Original Article

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Microbiology Section

Bacterial Contamination of Adult House Flies (*Musca domestica*) and Sensitivity of these Bacteria to Various Antibiotics, Captured from Hamadan City, Iran

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ABSTRACT

Introduction: House flies (*Musca domestica*) have been known as a mechanical vector in spreading infectious diseases such as cholera, shigellosis, salmonellosis and skin infections.

Aim: To investigate the bacterial contaminations of house flies and determine the resistance of these bacteria against various antibiotics.

Materials and Methods: An analytical descriptive cross-sectional study was conducted. The study was performed from July to September 2015 in Hamadan University of Medical Sciences, Hamadan, Iran. A total number of 300 house flies were collected from four places, 75 flies from each place, including two educational hospitals belonging to Hamadan University of Medical Sciences, a fruit and vegetables center, and a livestock slaughter. The body surface of house flies was washed using the physiological sterile serum and the obtained solution was centrifuged at 2000 rpm for five minutes. The identification of bacteria was carried out using the phenotypic methods. The resistance of bacteria against various antibiotics was determined using the disk diffusion approach. Data were analysed by the employment of SPSS software package version 20.0.

Results: A total number of 394 bacterial strains were isolated from 275 house flies. The most prevalent type of bacteria was *Bacillus* spp which was detected in 31.1% of house flies. Moreover, *Staphylococcus* spp. (22.9%), *Escherichia coli* (11.6%) were other prevalent species, whereas, *Enterococcus* spp. was the least prevalent type of bacteria in the collected house flies. In terms of resistance to antibiotics, it was identified that bacteria extracted from house flies which were collected from hospital environments were more resistant to antibiotics compared with the resistance of bacteria extracted from house flies which were collected from non hospital environments. The maximum bacterial isolation was found in houseflies from hospital No.1 environment from around the accumulation of garbage.

Conclusion: It is a well-known fact that house flies are a source of bacterial contamination and can act as a mechanical carrier and cause bacterial diseases. It can be postulated that house flies play a major role in spreading antibiotic resistant bacteria. However, the flies from hospital environments were more contaminated, mainly because the people referring to health center are normally ill and carrier of many pathogens. Further, hospital environments should be controlled using administrative procedures.

Keywords: Kirby bauer, *Staphylococcus aureus*, Tetracycline

INTRODUCTION

House fly, with the scientific name of *Musca domestica* Linnaeus, belongs to the Diptera order and Muscidae family [1] and has been known as an important medical insect all around the world [2]. These are day active insect species normally found around surroundings of human. House flies are able to transport various pathogens from one place to another, posing humans to the risk of various diseases [3,4]. Some of microorganisms living in the body or on the body surface of these flies can remain viable as long as 35 days [5,6].

House flies are able to transport pathogenic agents by attaching them to their mouth, body surface, foot, wings and so on [7]. Many Diptera play a significant role in the transmission of bacteria and parasites and can harbor many different species of pathogenic microorganisms and is known to play a role in the epidemiology of many infectious diseases [8]. Moreover, they always are in a direct contact with the sewage and garbage, hence, it is possible for pathogenic agents to be transported by house flies from such polluted areas to the places where humans live [9]. House flies have been recognized by the US Food and Drug Administration (FDA) as a major agent in spreading diseases such as cholera, shigellosis, and salmonellosis [10].

The most important microorganisms that can be carried by house flies includes intestinal infections (such as dysentery, diarrhea, typhoid, cholera and specific helminthic infections), eye infection (such as trachoma and conjunctivitis), certain skin infections (such as yaws, cutaneous diphtheria, some fungal infections and leprosy), as well as polio [11]. Besides, it has been recently identified that house flies can act as a potential carrier of the bird flu virus, a serious threat to humans health, livestock, and livestock industry all around the world [12]. Moreover, a significant correlation has been reported between the prevalence of gastrointestinal diseases, such as diarrhea, and a seasonal increase in population of house flies, which can be prevented by controlling the population of such flies using different approaches [13,14].

In this regard, several studies have demonstrated that house flies are a carrier of *Salmonella* spp (the cause of typhoid, food poisoning, and diarrhea) from slaughter houses to the fruit and food markets as well as residential areas [15,16]. According to the studies so far carried out in various countries, the use of effective measures for controlling the population of house flies would reduce the prevalence of gastrointestinal symptoms such as diarrhea, and eye infections such as trachoma [17].

One of the most important obstacles that the world faces in fighting against microorganisms is that they are increasingly becoming resistant to antibiotics that are available in the markets. The resistance of various bacteria to antibiotics is encoded by various genes which also are able to transfer between bacterial horizontally [18,19]. House flies can mechanically transport antibiotic resistant bacteria from hospital environments to non hospital area and create serious problems for residents in non hospital area.

According to the aforementioned issues, the present study was set to investigate the types and prevalence of bacteria living on the body surface of house flies from various hospital and non-hospital environments in Hamadan, Iran. In addition, the resistance of the identified bacteria against some commercially available antibiotics were studied.

MATERIALS AND METHODS

Collecting House Flies

This is an analytical descriptive cross-sectional study. The study was conducted from July 2015 to September 2015 in Hamadan, Iran. A total number of 300 house flies were collected from accumulated of garbage from four places, 75 flies from each place including; hospital No.1, hospital No.2, a fruit and vegetables center, and a livestock slaughter.

The flies were captured using a sterile sweeping net and immediately shipped to the microbiology laboratory, located in the medical faculty of Hamadan University of Medical Sciences, in separate special glass containers. In the next step, using a stereo-microscope the morphological characteristics of various body parts of flies, including antenna, vein, arista hair, and forehead furrows, were assessed in order for determining the identity of the captured flies and those which belonged to *Musca domestica* species were included in the study [20].

Extraction of Bacteria

For extracting the bacteria, the body surface of the flies was washed with the sterile physiological serum. In the next step, the solutions obtained from the previous step were centrifuged at 2000 rpm for five minutes. Then, the sediments remained at the bottom of the centrifuge tubes were cultured on the various mediums such as Eosin Methylene Blue (EMB) and blood agar (Merck, Germany), incubated in 37°C for 24 hours. In the next step, for each colony, all differentiation tests were conducted to specify the type of each bacteria. For identification of Gram-negative bacteria Gram stain, fermentation of sugars, motility test, Triple Sugar Iron (TSI) (Merck), Sulfide Indole Motility (SIM) (Merck), and Simmon citrate (Merck) tests were applied. For identification of Gram-positive bacteria, Gram stain, catalase test, oxidase test, fermentation of sugars (mannitol), and sensitivity to antibiotics were employed [6].

Antibiogram

The resistance of bacterial strains isolated from house flies was investigated using the Kirby-Bauer disk diffusion method. A control strain of *Pseudomonas aeruginosa* ATCC 27853 was used for quality control of susceptibility testing [21]. A suspension with turbidity equal to 0.5 McFarland standards was provided for each bacterial strain. Then, the suspensions were placed in Mueller-Hinton agar medium and exposed to the different disks of antibiotics and incubated for 24 hours at 37°C. It is worth mentioning that all antibiotic disks were purchased from Mast Group Ltd., (England) and all included antibiotic disks were erythromycin (15 µg), ampicillin (30 µg), tetracycline (30 µg), kanamycin (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (75/23 -25/1 µg), gentamycin (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), and cefotaxime (30 µg), for Gram negative bacteria and erythromycin (15 µg), ampicillin (30 µg), tetracycline (30 µg), kanamycin (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (75/23 -25/1 µg), gentamycin (30 µg), ciprofloxacin (5 µg), cefalotin (30 µg), and cefotaxime (30 µg) for Gram positive bacteria. After this period of time, the diameter of growth inhibition zone of each sample was measured and categorized in three distinct groups of sensitive, intermediate, and resistant based on guidelines recommended by CLSI (M100-S23. 2014) [22]. Data were analysed using SPSS software package developed by IBM cooperation.

RESULTS

The Captured House Flies and Extracted Bacterial Strains

A total number of 300 house flies were collected from four places. From these flies, 25 flies (included 2 flies in hospitals and 23 flies in the fruit and vegetables centers and livestock slaughter) were free of any bacteria and 394 bacteria were isolated from 275 flies. The frequencies of each bacterial strain in various places are summarized in [Table/Fig-1]. In overall, *Bacillus* spp. and *Staphylococcus* spp. were the most prevalent types of bacteria observed in various places, constituting 31.1% and 22.9% of all identified strains, respectively. In contrast, *Enterococcus* spp. was the least prevalent type of bacteria only found in hospital No.2. No *Salmonella and Shigella* spp was isolated in the present study.

In overall, the flies significantly differed in respect to bacterial contamination. The frequency of bacteria were higher in flies captured from hospital environments; totally 394 bacterial strains were isolated from 275 house flies (148 flies in hospitals and 127 flies in the fruit and vegetables centers and livestock slaughter). Out of 394 isolates, 231 strains isolated in hospitals and 163 strains isolated from the fruit and vegetables centers and livestock slaughter. In the hospitals, in 76 flies-one type of bacteria, in 62 flies-two types of bacteria, in 9 flies-three types of bacteria, and in 1 flies-four types of bacteria were isolated. In the fruit and vegetables centers and livestock slaughter, in 91 flies-one type and in 36 flies-two types of bacteria were isolated.

STATISTICAL ANALYSIS

Statistical analysis revealed that there was a significant relationship between the level of bacterial contamination and the type of places where these bacteria were collected (p-value<0.05).

Bacteria	Place				Total No (%)
	Hospital No. 1	Hospital No. 2	Slaughter	Fruit center	
E. coli	13	11	13	9	46 (11.6)
Klebsiella spp.	7	25	5	0	37 (9.3)
Citrobacter spp.	8	3	0	0	11 (2.7)
Enterobacter spp.	17	0	0	0	17 (4.3)
Staphylococcus aureus	15	10	0	0	25 (6.3)
CoNS	6	0	43	16	65 (16.6)
Bacillus spp.	40	29	20	33	122 (31.1)
Proteus spp.	9	8	20	4	41 (10.3)
Pseudomonas spp	21	7	0	0	28 (7.0)
Enterococci spp	0	2	0	0	2 (0.5)
Total	136	95	101	62	394 (100)

[Table/Fig-1]: The prevalence of various bacterial isolated from different places.
CoNS: Coagulase-Negative *Staphylococci*

Sensitivity of Bacteria to Antibiotics

The results of antibiogram tests are summarized in [Table/Fig-2,3]. According to these results, bacterial isolated from hospital environments had a significantly higher level of resistance against various antibiotics than the resistance of bacterial isolated from non hospital environments (p-value<0.05).

DISCUSSION

In recent years, house flies have been recognized as a potential agent to mechanically transport pathogenic bacteria [23]. One of the objectives of the present study was to address this question that which type or types of bacteria are likely to be transported by these flies.

The results of our study demonstrated that all house flies were capable to carry at least one type of bacteria. Moreover, it was

Bacteria	Antibiotic										
	T No. (%)	GM No. (%)	K No. (%)	CTX No. (%)	CF No. (%)	AM No. (%)	C No. (%)	CP No. (%)	FM No. (%)	SXT No. (%)	E No. (%)
E. coli	6 (25)	2 (8.3)	8 (33.3)	19 (79.1)	ND	11 (45.8)	0 (0)	17 (70.8)	5 (20.8)	5 (20.8)	12 (50)
Klebsiella spp.	10 (31.2)	0 (0)	3 (9.3)	32 (100)	ND	18 (56.2)	11 (34.3)	26 (81.2)	21 (65.6)	0 (0)	20 (62.5)
Citrobacter spp.	0 (0)	0 (0)	0 (0)	6 (54.5)	ND	11 (100)	0 (0)	3 (27.2)	0 (0)	0 (0)	11 (100)
Enterobacter spp.	0 (0)	0 (0)	0 (0)	11 (64.7)	ND	15 (88.2)	2 (11.7)	6 (35.2)	16 (94.1)	1 (5.8)	15 (88.2)
Proteus spp.	6 (19.3)	0 (0)	2 (6.4)	10 (32.2)	ND	7 (22.5)	0 (0)	20 (64.5)	0 (0)	5 (16.1)	7 (22.5)
Pseudomonas spp.	4 (5.7)	3 (4.3)	4 (5.7)	46 (66.6)	ND	7 (10.1)	1 (1.4)	30 (43.4)	1 (1.4)	0 (0)	2 (2.8)
Bacillus spp.	12 (70.5)	1 (5.8)	2 (11.7)	13 (76.4)	3 (17.6)	0 (0)	11 (64.7)	11 (64.7)	ND	0 (0)	15 (88.2)
Staphylococcus aureus	4 (16)	1 (4)	4 (16)	8 (32)	5 (20)	4 (16)	1 (4)	15 (60)	ND	1 (4)	2 (8)
Enterococci spp.	0 (0)	0 (0)	0 (0)	2 (100)	2 (100)	1 (50)	0 (0)	1 (50)	ND	0 (0)	0 (0)

[Table/Fig-2]: The antibiotic resistance of bacterial isolated from hospital environments.
T=Tetracycline, GM= Gentamycin, K=Kanamycin, CTX=Cefotaxime, CF=Cephalothin, AM=Ampicillin, C=Chloramphenicol, CP=Ciprofloxacin, FM=Nitrofurantoin, SXT=Trimethoprim-Sulfamethoxazole, E=Erythromycin ND= Not done

Bacteria	Antibiotic										
	T No. (%)	GM No. (%)	K No. (%)	CTX No. (%)	CF No. (%)	AM No. (%)	C No. (%)	CP No. (%)	FM No. (%)	SXT No. (%)	E No. (%)
E. coli	0 (0)	0 (0)	1 (4.5)	20 (90.9)	ND	2 (9)	1 (4.5)	15 (68.1)	4 (18.1)	0 (0)	1 (4.5)
Klebsiella spp.	0 (0)	0 (0)	2 (40)	5 (100)	ND	0 (0)	2 (40)	5 (100)	2 (40)	0 (0)	2 (40)
Proteus spp.	1 (1.6)	1 (1.6)	3 (5)	32 (54.2)	ND	0 (0)	0 (0)	17 (28.8)	2 (3.3)	2 (3)	8 (13.5)
Pseudomonas spp.	1 (1.8)	2 (3.7)	0 (0)	41 (75.9)	ND	2 (3.7)	1 (1.8)	18 (33.3)	0 (0)	0 (0)	2 (3.7)
Bacillus spp.	3 (12.5)	2 (8.3)	4 (16.6)	20 (83.3)	4 (16.6)	2 (8.3)	3 (12.5)	10 (41.6)	ND	0 (0)	18 (75)
CoNS	10 (16.9)	0 (0)	8 (13.5)	22 (37.3)	12 (20.3)	6 (10.2)	0 (0)	28 (47.4)	ND	9 (15.2)	10 (16.9)

[Table/Fig-3]: The antibiotic resistance of bacterial isolated from non hospital environments.
CoNS=Coagulase-Negative Staphylococci. T=Tetracycline, GM= Gentamycin, K=Kanamycin, CTX=Cefotaxime, CF=Cephalothin, AM=Ampicillin, C=Chloramphenicol, CP=Ciprofloxacin, FM=Nitrofurantoin, SXT=Trimethoprim-Sulfamethoxazole, E=Erythromycin ND= Not done

observed that house flies collected from hospital environments carried a significantly higher number of bacteria than those flies collected from non hospital environments (p-value<0.05). In overall, 231 bacterial strains were found in house flies collected from hospital environments and 163 bacterial strains were found in those collected from non hospital environments [Table/Fig-1]. The previous study has demonstrated that the types and number of bacteria that may be transported by house flies differ from one country to another [24]. However, the important point in this regard is that all these studies have emphasized that house flies are an important carrier of pathogenic bacteria.

De Jesús AJ et al., conducted a study on house flies captured from various food products; they reported that the most prevalent type of bacteria transported by these flies was *E. coli* [23]. However, this finding is not in line with what we observed in the present study. This contradiction can be attributed to the different places where the flies were captured in these two studies. In another study, Liu Y et al., collected a total number of 1228 house flies from an airport located in Shanghai, China [25]. They extracted only 48 bacterial strains from the captured flies which is totally different to what observed in the present study, both in terms of numbers and types of bacteria, hence, it can be concluded that the type and number of bacteria may be carried by house flies to a high degree is a function of place where these flies are captured. It is explained by Akhtar M et al., that house flies are an important carrier of various types of bacteria, particularly *Staphylococcus epidermidis*, *Staphylococcus lantos*, *Streptococcus sanguinis*, and various species of *Bacillus yersinia*, which is in agreement with the results of the present study [26]. In the same vein, Rosef O and Kapperud G conducted a study on house flies living in poultries and pig farms. They reported that there was a considerable number of different species of *Campylobacter* bacteria on the body surface and in stomach contents of these flies [27]. However, these results cannot be compared with the results of the present study, because we did not attempt to extract this type of bacteria from the captured flies and concentration was on routine bacteria. In this regard, several studies also have been done by Sulaiman S et al., in Malaysia [28,29]. They reported that diversity of bacteria was higher in flies captured from poultries which is not comparable with the results of the present study, because we found that the number and types of bacteria was higher in flies captured from hospitals than non hospital environments with a lower level of general hygiene. The flies investigated in the present study have been collected from the vicinity of trash cans and where hospital wastes were collected for disposing. A poor level of sanitation normally existed in such areas.

Another objective of the present study was to evaluate the sensitivity of the isolated bacteria to various commercially available antibiotics. The results of this set of investigations showed that the resistance of bacterial isolated from hospital environments was significantly higher than those of bacterial isolated from non hospital environments (p-value<0.05). Recent studies demonstrated that house flies play an important role in spreading antibiotic resistant genes among bacteria. Petridis M et al., reported that horizontal transfer of resistant genes and virulence can take place in the gastrointestinal tracts of house flies [30]. Furthermore, Akhtar M et al., illustrated that plasmids can mediate the horizontal transfer of resistant genes in the gastrointestinal tracts of house flies [26]. This issue

demonstrates that house flies not only are a mechanical carrier of bacteria, but also they can act as a biological agent by providing an environment in their bodies for growth and reproduction of various bacterial strains. Bacteria isolated from house flies which were captured in hospital environments showed a high level of resistance against all antibiotics other than gentamycin and cephalothin (p-value <0.05). These results are different to those of Liu Y et al., who reported that all bacteria were resistant against such antibiotics as amoxicillin [25], tetracycline, cephalothin, and cefuroxime, while sensitive to meropenem and imipenem. One of the main reasons why the results of these studies differ from each other is because of the geographical and hygiene level differences between countries where these two studies were carried out. Moreover, the higher level of resistance in bacteria isolated from hospital house flies was expectable, because hospital house flies contain virulence genes which make them able to induce diseases in hospital environments [31,32]. Furthermore, these bacteria can genetically transfer these resistant genes to other bacteria within or out of their species.

LIMITATION

Because a wide range of diagnostic tests was required to determine each strain, but it was not practical and financially feasible for the present study so, we decided to stop the differentiation tests at the species level in some isolates.

CONCLUSION

Houseflies are a source of bacterial contamination and act as a mechanical carrier of such flies. Moreover, they can induce some bacterial diseases and this is a well-known fact. According to the results of the present study, it can be postulated that house flies play a major role in spreading antibiotic resistant bacteria. However, the flies from hospital environments were more contaminated, mainly because the people who refer to health centers are normally ill and carry various pathogens. Accordingly, it is highly necessary to control such flies effectively. Further, hospital environments should be controlled using administrative procedures.

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