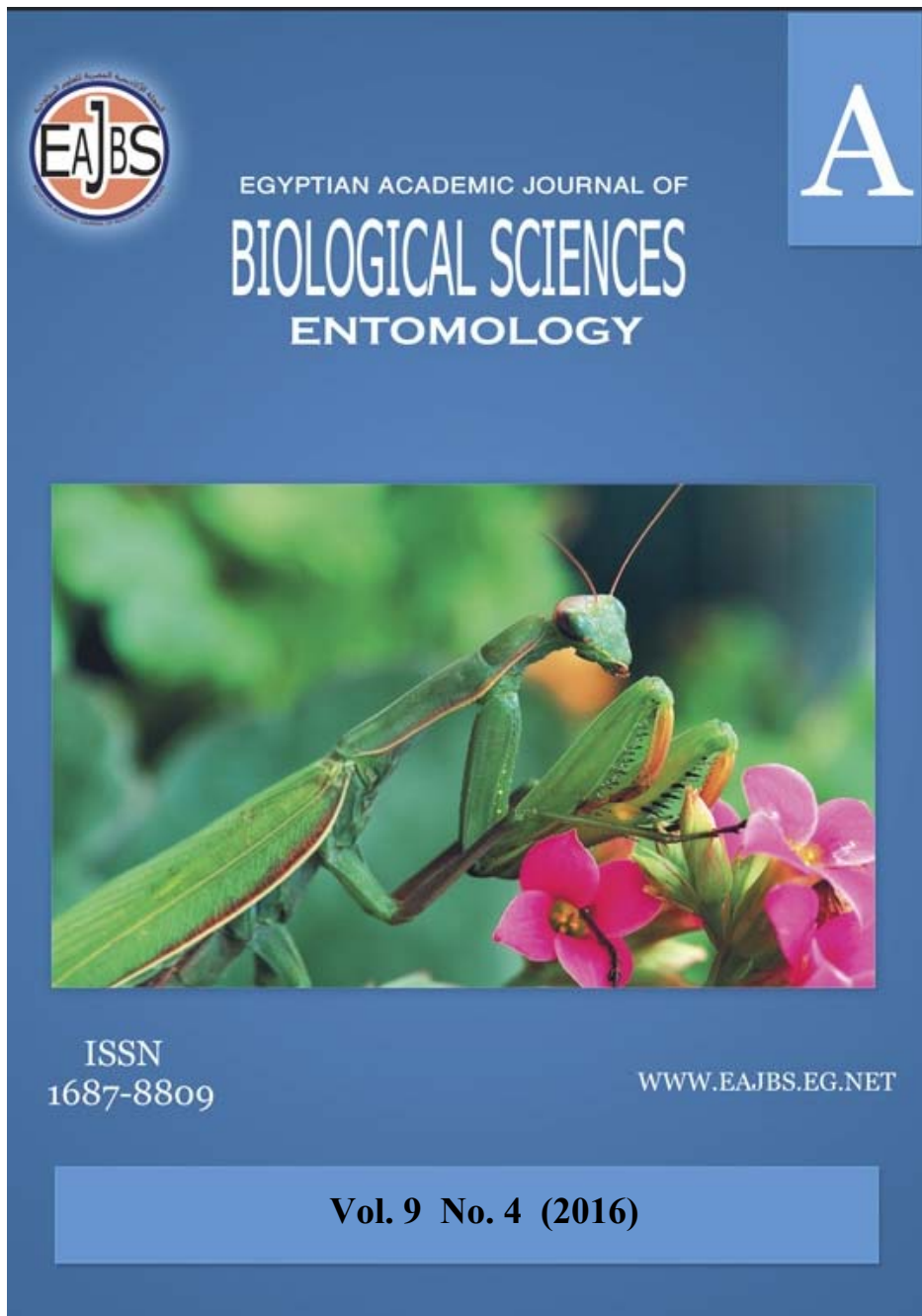


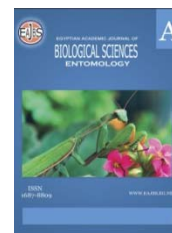
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Isolation and Identification of Bacterial Species Associated with Non- Biting Flies in Egypt

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ABSTRACT

Thirteen different species of bacteria were isolated and identified from the external body surface of *Musca domestica* collected from carrions and garbage dumps at Al Mansoureyah, Giza, Egypt. The identified bacteria species were season and habitat dependent. The most frequent bacteria isolated from house flies collected from carrion or garbage in all seasons were *Staphylococcus* spp. and *Esherichia coli*. The total count of bacteria species was higher in summer season than in other seasons. Also, this count was higher on flies collected from carrion than on flies collected from garbage dumps. *Chrysomya albiceps* flies carried (11) different bacterial species on their external body surface all over the four seasons. Bacteria species were season and habitat dependent. The most frequent bacteria isolated from *C. albiceps* flies collected from carrion or garbage dumps in all seasons were *Staph.*spp. . The highest total bacterial count was recorded during spring season for bacteria isolated from *Chrysomya* flies collected from carrion. Four different bacterial species were identified on the external body surface of *Sarcophaga carnaria* in autumn 2014. The most frequent bacteria species isolated were *Staphylococcus* spp. Three different bacterial species were identified on the external body surface of *Lucilia sericata* in autumn 2014. The most frequent bacteria species was *Staph.* spp.. In general, 36 bacterial isolates from the different flies tested were identified. Bacteria species isolated from the external body surface of *C. albiceps*, *S. carnaria* and *L. sericata* were identified for the first time. The highest number of bacteria species (13) were isolated from *M. domestica* followed by (11) species from *C. albiceps*.

INTRODUCTION

Non-biting flies, particularly the common house fly (*Musca domestica*), have sporadically been described as possible mechanical vectors of nosocomial infections because they may carry human pathogens (Faulde *et al.*, 2001 and Nmorsi *et al.*, 2007). Domestic filth flies (families; Sarcophagidae (flesh flies), Muscidae (house flies) and Calliphoridae (blow flies and bottle flies) have evolved to live in close association with man (Synanthropic flies) as annoying pestiferous scavengers (Greenberg, 1971 and 1973). Filth flies breed in animal manure and human excrement, garbage, animal bedding and decaying organic matter (Greenberg, 1973). Transmission of human pathogens by adult flies occurs via A) mechanical dislodgment from the exoskeleton; B) fecal deposition; C) regurgitation (Greenberg, 1973).

Non-biting flies such as house flies, flesh flies and blow flies, which develop in decaying organic materials, may transmit antibiotic resistance bacteria from the manure of animals and other decaying organic substrates to residential setting. The habitats in which they develop, their dependence on a live microbial community, their feeding mechanism (regurgitation), their attraction to human food and their ability to fly long distance make these flies a very good candidate for dissemination of fecal bacteria, including human and animal pathogens (Graczyk, *et al.*, 2001, Zurek *et al.*, 2000; Alam and Zurek, 2004).

There have been no studies on the carriage of pathogenic bacteria by flesh flies and blow flies in Egypt. This study is considered the first report of bacterial isolation and identification from *Sarcophaga carnaria* and *Chrysomya albiceps* in Egypt.

MATERIALS AND METHODS

Collection of non- biting flies:

House flies and other non-biting flies were captured by a nylon net from different breeding media such as garbage, decaying organic matters, manure and carrions of different animals (e.g. horse, donkey, dog) at Al Mansoureyia, Giza Governorate, Egypt. The collection of flies was carried out during the different seasons of year, 2014-2015; at 5/10 / 2014, 5 / 1 / 2015, 5 / 4 / 2015, 5/7/ 2015, were collected the *Sarcophaga carnaria* and *Lucilia sericata* in autumn 2014, while *Musca domestica* and *Chrysomya albiceps* collected in all seasons. Flies were caught from the selective habitats during the study period from 10 a.m. To 2 p.m. when flies are active. The collected flies were placed into sterile container, and flies were transferred immediately to Entomology laboratory at the Department of Zoology and Entomology, Faculty of Science, Al-Azhar University (Cairo), Egypt. Then fly tubes were placed in freezer for killing. The flies were identified to species level.

After identification, 1 ml of sterile physiological saline solution was added to each vial, which was shaken vigorously for 1 min. with the fly remaining inside. The fly was then removed from the saline, and was checked for bacteria dislodged from the external surfaces of the fly.

The averages of ambient temperature and relative humidity in the four seasons of the study in Giza Governorate (from autumn, 2014 to Summer, 2015) were obtained monthly from meteorological station of Kobri El-Kobba in Cairo, Egypt. The flies collected were identified as; *Musca domestica*, *Chrysomya albiceps*, *Sarcophaga carnaria* and *Lucilia sericata*.

Bacterial isolation and identification.

Different bacterial species from the external body surface of non-biting flies were isolated by using the normal isolation technique. Bacterial colonies presenting morphological differences were picked and streak on new blood agar plate's. The cultures were then observed daily for growth and all bacteria colonies subculture on to corresponding media and further incubated until pure colonies were obtained. The bacteria were identified to the genus level by morphological, physiological, biochemical test according to Bergy's manual of systematic bacteriology 2005, 2009 and confirmed this identification by using Biomerieux Vitek2 System.

Bacterial Count.

Serial dilutions of a subsample of each bacterial suspension were prepared in sterile saline. Each dilution was then inoculated onto two plates of plate-count agar and incubated overnight at 37 °C. Colony forming units (CFU/ ml) were then counted so that the total numbers of bacteria recovered from the external surface of each fly

could be estimated. Briefly, Non-biting flies individually were shaken thoroughly in sterile saline solution (2 ml) for 2 min. The suspension was then serially diluted and inoculated on plate count agar. Plates were incubated for 24 h at 37 °C (Koneman *et al.* 1992).

RESULTS

Bacterial strains isolated from the external body surface of:

Musca domestica

The bacterial strains isolated from the external body surface of *Musca domestica* adults collected from different habitats in the different seasons, and their total counts are represented in Table (1).

The show from the data given in Table (1) the adults of *Musca domestica* collected from carrion and garbage dumps at Al Mansoureira were found to carry five species of bacteria in autumn 2014 (average temp. 20.3 and average R.H. 56.3). The flies collected from carrion carried the Gram-negative bacteria; *Pseudomonas fluorescens* and Gram - positive bacteria; *Aerococcus viridans* which total count of 195×10^{-2} C.F.U./ml , while the bacterial species isolated from flies collected from the garbage dumps were Gram-positive bacteria . These bacterial species were identified as; *Kocuria rosea*, *Staphylococcus lentus* and *Micrococcus lentus / laylae* with total count of 185×10^{-2} C.F.U./ml.

Table 1: Bacterial species isolated from the external body surface of *Musca domestica* collected from various habitats during the different season.

Season	N. of Flies tested	Habitat of flies	Total count CFU / ml	Bacterial identified
Autumn 2014 Average Temp. (20.3) Average R.H. (56.3)	3	Carrion	195×10^{-2}	<i>Aerococcus viridans</i> <i>Pseudomonas fluorescens</i>
		Garbage dumps	185×10^{-2}	<i>Kocuria rosea</i> <i>Staphylococcus lentus</i> <i>Micrococcus lentus/laylae</i>
Winter 2015 Average Temp. (15.6) Average R.H. (50.4)	3	Carrion	149.3×10^{-3}	<i>Escherichia coli</i> <i>Enterobacter aerogenes</i>
		Garbage dumps	167.3×10^{-3}	<i>Staphylococcus sciuri</i> <i>Staphylococcus lentus</i>
Spring 2015 Average Temp. (24) Average R.H. (46.7)	3	Carrion	125×10^{-2}	<i>Staphylococcus sciuri</i> <i>Staphylococcus simulans</i>
		Garbage dumps	65×10^{-2}	<i>Leuconostoc pseudomesentroides</i>
Summer 2015 Average Temp. (29.3) Average R.H. (51.6)	3	Carrion	207.3×10^{-2}	<i>Lactobacillus delbrueckii</i> <i>Pseudomonas veronii</i>
		Garbage dumps	178×10^{-2}	<i>Kocuria kristinae</i> <i>Escherichia coli</i>

In winter 2015 (average temp. 15.6 and R.H. 50.4) four species of bacteria were isolated from the external body surface of flies; two Gram – negative bacteria ; namely *Escherichia coli* and *Enterobacter aerogenes* from flies collected from carrion with total count of 149.3×10^{-3} C.F.U./ml, while the other two bacterial species were Gram-positive bacteria namely; *Staphylococcus sciuri* and *Staph. lentus* and were isolated from flies collected from garbage dumps with total count of 167.3×10^{-3} C.F.U./ml .

In spring 2015 (average temp. 24 and R. H. 46.7) three species of bacteria were isolated from flies collected from carrion and garbage dumps; two Gram – positive species namely; *Staph. sciuri* and *Staph. simulans* with total count of 125×10^{-2} C.F.U./ml , while only one species namely , *Leuconostoc sp.* (Gram-positive) with total count of 65×10^{-2} C.F.U./ml .

In summer, 2015 (average temp. 29.3 and average R.H. 51.6) four species of bacteria were isolated from the external surface of flies ; two bacterial species, Gram – positive and gram - negative bacteria namely ; *Lactobacillus delbrueckii* and *Pseudomonas veronii* respectively, with total count of 207.3×10^{-2} C.F.U./ml from flies collected from carrion. The other two species were identified as *Kocuria kristinae* (Gram – positive) and *E.coli* (Gram – negative) with total count of 178×10^{-2} C.F.U./ml from flies collected from garbage dumps.

From the aforementioned results it is appeared that this study showed that 13 species of bacteria were isolated from the external body surface of *M. domestica* (Table 1).

The bacteria isolated from flies collected from different habitats (Carrion and garbage dumps) at Al Mansoureyia in the different seasons are represented in Table (1). As shown from this table, the bacteria species were season and habitat dependent.

The most frequent bacteria isolated from house flies collected from carrion or garbage dumps in all seasons were *Staphylococcus sp.*, and *Esherichia coli*. Also, the results recorded an increase of total count for bacterial isolates from flies during summer season. Moreover, the total bacterial count on flies collected from carrion was higher than that of bacteria isolated from flies collected from garbage dumps.

Chrysomya albiceps

The bacterial strains from the external body surface of *Chrysomyaalbiceps* collected from different habitats in the different seasons and their total counts are represented in Table (2).

As shown from data given in Table (2) the adult of *Chrysomya albiceps* collected from carrion at Al Mansoureyia was found to carry two species of bacteria in autumn 2014 (average temp. 20.3 and R.H. 56.3). These two species were identified as Gram-positive bacilli namely; *Erysipelothrix rhusiopathiae* and Gram-negative; *Esherichia coli* with total count of 184.3×10^{-2} C.F.U./ml.

In winter 2015 (average temp. 15.6 and R.H. 50.4) three species were isolated from *Chrysomya* collected from carrion. These bacteria species were identified as: the Gram-negative, *Klebsiella pneumonia*, the Gram-positive; *Kocuria rosea* and *Staphylococcus lentus*. The two bacterial species; *K. pneumonia* (Gram-negative) and *Enterobacter aerogenosa* (Gram-negative) were identified on *Chrysomya* collected from garbage dumps.

In spring 2015 (average temp. 24 and R.H. 46.7) two Gram-positive bacteria were isolated from flies collected from carrion; these two species were identified as *Staphylococcus haemolyticus* and *Staph. sciuri* with total count of 711×10^{-2} C.F.U./ml . Also, the Gram – positive, *Streptococcus thoraltensis* with total count of 164×10^{-2} was isolated from flies collected from garbage dumps.

In summer, 2015 two bacterial species (one gram negative and one gram positive) were isolated from flies collected from carrion. These two species were identified as *Pseudomonas fluorescens* and *Enterococcus faecium* respectively, with total count of 215×10^2 C.F.U./ml.

From the aforementioned results it is appeared that the *C. albiceps* carries 11 different species of bacteria and the external body surface all over the four seasons.

As shown from Table (2), the isolated bacteria species were season and habitat dependent.

Table 2: Bacterial species isolated from the external body surface of *Chrysomya albiceps* collected from various habitats during the different seasons.

Season	N. of flies tested	Habitat of flies	Total count CFU / ml	bacteria identified
Autumn 2014 Average Temp. (20.3) Average R.H. (56.3)	3	Carrion	184.3×10^2	<i>Erysipelothrix rhusiopathiae</i> <i>Escherichia coli</i>
Winter 2015 Average Temp. (15.6)	3	Carrion	114×10^3	<i>Klebsiella pneumonia</i> <i>Kocuria rosea</i> <i>Staphylococcus lentus</i>
Average R.H. (50.4)	3	Garbage dump	190.3×10^3	<i>Klebsiella pneumoniae ssp pneumoniae</i> <i>Enterobacter aerogens</i>
Spring 2015 Average Temp. (24) Average R.H. (46.7)	3	Carrion	711×10^2	<i>Staphylococcus haemolyticus</i> <i>Staphylococcus sciuri</i>
	3	Garbage dump	164×10^2	<i>Streptococcus thoraltensis</i> <i>Streptococcus thoraltensis</i>
Summer 2015 Average Temp. (29.3) Average R.H. (51.6)	3	Carrion	215×10^2	<i>Pseudomonas fluorescens</i> <i>Enterococcus faecium</i>

The most frequent bacteria isolated from *Chrysomya* collected from carrion or garbage dumps in all seasons were *Staphylococcus spp.*

The highest total bacterial count was recorded during the spring season for bacteria isolated from *Chrysomya* collected from carrion.

Sarcophaga carnaria

The bacterial strains isolated from the external body surface of *Sarcophagacarnaria* adults collected from a carrion at Al Mansoureyia during autumn 2014 (average temp. 20.3 and R.H. 56.3) and their total count are represented in Table (3).

As shown from data given in the table, the adults of *S. carnaria* collected from carrion were found to four species of bacteria namely; *Corynebacterium glutanicum*, *Morganella morganii*, *Staphylococcus sciuri* and *Staphylococcus lentus* with total count of 182.6×10^2 C.F.U./ml.

The most frequent bacteria species isolated were *Staphylococcus spp.*

Lucilia sericata

Table 3: Bacterial species isolated from the external body surface of *Sarcophaga carnaria* collected from various habitats during the different seasons.

Season	Number of flies tested	Habitat of flies	Total count CFU / ml	bacteria identified
Autumn 2014 Average Temp. (20.3) Average R.H. (56.3)	3	Carrion	182.6x 10 ⁻²	<i>Corynebacterium glutanicum</i> <i>Morganella morganii ssp morgani</i> <i>Staphylococcus scuri</i> <i>Staphylococcus lentus</i>

The bacterial strains isolated from the external body surface of *Lucilia sericata* adults collected from a carrion at Al Mansoureya during autumn 2014 (average temp. 20.3 and R.H. 56.3) and their total count are given in Table (4).

As shown from data given in the table three different gram-positive bacterial species were identified namely; *Erysipelothrix rhusiopathiae*, *Staphylococcus oxylosus* and *Staphylococcus epidermidis* with total count of 115 x 10⁻² .

The most frequent bacteria species was *Staphylococcus ssp.*

Table 4: Bacterial species isolated from the external body surface of *Lucilia sericata* collected from various habitats during the different seasons .

Season	Number of flies tested	Habitat of flies	Total count CFU / ml	Bacteria identified
Autumn 2014 Average Temp. (20.3) Average R.H. (56.3)	3	Carrion	115 x 10 ⁻²	<i>Erysipelothrix rhusiopathiae</i> <i>Staphylococcus oxylosus</i> <i>Staphylococcus epidermidis</i>

DISCUSSION

Mechanical transmission of various pathogenic agents such as bacteria by house fly, *Musca domestica* has been confirmed (Crazyk *et al.*, 2001 and Thaddens *et al.*, 2005). The biology and ecology of *M. domestica* make it an ideal mechanical vector of human and animal pathogens .Due to highly anthropophagic behavior of this cosmopolitan species, wide variety of habitats must be investigated for fly pathogenic inoculations where the close relations with flies and human are easily possible. Decaying organic materials, cattle barns, poultry houses, slaughter houses and hospitals are sites where house flies can reproduce (peter *et al.*, 2007). However, little information about bacteria carried by other non-biting flies such as *Chrysomya*, *Sarcophaga* and *Lucilia* flies. These flies reproduce on carrion and flesh.

Insects such as house flies that develop in decaying organic materials may transmit antibiotic-resistant bacteria from manure of animals and other decaying organic substances to residential setting.

This study is the first report of bacterial infection of *Chrysomya*, *Sarcophaga* and *Lucilia* flies.

Bacterial species isolated from the external body surface of non-biting flies:***Musca domestica***

The present study showed that 13 bacterial species of bacteria were isolated from the external body surface of *M. domestica* collected from dumps and carrion during the four seasons of the year. The most frequent bacteria were *Staphylococcus spp.* and *Esherichia coli*. The isolated bacterial species were season and habitat dependent. The total count of all bacterial species on house flies was increased in summer season and on flies collected from carrion. These results are in accordance with other reports which deal with the importance of house flies in carrying various pathogens (Koura and Kamel, 1990; Gurbel *et al.*, 1997; Kobayashi *et al.*, 1999; Pai *et al.*, 2003). Ahmed *et al.* (2013) isolated 6 species of bacteria from *M. domestica* collected from garbage dumps, cattle barns, poultry houses, slaughter houses and hospitals in Iraq. The most frequent bacterial species were *Staph. spp.*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella spp.* and *Klebsiella ssp.*

The results of this study indicated that *M. domestica* could play a great role as mechanical carrier of pathogenic bacteria such as *E. coli*, *Staph. ssp.* These results are in harmony with these obtained by Moosa- Kazemi *et al.* (2010) and Ahmed *et al.* (2013).

The increase of total bacterial count on the external body surface of *M. domestica* in summer is attributed to hot weather which prepares suitable conditions for house fly activities.

Chrysomya albiceps

The present study identified 11 bacterial species from the external body surface of *Chrysomya* flies during the four seasons of year .Also, it showed that the isolated species were season and habitat dependent. The most frequent bacteria isolated from *Chrysomya* collected from carrion or dumps were *Staphylococcus spp.* The highest total bacterial count was recorded during season, this may attributed to high activity and reproductively in spring season.

Sarcophaga carnaria

This study revealed and identified 4 bacteria species from the external body surface of *S. carnaria* flies. Also, the most frequent bacteria species was *staph. ssp.*

Lucilia sericata

The present study identified three different bacteria species on the external body surface of *Lucilia* flies collected from carrion. The most frequent bacteria species was also *Staph. ssp.*

CONCLUSION**The present study revealed that:**

- 1- Non-biting flies namely; *Musca domestica*, *Chrysomya albiceps*, *Sarcophaga carnaria* and *Lucilia sericata* collected from decaying organic materials and carrions carry various pathogenic bacterial species on their external body surface.
- 2- Bacterial species which isolated from the external body surface of *Chrysomya*, *Sarcophaga* and *Lucilia* flies were identified for the first time.

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ARABIC SUMMERY

عزل و تعريف أنواع البكتريا المصاحبة للذباب غير الماص للدم فى مصر

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تم عزل ثلاثة عشر أنواع مختلفة من البكتريا والتعرف عليها من سطح الجسم الخارجى للذباب المنزلية المجمع من الجيفة و أكوام الزبالة فى المنصورية - جيزة - مصر. وقد اعتمد نوع البكتريا التى تم تعريفها على الموسم وأيضا على البيئة التى جمعت منها . كانت أكثر أنواع البكتريا شيوعا المعزولة من الذباب ، وقد *Staphylococcus Sp., E. coli* المجمع من أكوام الزبالة هى الـ كان العدد الكلى لأنواع البكتريا فى فصل الصيف أعلى منه فى المواسم الأخرى ، كما كان أيضا هذا العدد أعلى فى البكتريا المجمع من الجيف منه على أكوام الزبالة.

كما وجد أن الذباب الزرقاء تحمل احدى عشر نوعا من البكتريا على السطح الخارجى للجسم فى الاربع مواسم و قد وجد أيضا أن هذه الانواع تعتمد على الموسم والبيئة التى جمعت منها. و قد كانت البكتريا الأكثر شيوعا المعزولة من *Staph. SP.* الذباب الزرقاء المجمع من الجيف أو أكوام الزبالة كانت هى الـ و كان أعلى تعداد لأنواع البكتريا المعزولة من هذا النوع من الذباب فى موسم الربيع و كان من الذباب المجمع من الجيف . أيضا تم عزل أربعة أنواع مختلفة من البكتريا من السطح الخارجى لجسم ذبابة اللحم فى خريف - ٢٠١٤ . أيضا تم جمع ثلاثة أنواع فقط من السطح *Lucilia sericata* الخارجى لجسم ذبابة الـ *Staph. Sp.* فى خريف - ٢٠١٤ ، وقد كانت البكتريا الأكثر شيوعا هى الـ عموما تم عزل ستة وثلاثون نوع من البكتريا من الذباب المختلف الذى تم دراسته. و قد كانت أنواع البكتريا المعزولة من السطح الخارجى لاجسام : قد تم عزلها والتعرف *C. albiceps, S. carnaria and L. sericata* لأول مرة فى مصر. وقد دلت نتائج البحث على أن أكثر من البكتريا (١٣) قد تم عزله من الذباب المنزلية يليه عدد (١١) نوع من البكتريا تم عزلها من الذبابة *C. albiceps* الزرقاء