envy- been isolated from the digestive tract of flies (Butler JF *et al.* (2010)). It has been reported that viable *H. pylori* organisms are released in feces from infected individuals (Grubel, P. and D.R Cave (1997)). Many patients develop a period of hypochlorhydria or achlorhydria shortly after the acute on set of infection with *H. pylori* (Vazirianzadeh, B.S *et al.* (2008)). By analogy, this suggests that during the hypochlorhydric stage of *H. pylori* infection, there may be enhanced excretion of viable bacteria in feces, thus providing an opportunity for flies to access viable organisms. Our data indicate that flies are able to ingest *H. pylori* during feeding on contaminated material. After *H. pylori* are swallowed by a fly, it faces a challenging environment. It passes through three distinct gut regions: the foregut, consisting of the mouth, pharynx, esophagus, and crop; the midgut, with stomach; and the hindgut, comprising the ileum, colon, rectum, and anus. *H. pylori* is exposed to substantial pH changes during passage through the midgut, as has been shown in insects of the order *Diptera*, the insect order to which houseflies belong (Behar A, *et al.* (2008)). The for midgut has a pH of 6.1, the mid-midgut has a pH of 3.1, and the hind midgut has a pH

of 6.8. The low pH in the mid-midgut of flies is extraordinary, since those insects are the only invertebrates to display such an acidic region in their alimentary tract (McGaughey, J and D. Nayduch (2009). Furthermore, oxyntic cells have been described in this mid-midgut region of houseflies. The oxyntic cells were morphologically similar to the parietal cells from the mammalian stomach (Vazirianzadeh, B.S *et al*. (2008). The low pH enhances the actions of pepsin and lysozyme, which are also found in the fly's mid-midgut (Behar A *et al.* (2008), Vazirianzadeh, B.S *et al.* (2008)), in order to lyses most of the ingested bacteria to be used as food. Since insects preceded humans on earth by at least 400 million years (Butler JF *et al.* (2010)), we speculate that *H. pylori* evolved its unique mechanisms of survival in acidic environments, such as the insect stomach, millions of years beforefinding a new ecological niche, the mammalian stomach. We postulate that *H. pylori* is acquired from human excrement by the housefly, which then, while crawling on human food, contaminates it with either regurgitated material and/or feces. The food, swallowed by a susceptible individual, then deposits *H. pylori* on the gastric mucosa, thus reestablishing infection. In the present study, houseflies were exposed to *H. pylori* under conditions that would not occur naturally. Although the initial bacterial load of these flies was decreased by washing the pupae in phenol solution, it has been shown that about 85% of such flies are still heavily colonized with bacteria at the moment of emergence from the pupil case (Brady CL, (2009), Broderick NA, (2004)). In the presence of these other bacteria, *H. pylori* were still able to colonize these flies. Although it remains to be determined if flies in their natural setting can transmit *H. pylori*, we suggest that they should be considered prime suspects for the transmission of *H. pylori*, particularly in warm and developing countries, where sanitary and domestic facilities are poor. This may explain the fact that *H. pylori* infection is almost universal among adults in developing countries, the prevalence already being 50% by age5 years (Butler JFm (2010)). The easy access for flies to outside toilets or open sewers, and hence untreated sewerage, and their ability to carry *H. pylori* could explain the high prevalence of infection in the developing world. The use of closed sewage systems would break the chain of transmission. (Yap, K.L *et al.* (2008)). The age of acquisition of infection is predominantly in childhood, and since infection is usually life long, the prevalence at a specific agree fleets childhood prevalence rates for that cohort. Our conclusions conflict with those of Grubel*et al.* (Grubel, P and D.R Cave (1997)) who suggested that *M. domestica* could be a vector for the spread of infection and also serve as a reservoir for *H. pylori*. The fact that we were unable to recover *H. pylori* from houseflies that were exposed to stool containing approximately 9 3107 CFU of *H. pylori* per g of stool indicates that even higher levels of viable organisms must be present in nature to ensure positive recovery from flies. Previous data have shown that such high levels of viable *H. pylori* may not be present in the extra gastric environment (Benson MJ *et al.* (2004, Vazirianzadeh, B.S *et al.* (2008)). Therefore, it appears unlikely that the domestic housefly is a vector for transmission of *H. pylori* infection or is an extra gastric reservoir of *H. pylori*. In communities with good provision for sewerage and waste disposal, flies should not, and cannot, be a health problem but the presence of flies would indicate sanitary deficiency and unhygienic condition. Possible breeding sites for flies should be eliminated and flies should be prevented from gaining access to contaminate human materials.

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ARABIC SUMMERY

الكشف عن البكتريا المعوية الممرضة Enteric Pathogenic Bacteria والتي تنتقل بواسطة الذبابة المنزلية (*House Fly(Musca Domestica*) في الرياض

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المملكة العربية السعودية

تلعب الحشرات دورا مهما في انتقال الكثير من الأمراض وخصوصا الأمراض المعوية والتي يتأثر بها كلا من الأطفال وكبار السن وأيضا المرضى الذين يعانون من أمراض نقص المناعة أو السرطان وكذلك الأمراض المزمنة. حيث أن الذبابة المنزلية ليست فقط مزعجة و لكنها تعمل كناقل ميكانيكي لكثير من الكائنات الدقيقة المسببة للأمراض و التي تشمل البكتيريا ، الأوليات ، الديدان، الفطريات، الفيروسات بين البشر و الحيوانات.لذلك كان الهدف من هذه الدراسة الكشف عن البكتريا المعوية الممرضة التي تُنقل بواسطة الذبابة المنزلية *Musca Domestica* للإنسان .حيث تم استخدام الطرق الميكروبية لتعريف البكتريا المعوية الممرضة وفي هذه الدراسة تم تجميع 200 عينة من الذبابة المنزلية من شرق و غرب و وسط مدينة الرياض لعزل البكتريا منها ،حيث تم عزل أنواع متعددة من البكتريا المعوية. حيث تم عزل بكتريا *Escherichia coli* من 85 ذبابة بنسبة 42.5% . بينما عزلت البكتريا المعوية الممرضة والتى تسمى بالبكتريا الحلزونية Enteric pathogenic *Helicobacter pylori* من 63 ذبابة بنسبة.31.5%.
و هناك أنواع بكتيريا أخرى معزولة و هي *Stophylococci aureus* من 21 ذبابة بنسبة 10.5% ، *Pseudomanas sp*من 20 ذبابة بنسبة. 10% بينما.*Proteus spp* تم عزلها من 11 ذبابة بنسبة .5.5%. و تشير نتائج هذه الدراسة أن الذبابة المنزلية *Musca Domestica* ليست مزعجة فقط و لكنها تمثل خطرا على الصحة العامة و بالتالي يجب التحكم في أعدادها.