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Frequency (Occurrence) and seasonal variation of bacterial species on the external surface of non-biting flies in Egypt

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Abstract

The feeding and reproductive habits of non-biting synathropic flies such as house flies and blow flies make them important mechanical vectors of pathogenic bacteria to human. The aim of this study was to isolate and identify bacteria found in natural association with adults of ***Musac domestica***, ***Chrysomya albiceps, Sarcophaga carnaria*** and ***Lucilia sericata*** collected from different habitats (garbage and carrion) in different seasons of the year. The results indicated that 8 bacterial species were isolated from the external surface of ***M. domestica*** collected from carrion, while 7 bacterial species were isolated from flies collected from garbage: 5 bacterial species were found in autumn-2014, 4 bacterial species were found in winter season, 2015, 3 species were found in spring- 2015 and 4 species were found in summer-2015. Most of bacteria species isolated from the external surface of ***C. albiceps*** were found on flies collected from carrion; 2 , 4 , 3 and 2 bacterial species were found on flies tested in autumn-2014, winter, spring and summer-2015; respectively. The bacteria species; ***Staphylococcus lentus*** and ***Staphylococcus sciuri*** had the highest frequency on the flies tested. Four different bacterial species were identified on the external body surface of ***S. 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 ***L. sericata*** in autumn 2014; the most frequent bacteria species was ***Staphylococcus*** spp.

Keywords: Bacterial strains, Frequency, Non-biting flies, Seasonal variation.

1. Introduction

Mechanical transmission of various pathogenic agents such as bacteria by house fly, ***Musca domestica***, has been confirmed (**Crazyk *et al.*, 2001** and **Thaddeus *et al.* ,2005**). The biology and ecology of ***M. domestica*** mak it an ideal mechanical vector of human and animal pathogens. Due to highly anthropophagic behavior of this cosmopolitan species, wide variety of habitat investigated for fly pathogenic inoculations where the close relations with flies and human are easily possible. Decaying organic materials, cattle borns, poultry houses, slaughter houses and hospitals are sites where house flies can reproduce (**Peter *et al.* , 2007**). However, few information about other non-biting flies such Chrysomya, Sarcophaga and Lucilia flies in pathogens transmission is known. These non-biting flies reproduce on carrions and flesh. Insects such house flies ***Musca domestica, Chrysomya albiceps, Sarcophaga carnaria*** and ***Lucilia sericata*** that develop in decaying organic material may transmit antibiotic-resistant bacteria from manure of animals and other decaying organic substances to residential setting.

Studies of bacterial communities associated with insects have been of great interest to many researchers. Insects provide an important source of bacterial diversity and a potentially ideal model for studying bacteria-host interactions (**Wei *et al.*, 2014**)

This study is the first report of bacterial infection of ***Chrysomya albiceps, Sarcophaga carnaria*** and ***Lucilia sericata*** in Egypt , and it has been conducted with the aim of isolating and identifying bacteria on the surface of ***Musca domestica, Chrysomya albiceps, Sarcophaga carnaria*** and ***Lucilia sericata*** collected from different habitats in different seasons of the year to unstained the relationship between bacteria species, fly host and season.

2. Materials and Methods

1-Collection of non- biting flies:

House flies and other non – biting flies were captured by a naylon net from different breeding media such as garbage, decaying organic matters, manure and carrions of different animals (e.g. horse, donkey, dog) at Al Mansoureya, 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. 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 -Cairo, Egypt. The flies collected were identified as; ***Musca domestica, Chrysomya albiceps, Sarcophaga carnaria*** **and** ***Lucilia sericata***.

2-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.

3. Results

1-Frequency (occurrence) of bacteria species on the external body surface of non-biting flies:

Data given in Table (**1**) indicate the occurrence of the different bacterial species on the external body surface of the non-biting flies namely ; ***Musca domestica*** , ***Chrysomya albiceps*** , ***Sarcophaga carnaria*** and ***Lucilia sericata*** collected from different habitats (carrion or garbage dumps through the different seasons of the year 2014 –2015 .

AS shown from the table each fly was characterized by carrying special bacterial species. In general it is appeared that the bacterial species namely; ***Staphylococcus lentus*** and ***Staph. sciuri*** had the highest frequency on the flies tested, where they occurred frequently on the external body surface of ***M. domestica*** , ***C. albiceps*** and ***S. carnaria*** . This was followed by the bacteria species namely; ***Pseudomonas fluorescence***, ***Kocuria rosea***, ***Esherichia coli*** and ***Enterobacter aerogenes*** which were carried by ***M. domestica*** and ***C. albiceps*** and ***Morganella morganii*** which was carried by ***M. domestica*** and ***S. carnaria*** .

The bacteria species ***Erysipelothrix rhusiopathiae*** was isolated only from the blow flies; ***C. albiceps*** and ***L. sericata***.

From the aforementioned results it is appeared that flies, ***M. domestica*** , ***C. albiceps*** and ***S. carnaria*** have the biggest ability to transmit different bacterial species.

Table (1): Frequency (occurrence) of bacterial species on the external body surface of non-biting flies:

Number	*M. domestica*	*C.albiceps*	*S. carnaria*	*L. sericata*
Aerococcus viridans	+	_	_	_
Pseudomonas fluorescens	+	+	_	_
Kocuria rosea	+	+	_	_
Staphylococcus lentus	++	+	+	_
Micrococcus lentus/lylae	+	_	_	_
Lactobacillus delbrueckii	+	_	_	_
Escherichia coli	++	+	_	_
Enterobacter aerogenes	+	+	_	_
Staphylococcus sciuri	++	+	+	_
Staphylococcus simulans	+	_	_	_
Leuconostoc pseudomesentroides	+	_	_	_
Pseudomonas veronii	+	_	_	_
Kocuria kristinae	+	_	_	_
Morganella morganii	_	_	+	_
Erysipelothrix rhusiopathiae	_	+	_	+
Klebsiella pneumonia ssp pneumniae	_	++	_	_
Staphylococcus haemolyticus	_	+	_	_
Streptococcus thoraltensis	_	++	_	_
Enterococcus faecium	_	+	_	_
Corynebacterium glutanicum	_	_	+	_
Erysipelothrix rhusiopathiae	_	_	_	+
Staphylococcus oxylosus	_	_	_	+
Staphylococcus epidermidis	_	_	_	+

+ one organism occurrence, ++ two organisms occurrence, - No organism occurrence.

2-Seasonal variation of bacterial species isolated from the external body surface of non-biting flies:

2.1- *Musca domestica*

Data given and illustrated in Table (2) indicate the different bacteria species isolated from ***M. domestica*** collected from garbage or carrion during different seasons of the year 2014-2015.

As shown from the results Fig. (1), each bacteria species was found in special breading site and during definite seasons of year. For example the bacterial species; ***Aerococcus viridans, Pseudomonas fluorescens, Escherichia coli, Enterobacter aerogenes , Staphylococcus sciuri , Staphylococcus simulans, Lactobacillus delbruecki*** and ***Pseudomonas veronii*** were isolated and identified from flies

collected from carrion. While, ***Kocuria rosea, Staphylococcus lentus, Micrococcus lentus/lylae , Staphylococcus sciuri, Escherichia coli, Leuconostoc pseudomesentroides, Kocuria kristinae*** were isolated and identified from flies collected from garbage. ***A.viridus, Pseudomonas fluorescens, K. rosea, Staph. lentus*** and ***M. lentus*** bacteria were found during autumn, 2014. ***Staph. lentus***, ***E. coli***, ***Enterobacter aerogenes*** and ***Staph. sciuri*** were found during winter season, 2015. ***Staph. sciuri*** , ***Staph. simulans*** and ***Leuconostoc pseudomesentroides*** were found during Spring, 2015. ***E. coli***, ***L. delbrueckii, Pseudomonas veronni*** and ***Klebsiella kristinae*** were found during summer, 2015.

Table (2): Seasonal variation of bacterial strains isolated from the external surface of *M.domestica* adult collected from carrion ■ and garbag □.

Number	Autumn 2014	Winter 2015	Spring 2015	Summer 2015
Aerococcus viridans	■	–	–	–
Pseudomonas fluorescens	■	–	–	–
Kocuria rosea	□	–	–	–
Staphylococcus lentus	□	□	–	–
Micrococcus lentus/lylae	□	–	–	–
Escherichia coli	–	■	–	□
Enterobacter aerogenes	–	■	–	–
Staphylococcus sciuri	–	□	■	–
Staphylococcus simulans	–	–	■	–
Leuconostoc pseudomesentroides	–	–	□	–
Lactobacillus delbrueckii	–	–	–	■
Pseudomonas veronii	–	–	–	■
Kocuria kritinae	–	–	–	□

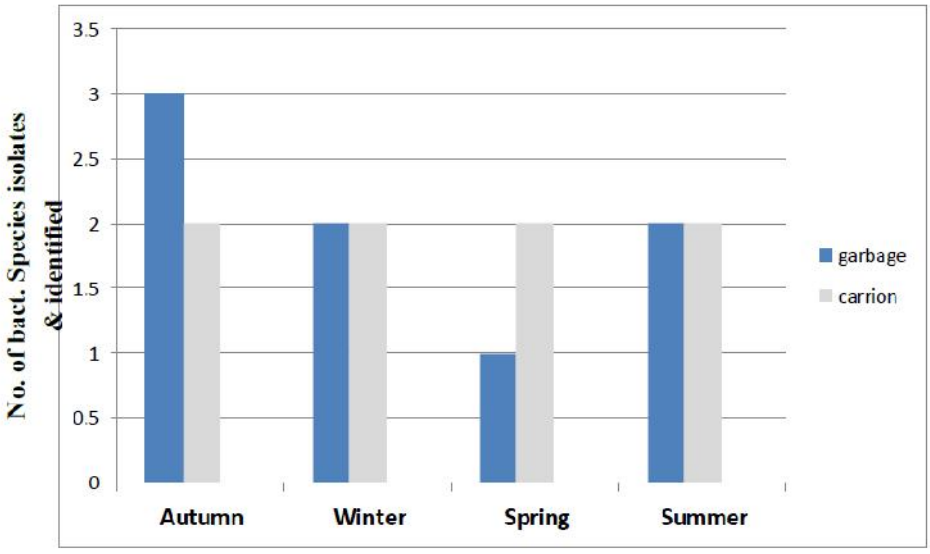

Fig. (1) : Number of bacterial species isolated from *M. domestica* collected from garbage and carrion in different seasons.

2.2- *Chrysomya albiceps*

Data given and illustrated in Table (3) indicate the different bacterial species isolated from ***C. albiceps*** collected from garbage or carrion during different seasons of the year 2014-2015.

As shown from the results Fig. (**2**), the majority of bacterial species were isolated from flies collected from carrion except ***Klebsiella pneumonia***, ***Enterobacter aerogenes*** and ***Streptococcus thoraltensis*** were isolated from flies collected from garbage.

Table (3): Seasonal variation of bacterial strains isolated from the external surface of *C. albiceps* adult collected from carrion ■ and garbage □ .

Bacteria Sp.	Autumn 2014	Winter 2015	Spring 2015	Summer 2015
Erysipelothrix rhusiopathiae	■	–	–	–
Escherichia coli	■			
Klebsiella pneumonia ssp pneumniae	–	■□	–	–
Kocuria rosea	–	■	–	–
Staphylococcus lentus	–	■	–	–
Enterobacter aerogenes	–	□	–	–
Staphylococcus haemolyticus	–	–	■	–
Staphylococcus sciuri	–	–	■	–
Streptococcus thoraltensis	–	–	□	–
Pseudomonas fluorescens				■
Enterococcus faecium	–	–	–	■

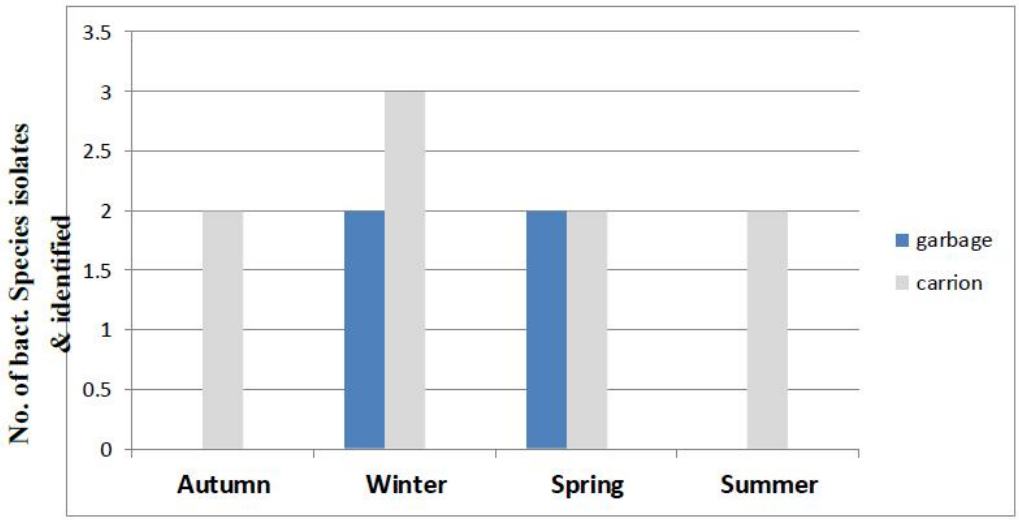

Fig. (2): Number of bacterial species isolated from *Chrysomya albiceps* collected from garbage and carrion in different seasons.

Bacteria, ***Erysipelothrix rhusiopathiae*** and ***E. coli*** were found on flies during autumn, 2014. ***Klebsiella pneumonia*** , ***Kocuria rosea***, ***Staph.lentus*** and ***Enterobacter aerogens*** were isolated from flies during winter, 2015. ***Staph. haemolyticus, Staph. sciuri*** and ***Streptococcus thoraltensis*** were isolated during spring, 2015. ***Pseudomonas fluorescens*** and ***Enterococcus faecium*** were found on flies during summer, 2015.

From the aforementioned results it is obvious that the isolated bacterial species from ***M. domestica*** and ***C. albiceps*** flies are season of year dependent. Also, the type of these species is fly host and habitat of host dependent.

4. Discussion

In the present study bacterial isolates from the external body surface of the non-biting flies namely; ***Musca domestica, Chrysomya albiceps***, ***Sarcophaga carnaria*** and ***Lucilia sericata*** collected from different habitats have been done for the first time. The results showed that the bacterial species; ***Staphylococcus lentus*** and ***Staph. sciuri*** had the highest frequency on the flies tested. They occurred on the external body surface of ***M. domestica***, ***C. albiceps*** and ***S. carnaria***; followed by the bacteria species namely; ***Pseudomonas fluorescens , Kocuria rosea , E. coli and Enterobacter aerogens*** which were isolated from ***M. domestica , C. albiceps*** . ***Morganella morganii*** was isolated only from ***M. domestica*** and ***S. carnaria***. The bacteria species ***Erysipelothrix rhusiopathiae*** was isolated only from the blow flies; ***C. albiceps*** and ***L. sericata***. The great number of pathogenic bacteria that isolated from ***M. domestica*** in this study was accordance with those of **Ugbogu *et al.*** (**2006**), where house flies are probably the most important nuisance insect pest and mechanical vector of pathogens.

Most of frequent bacteria isolated from the tested flies were medically important including ***Staphylococcus lentus, Staph. sciuri , Pseudomonas*** **sp.,** ***Kociuria resoae, E. coli*** **and** ***Enterobacter aerogens***. These findings are in accordance with **Babak *et al.* (2008)**, **Moosa-Kazemi *et al.* (2010)** and **Ahmed et al. (2013)**, who isolated and identified these bacterial species from the external surface of ***M. domestica.***

Regarding the effect of season on bacterial communities associated with flies tested, the present study has shown that climatic conditions of the different season played a role in shaping fly – associated bacterial communities, where definite bacterial species were carried by each fly in each season. This finding was in agreement with **Wei *et al.* (2014)** working on green bottle flies. For example ***Staphylococcus spp.*** was isolated from ***M. domestica*** and ***C. albiceps*** in spring. This bacteria species were also isolated from other insects in spring **(Corby – Harries *et al.* 2007 ; Osei –Poku *et al.* 2012 and Wei *et al.* 2014).**

The present study suggests that flies; ***M. domestica , C. albiceps*** and ***S. carnaria*** are the most capable non-biting flies tested to transmit mechanically different pathogenic bacteria to human and animal.

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