

Isolation and Identification of Bacterial Isolates from House Flies in Sulaymaniya City

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Received on: 6/2/2012 & Accepted on: 24/6/2012

ABSTRACT

The objective of this study was to isolate and identify bacteria found in natural association with adults of *Musca domestica*. In this study adults of *Musca domestica* were collected from four sites in Sulamani city, Kudistan, Iraq to isolate and identify bacteria on the external surface of house flies . Bacteria from fly samples isolated using the conventional isolation technique were *E.coli* , *Pseudomonas aeruginosa* , *Staphylococcus albus* , *Staphylococcus aureus* , *Salmonella* sp. and *Klebsiella* sp. The results of current study confirm that the houseflies are much more than nuisance and they pose potentially serious health risks. Consequently, the population of houseflies has to be controlled.

Key Words: *Musca domestica* , Vector, bacteria, antibiotic ,resistance.

عزل وتشخيص عزلات بكتيرية من ذباب المنزل في مدينة السليمانية

الخلاصة

تهدف الدراسة الى عزل وتشخيص البكتيريا الموجودة في تداخل طبيعي مع الحشرة اليافعة *Musca domestica* . في هذه الدراسة تم جمع الحشرات اليافعة لـ *Musca domestica* من اربعة مواقع في مدينة السليمانية ، كردستان العراق لغرض عزل وتشخيص البكتيريا من السطح الخارجي لذباب المنزل . تم عزل البكتيريا من عينات الحشرات باستخدام تقنية العزل التقليدية وهي *E .coli* , *Pseudomonas aeruginosa* , *Staphylococcus albus* , *Staphylococcus aureus* , *Salmonella* sp. and *Klebsiella* sp. . اكدت نتائج الدراسة الحالية ان ذباب المنزل اكثر خطورة على الصحة من الحشرات خارج المنزل . والنتيجة نجد من الضروري السيطرة على تجمعات حشرة الذباب .

INTRODUCTION

The house fly, *Musca domestica* L. is well-known cosmopolitan pest. It has a worldwide distribution and is found throughout the country in close association with human activities. It receives the common name of house fly by virtue of being the most common fly found in and around houses in addition to being a nuisance pest [1]. It is a vector of many pathogenesis carrier of over 100 different pathogenic organisms including organisms for diseases, typhoid, cholera, bacillary dysentery, ophthalmia neonatorum and infantile diarrhea as well as parasitic worms [2,3].

House flies *Musca domestica* are of medical and veterinary importance due to their capacity to act as mechanical vectors of microorganisms originating in animal manure and other decaying organic substrates [4]. However, its classification as a "disease causing fly" as follows (1) its confirmed association with food-borne pathogenus *Escherichia coli*, *Salmonella*, and *Shigella*, (2) the fact that it is ecologically associated with humans (synanthropic), (3) its association with domestic environments (endophilic), (4) its equal attraction to excrements and human food sources, and (5) its municative behavior that allows the house fly to easily move from heavily contaminated to human populated areas [5].

Houseflies *Musca domestica* have been shown to be carriers of several species of bacteria; this is because of their close association with decaying organic matters, garbage and faeces [6]. The hairy proboscis and feet with glandular hairs and pads that secrete sticky material enable the flies to pick up the pathogens on to their bodies. In addition the regurgitation of vomits and deposit of faecal droplets during feeding process contribute to the flies' ability to spread the pathogens [7,8]. The common house fly is a medically-important insect worldwide [9,10,11]. House flies have been implicated as vectors or transporters of various human pathogenus, including *Vibrio cholerae*, *Entrobacteriaceae* pathogenus, *Staphylococcus aureus*, and *Pseudomonas* spp. [5, 9, and 12]. Transmission takes place when the fly makes contact with people or their food. As many as 500000 microorganisms may swarm over its body and legs [13]. A number of researchers have studied and isolated pathogenus, such as *Vibrio cholera* [9], *Escherichia coli* 0157:H7 [2], *Salmonella* and *Compylobacter* [14, 15, and 16] from house flies and reported them as a potential source for transmission and spread of these pathogenus. While feeding or resting, house flies often defecate, leaving fly specks and organisms passing through their digestive system [2]. This a simple mechanical transfer of microbes by vector whose behavior places the contaminants from decayed and diseased source they visit [6]. According to [17], flies can contaminate clean surfaces with approximately 0.1mg of food per landing. The organisms associated with house flies number in the hundreds and commonly include dysentery-causing and tissue-infecting agents such as *Bacillus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Shigella* spp., *Esherichia coli*, *Bacillus anthracis*, *Chlamydiales*, *Corynebacterium* spp., and other parasitic organisms [5,18]. Synanthropic flies are major epidemiologic factors responsible for spread of acute gastroenteritis, trachoma among infants and young children in developing countries

and transmission of nosocomial infections with multiple antibiotic-resistance bacteria in hospitals environment [10].

Insects, such as houseflies, that develop in decaying organic material may transmit antibiotic-resistance bacteria from the manure of animals and other decaying organic substrates to residential setting. The habitats in which it develops (e.g., manure), its dependence on a live microbial community, its feeding mechanism (regurgitation), its attraction to human food, and its ability to fly long distance make this insect a very good candidate for dissemination of fecal bacteria, including human and animal pathogens [10,19,20].

In the present study we collected house flies from residential area of four locations in Sulamani city and isolated human pathogenic bacteria from the external surfaces of this flies. Afterward, we determined the susceptibility of the bacterial strains to different antibiotics.

MATERIAL AND METHODS

Collection of flies

The study was confined to the city of Sulamani. House –flies were collected in batches of 10 flies each from four different places (i.e. slaughter houses, garbage dumps, restaurant dumps, hospital dumps). Flies were caught from the selective sites during the period of study with sterilized nets and from 9:00 a.m to 1:0 p.m., when the flies are active. The collected flies were placed in to sterile 150 ml container, and flies were transferred immediately to bacteriological laboratory.

BACTERIAL ISOLATION

Culture Media

The Following media were used in culturing the bacteria, Cetrimide agar, Manitol salt agar, Chocolate agar, MacConky agar, EMB agar, API-20E system.

Isolation of bacteria from flies

Bacteria from fly sample collections were isolated using the normal isolation technique. The flies were anesthetized by keeping them at 4C° for 5 minute [21]. Each flies transferred individually with sterile tweezers to agar plate, flies were kept alive on plates where they moved over the surface while feeding, walking. After 1 hour at room temperature, the flies were removed and the plates were incubated at 37C° for 24 hours [6]. 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 [7]. The bacteria were identified to the genus level by colony morphology, texture and Gram staining and species level for some of them by using API-20E system. Antibiotic susceptibility test was performed and the following antibiotics were used in this study: Tetracycline, Erythromycin, Ciprofloxacin, Gentamycin, Ampicillin, Amoxicillin, Penicillin, Nalidixic acid, Ferodantin, Amikacin, Rifadin [10].

Results

From the 40 house flies collected from slaughter house, garbage dumps, hospital dumps, and restaurant dumps at Sulamani city, house flies were found to carry several species of bacteria on the external surface. This study showed that six species of bacteria were isolated from *Musca domestica*. The bacteria isolated from these sites as represented in Table (1).

The most frequent bacteria isolated from houseflies coming from all locations of the city were *Pseudomonas*, *Staph. aureus*, *Staph. albus*, and *E.coli* while the least present bacteria were *Klebsiella sp.* and *Salmonella sp.*(Table - 1). A total 40 house flies collected from slaughter houses, garbage dumps, restaurant dumps, hospital dumps. In general, flies may transport agents of diseases on and within their bodies. In this work, only the exterior (external surface) transmission of microorganism was studied. As mechanical vectors, this fly transferred microorganism by means of their leg or/and mouth parts. This was also reported from many other authors [15]. All flies investigated transmitted more than microorganisms were identified and demonstrated in Table (1). Figures (1, 2), show the primary screening of isolates from flies on selective media. Antibiotic resistance were tested for six bacteria cultures obtained from house flies to determine their disc sensitivity or resistance to 11 commonly used antibiotics Table (2). Among all the isolates from slaughter house *E.coli* and *Pseudomonas* resistance to Tetracycline, Erythromycin, Gentamycin, Ampicillin, Amoxicillin, Penicillin, Nalidixic acid, Ferodantin, and Rifadin, and they were sensitive to Amikacin, Ciprofloxacin respectively, while *Staph aureus* resistance to Erythromycin, Penicillin, Nalidixic acid, Ferodantin, Rifadin and sensitive to Tetracycline, Cipromycine, Gentamycin, Ampicillin, Amoxicillin, Amikacin.

The bacteria which were isolated from garbage dump, *E.coli* was resistance to Tetracycline, Amoxicillin, Penicillin, Ferodantin, Rifadin, and sensitive to Cipromycine, Ampicillin, Ciprofloxacin, Gentamycin, Nalidixic acid, Nalidixic acid. *Staph albus* resistance to all antibiotics and sensitive to Amikacin, *Staph.*

Aureus showed resistance to Erythromycin, Gentamycin, Ampicillin, Amoxicillin, Penicillin, Ferodantin, Rifadin and sensitive to Tetracycline, Ciprofloxacin, Nalidixic acid, Amikacin). Moreover the *Klebsiella* was resistance to the most antibiotics Erythromycin, Gentamycin, Ampicillin, Amoxicillin, Penicillin, Ferodantin, Amikacin, Rifadin and sensitive to Tetracycline, Ciprofloxacin, Nalidixic acid. All the bacteria that isolated from hospital dump were resistance to all antibiotics except *E.coli* sensitive only to Ciprofloxacin on other hand all isolated bacteria from restaurant dump show totally resistance to all antibiotics.



Figure (1): Adult house fly individually placed on agar plate for 1 hour.



Figure (2): Bacterial colonies obtained from house fly.

Table (1): Bacteria isolated from external body surface of *M. domestica* collected from various sites in Sulaymaniya, Kurdistan, Iraq.

Collection sites	Means of transmission	Type of bacterial isolated and identified
Slaughter house	External surfaces	E.coli ; Pusedomonas ; Staphylococcus aureus
Garbage dump	External surfaces	E.coli ; Staph. albus ; Staph..aureus ; Klebseilla sp.
Hospital dump	External surfaces	E.coli ; Pseudomons ; Staph. aureus ; Staph. albus ; Salmonella sp .
Restaurant dump	External surfaces	E-coli 2- Pseudomonas ; Staph auerus ; Staph. albus

Table (2): Antibiotic susceptibility tests of identified bacteria from slaughter house, garbage dumps, restaurant dump, hospital dump at Sulaymaniya city, Kurdistan, Iraq.

Collection Sites	Bacterial isolates	Types of Antibiotic*											
		Tet	Ery	Cip	Gen	Amp	Amd	Pen	NA	F	AK	RF	
Slaughter house	<i>E.coli</i>	R	R	R	R	R	R	R	R	R	R	S	R
	<i>Pseudo monas</i>	R	R	S	R	R	R	R	R	R	R	R	R
	<i>Staph. aureus</i>	S	R	S	S	S	S	R	R	R	R	S	R
Garbage dump	<i>E.coli</i>	R	R	S	S	R	R	R	S	R	S	R	R
	<i>Staph. albus</i>	R	R	R	R	R	R	R	R	R	S	R	R
	<i>Staph .aureus</i>	S	R	S	R	R	R	R	S	R	S	R	R
	<i>Klebsie lla</i>	S	R	S	R	R	R	R	S	R	R	R	R
Hospital dump	<i>E. coli</i>	R	R	S	R	R	R	R	R	R	R	R	R
	<i>Pseudo monas</i>	R	R	R	R	R	R	R	R	R	R	R	R
	<i>Staph albs</i>	R	R	R	R	R	R	R	R	R	R	R	R
	<i>Staph aureus</i>	R	R	R	R	R	R	R	R	R	R	R	R
	<i>Salmon ella</i>	R	R	R	R	R	R	R	R	R	R	R	R
Restaurant dump	<i>E.coli</i>	R	R	R	R	R	R	R	R	R	R	R	R
	<i>Psedo monas</i>	R	R	R	R	R	R	R	R	R	R	R	R
	<i>Staph. albus</i>	R	R	R	R	R	R	R	R	R	R	R	R
	<i>Staph .aureus</i>	R	R	R	R	R	R	R	R	R	R	R	R

*Tetracycline, Erythromycin, Ciprofloxacin, Gentamycin, Ampicillin, Amoxicillin, Penicillin, Nalidixic acid, Ferodantin, Amikacin, Rifadin. (S) = Sensitive, R=Resistance

DISCUSSION

The biology and ecology of *Musca domestica* make it an ideal mechanical vector of human and animal pathogens. Garbage, cattle barns, poultry houses, slaughter houses, and hospitals are sites where house flies can reproduce [22]. This study showed that six species of bacteria were isolated from *Musca domestica* (Table - 1). Our results are in accordance with other reports which highlight the importance of house flies in carrying various pathogens [23, 24, 25, 26].

Many scientists indicated that the external organs of *Musca domestica* (legs, wings, and mouth parts) constituted a large source of bacteria they isolated [24, 27]. The results of this study indicated that *Musca domestica* could play a great role as a mechanical carrier of bacteria in this study, most of the bacteria isolated were medically important, including *E. coli*, *Staph. aureus*, *Staph. albus*, *Pseudomonas aeruginosa*, *Klebsiella*, and *Salmonella*. These findings agree with the results of [28, 29] which presence *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Proteus mirabilis* on the external surface of house fly collected from slaughterhouse, zoo, and hospitals. There are more studies which confirm the role of house flies on transmission of different bacteria as a world wide agent transmission of bacteria is using different insect species and different methods of transmission. [30] isolated *Salmonella enteritidis*, *S. infantis* and *S. heidelberg* from house flies over poultry houses. 18 species of enteropathogenic bacteria from different cyclorrhaphan flies were isolated in Malaysia [31]. The isolates of *E. coli*, *Staphylococcus aureus*, *Pseudomonas* spp., *Proteus* spp., in Ahvaz city [29]. This confirms that the housefly's body can act as a mechanical vector either bacteria or many microorganisms. We examined the antibiotic susceptibility and resistance patterns of six bacteria obtained from the houseflies by using 11 commonly used antibiotics (Table 2). The types of bacteria in general were resistance to all antibiotics. These findings are in agreement with the findings of other studies which show that the enter bacteria *Salmonella*, *E. coli*, *Klebsiella*, *Shigella* isolated from house flies collected in hospital were found to be resistant to significantly more of the commonly used antibiotics that were tested than the Enterobacteria isolated from the flies caught in the streets, and *Pseudomonas* isolates frequently showed resistance to multiple antibiotics [32]. Multiple resistances to antibiotics are common among *P. aeruginosa* isolated from different clinical sources in Iran [33]. Insects such as house flies *Musca domestica* L. that develop in decaying organic material may transmit antibiotic-resistant bacteria from manure of animals and other decaying organic substrates to residential settings [10, 19, 20]. Multidrug resistance in clinical isolates has become a serious problem due to a progressive decline in the number of antibiotics that are effective for treatment of human infections [34]. It has been suggested that there is a connection between the antibiotic resistance of food animal origin, the antibiotic resistance of clinical isolates, and community health [35, 36, 37]. However, this remains a controversial issue [38, 39] because the ecology of antibiotic resistance and virulence genes in the environment is not well understood. The present study indicates that the house fly *Musca domestica* poses a possible health risk to communities proved that the isolated strains of bacteria were resistance to various antibiotics. In

conclusion, we report that house flies collected in slaughter house garbage dump, hospital dump, and restaurant dump may be involved in the spread of antibiotic resistant bacteria and may increase the potential for human exposure to antibiotic resistant bacteria. It recommended that suitable steps must be taken to control the flies.

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